Clinica Chimica Acta

Vol. 530/S1 (2022)

Clinica Chimica Acta

International Journal of Clinical Chemistry and Diagnostic Laboratory Medicine

Aims and Scope

Clinica Chimica Acta publishes original Research Communications in the field of clinical chemistry and laboratory medicine, defined as the diagnostic application of chemistry, biochemistry, immunochemistry, biochemical aspects of hematology, toxicology, and molecular biology to the study of human disease in body fluids and cells. The objective of the journal is to publish novel information leading to a better understanding of biological mechanisms of human diseases, their prevention, diagnosis, and patient management. Reports of an applied clinical character are also welcome. Papers concerned with normal metabolic processes or with constituents of normal cells or body fluids, such as reports of experimental or clinical studies in animals, are only considered when they are clearly and directly relevant to human disease. Evaluation of commercial products have a low priority for publication, unless they are novel or represent a technological breakthrough. Studies dealing with effects of drugs and natural products and studies dealing with the redox status in various diseases are not within the journal's scope.

Development and evaluation of novel analytical methodologies where applicable to diagnostic clinical chemistry and laboratory medicine, including point-of-care testing, and topics on laboratory management and informatics will also be considered.

Typescripts submitted to *Clinica Chimica Acta* should not have been published previously and should not be under consideration for publication elsewhere. The agreement of all named authors to the submission must be affirmed. Relevant ethical approval must be noted for investigations involving human or animal subjects. Authors are invited to consult any member of the Editorial Board, if in doubt about any aspect of scope, format or content of a proposed paper.

Online submission of papers

Authors are requested to submit their manuscripts electronically, by using the EESubmit submission tool at http://ees.elsevier.com/cca/. After registration, authors will be asked to upload their article, an extra copy of the abstract, and associated artwork. The submission tool will generate a PDF file to be used for the reviewing process. The submission tool generates an automatic reply, which incorporates the manuscript number for future correspondence.

For a full and complete Guide for Authors, please go to: http://www.elsevier.com/locate/clinchim

Publication information: Clinica Chimica Acta (0009-8981). For 2022, volumes 524–537 are scheduled for publication. Subscription prices are available upon request from the Publisher or from the Elsevier Customer Service Department nearest you or from this journal's website (http://www.elsevier.com/locate/clinchim). Further information is available on this journal and other Elsevier products through Elsevier's website (http://www.elsevier.com). Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request. Claims for missing issues should be made within six months of the date of dispatch.

Author inquiries
You can track your submitted article at http://www.elsevier.com/track-submission. You can track your accepted article at http://www.elsevier.com/trackarticle. You are also welcome to contact Customer Support via http://service.elsevier.com.

Orders, claims, and journal inquiries: please contact the Elsevier Customer Service Department nearest you:

St. Louis: Elsevier Customer Service Department, 3251 Riverport Lane, Maryland Heights, MO 63043, USA; phone: (877) 8397126 [toll free within the USA]; (+1) (314) 4478878 [outside the USA]; fax: (+1) (314) 4478077; e-mail: JournalCustomerService-usa@elsevier.com

Oxford: Elsevier Customer Service Department, The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK; phone: (+44) (1865) 843434; fax: (+44) (1865) 843970; e-mail: JournalsCustomerServiceEMEA@elsevier.com

Tokyo: Elsevier Customer Service Department, 4F Higashi-Azabu, 1-Chome Bldg, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044, Japan; phone: (+81) (3) 5561 5037; fax: (+81) (3) 5561 5047; e-mail: JournalsCustomerServiceJapan@elsevier.com

The Philippines: Elsevier Customer Service Department, 2nd Floor, Building H, UP-Ayalaland Technohub, Commonwealth Avenue, Diliman, Quezon City, Philippines 1101; phone: (+65) 63490222; fax: (+63) 2 352 1394; e-mail: JournalsCustomerServiceAPAC@elsevier.com

Advertising information: If you are interested in advertising or other commercial opportunities please e-mail Commercialsales@elsevier.com and your inquiry will be passed to the correct person who will respond to you within 48 hours.

Sponsored Supplements and/or Commercial Reprints: For more information please contact Elsevier Life Sciences Commercial Sales, Radarweg 29, 1043 NX Amsterdam, The Netherlands; phone: (+31) (20) 485 2939 / 2059; e-mail: LSCS@elsevier.com.

Funding Body Agreements and Policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English Language Editing service available from Elsevier s WebShop (http://webshop.elsevier.com/languageediting/) or visit our customer support site (http://service.elsevier.com) for more information.

Illustration services

Elsevier's WebShop (http://webshop.elsevier.com/illustrationservices) offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medicalstyle images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper)

Printed by Printforce, Bedfordshire, U.K

IFCC WorldLab
SEOUL 2022
24TH INTERNATIONAL CONGRESS OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE
16TH ASIA-PACIFIC CONGRESS OF CLINICAL BIOCHEMISTRY

ABSTRACT BOOK

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Special Issue
WorldLab 2022

Advanced Technology
Analytical Technology
Atherosclerosis
Autoimmune Disease, Allergy
Biobanking
Bioinformatics, Data Management
Biomarker Discovery
Bone Metabolism
Cancer, Tumor Marker, Circulating Tumor Nucleic Acid
Cardiovascular Disease
Critical Care, Blood Gases, POCT
Education, Training
Endocrinology
Ethics Evidence Based Medicine, Guidelines
Gastrointestinal Disease
Geriatric Medicine
Hematology, Hemostasis
Hepatobiliary Disease
Immunodeficiency
Infection Control
Inflammation, Vascular Disease, Oxidative Stress
Inherited Disease, Metabolic Disorder
Kidney Disease, Urinalysis
Laboratory Information Technology
Laboratory Management
Laboratory Statistics
Method Evaluation, Reference Interval, Decision Levels
Microbiology, Infection Disease
Miscellaneous
Molecular Diagnostics, Genetic Testing
Neurodegenerative Disease
Neurological Disorder,
Nutrition, Vitamins, Trace Elements
Obesity, Metabolic Syndrome, Diabetes
Pediatric Medicine
Personalized Medicine, Pharmacogenetics
Quality Assurance, Accreditation
Rare Disease
Reproductive Medicine, Pregnancy
Sampling, Storage, and Physiological Variations
Sports Medicine
Standardization, Harmonization
Therapeutic Drug Monitoring, Toxicology
Transfusion Medicine
Transplantation Laboratory Medicine
Speaker Abstract
Educational Abstract

doi:10.1016/S0009-8981(22)01157-3

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

CONGRESS PRESIDENT

Won-Ki Min
University of Ulsan, Korea

CONGRESS ORGANIZING COMMITTEE

Organizing Committee Chair
Junghan Song
Seoul National University, Korea

Scientific Programme Chair
Sail Chun
University of Ulsan, Korea

Secretary General
Yeo-Min Yun
Konkuk University, Korea

President of KSCC
Jehoon Lee
The Catholic University of Korea, Korea

Secretary of KSLM
Sang Hoon Song
Seoul National University, Korea

IFCC Executive Board
Sunil Sethi
National University Hospital, Singapore

IFCC Corporate Member
Douglas Chung
Abbott Diagnostics Division, APAC

IFCC C-CC
Helen Martin
Healthscope Pathology, Australia

PCO
Stefano Montalbetti
MZ Events, Italy

SCIENTIFIC PROGRAM COMMITTEE

Chair
Sail Chun
University of Ulsan, Korea

Sung-Eun Cho
Green Cross Laboratories

Robert L. Fitzgerald
University of California, USA

Philippe Gillery
Hôpital Maison Blanche CHU de Reims, France

Daniel T. Holmes
St. Paul's Hospital, Canada

Graham Jones
St Vincent's Pathology in Sydney Australia, Australia

Soo-Youn Lee
Sungkyunkwan University, Korea

Yongwha Lee
Soon Chun Hyang University, Korea

Woochang Lee
University of Ulsan, Korea

Masato Maekawa
Hamamatsu University, Japan

Nader Rifai
Boston Children's Hospital, USA

Namhee Ryoo
Keimyung University, Korea

Cheng Bin Wang
Chinese PLA General Hospital, China

CONGRESS ORGANIZING SECRETARIAT

MZ EVENTS s.r.l.
Via Carlo Farini 81
20159 Milano (Italy)
Phone: +39 02 66802323
Fax: +39 02 6686699
E-mail: info@seoul2022.org

doi:10.1016/S0009-8981(22)01158-5

Clinica Chimica Acta 530 (2022) S1–S7

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Advanced Technology

M001

A novel biosensor for vitamin D detection based on resistance changes on a zinc oxide-copper oxide nano-crystalline composite

J. Kiely, R. Luxton, E. Sharif

Institute of Bio-sensing Technology, University of the West of England, Frenchay Campus, UK

Background-Aim

In the current study a biosensor based on ZnO-Cuo nano-crystals was used to investigate vitamin D captured antibody using a fast and low-cost fabrication method which applies colloidal distribution that is improved using sonication.

Methods

A uniform nano-crystalline sensor surfaces on glass cover slips utilising 1% concentrations of 1:2 zinc oxide and copper oxide nanocrystal suspensions was employed based on previous study (Lu article 15). Impedance spectroscopy was used to examine the sensor surfaces and to confirm the reproducibility of the dose response of vitamin D antigen. Changes in Resistance values based in 5 frequency readings, were used to establish dose dependent responses for Vitamin D antigen.

Results

A limit of detection of less than 24.1 ng/ml was demonstrated for nano-surfaces fabricated from concentrations of 1:2 ZnO/CuO.

Conclusions

The ZnO-CuO nano-crystalline sensor surfaces with and without vitamin D captured antibody on sensor surface were analyzed using scanning electron microscopy (SEM).

doi: 10.1016/j.cca.2022.04.291

M002

A shortened detection method was developed to reduce the turnaround time of amino acids on biochrom 30+ cation exchange HPLC

N. Yousufzai [a], H. Majid [b], A. Jamil [a], S. Abdul Mateen [a], S. Ahmed [b], L. Jafri [b], A. Habib Khan [b]

[a] *Biochemical Genetics laboratory, Aga Khan University, Karachi, Pakistan*
[b] *Department of Pathology and Laboratory Medicine, Aga Khan University, Pakistan*

Background-Aim

Due to high work load, the turnaround time (TAT) of amino acid would be reduced. So therefore, the running program of amino acid was shared with Biochrom company support, with their help some changes were done in program like flow rate and temperature. To optimize new program of amino acid (AA) to reduce the turnaround time using cat ion exchange high-pressure liquid chromatography (HPLC).

Methods

This intervention study was done at the biochemical genetic laboratory, Aga Khan University Hospital from January 2019 to Dec 2019. Quantitative analysis of AA was performed by cation exchange-high performance liquid chromatography on Biochrom 30+ Amino acid analyzer (Biochrom, US). The full AA profile was performed in 156 minutes. A shortened detection method was developed on Biochrom for separation and quantification amino acid using nor leucine as the internal standard using 200 × 4.6 mm physiological high performance column, standard lithium citrate buffers and standard physiological separation program. Optimization was done by accuracy and precision.

Results

The TAT of amino acids was reduced from 155.5 minutes to 131.5 and the throughput was improved from 9 samples/day to 11 samples/day with no significant effect noted on peak resolution or peak shape.

Precision study on HPLC showed cumulative CV of 6.74% (range 3.43–13.27) for 22 AA using control samples. For accuracy, samples of Proficiency testing and standards were run in triplicate. Cumulative accuracy of 22 Amino acid was 99.6 (range 96.8–101).

Conclusions

We optimized a short program for amino acid which led to increased throughput with no effect on overall AA analysis.

doi: 10.1016/j.cca.2022.04.292

M003

Cytogenetic mechanisms of the aortic coarctation formation and their relationship with late postoperative complications

D.S. Goncharov, G.A. Khugaev, A.V. Zubko

A.N. Bakulev National Medical Research Center of Cardiovascular Surgery, Moscow, Russia

Background-Aim

Coarctation of the aorta is adding up to about 6% among patients with CHD, and up to 10% among critical defects.

Methods

The first part of this research work is the analysis group of clinical samples by statistics methods. The second part of our research work is comprised of bioinformatical analysis and classification of samples. Conclusions based on available literature are processed by mathematical modeling techniques. PCR analysis of mutations pathogen significant genes. Light-optical microscopy of tinctorial lines of histological samples. The study of cell markers and substrates of the intercellular matrix of histological samples of interest is carried out by immunofluorescence microscopy and ELISA.

Results

This pathology of the aortic wall in the early stages of embryogenesis originates in the regulatory links of the elastogenesis and the formation of the architectonics of elastin matrix of the intimal layer and intimal-medial border. Taking into account all the syndromic variables and bioinformatic search for polygenic determinants, a histological study of the morphology of the vascular wall was performed. It was shown that structural disorganization of the intimal layer, attrition of the intimal-medial border, weak expression of elastino / collagen fibers correlate with the increase in the share of the cellular component in the intimal layer. The study is currently focused on assessing architectonics of elastin matrix and searching for genetic polymorphisms of genes such as fibulin-5 (integrin ligand), NOTCH1, genes of the mesenchymal-epithelial reorganization of the cytoskeleton (Rac 1, Cdc 42).

Conclusions

Thus, the purpose of this work is to search for the pathology activation resulting in altered elastin-mesenchymal interaction. According to the literature, it is quite possible that the existing violations of the gene regulation of the processes of differentiation and elastogenesis are reversible from the standpoint of attracting selective micro-RNA interference as a mechanism of secondary gene therapy.

doi: 10.1016/j.cca.2022.04.293

M004

Data mining: Seasonal fluctuations in thyroid stimulating hormone and lipid profiles

D. Wang

Peking Union Medical College Hospital, China

Background-Aim

Thyroid stimulating hormone (TSH) is associated with lipid metabolism. In this study, we aimed to evaluate the seasonal variation in TSH and lipid profiles based on clinical big data.

Methods

A total of 20,444 individuals who visited Peking Union Medical College Hospital for routine health check-ups from 2014 to 2018 were enrolled for retrospective analysis. Demographic, medical history, common biochemical analytes, and thyroid related tests data were obtained. A Kruskal-wallis H analysis was used to compare the differences in total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), and low- density lipoprotein cholesterol (LDL-C) by TSH quartiles. We analysed correlation between TSH and lipid profiles as well as with seasons and temperature.

Results

TC and LDL did not vary significantly with TSH concentration; however, TG and LDL-C did. TSH concentration showed weak positive correlation with serum TC, TG, and HDL-C but not with LDL-C. Serum TC concentration was positively correlated with TG, HDL-C, and LDL-C. TG was positively correlated with LDL-C but negatively correlated with HDL-C. HDL-C was negatively correlated with LDL-C. The TSH and lipids showed similar seasonal fluctuations, reaching their peaks in winter and minimums in summer. The concentrations of TSH, TC, TG, HDL-C, and LDL-C were all negatively correlated to median temperature.

Conclusions

Seasonal variation was observed in both TSH and lipids. TC, TG, HDL-C, and LDL-C were negatively correlated with temperature.

doi: 10.1016/j.cca.2022.04.294

M005

Establishing healthy distribution for thyrotropin receptor antibodies, thyroid stimulating immunoglobulin and thyroid stimulating blocking antibody for individuals in Beijing, China

C. Ma, X. Cheng, Y. Hu, A. Song, L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China

Background-Aim

Autoimmune thyroid disease involves thyrotropin receptor antibodies (TRAb), thyroid stimulating immunoglobulin (TSI) and thyroid stimulating blocking antibody (TSBAb), which are of great value in the diagnosis of disease. However, the distribution of the three antibodies in the health individuals has not been established. This study will establish a healthy distribution of the three antibodies in the Chinese population with suitable methods to provide theory basis for diagnosis of autoimmune thyroid disease

Methods

In total, 120 apparently healthy individuals were included in this study by questionnaire. Thyrotropin binding inhibitor immunoglobulin (TBII) assay was used for the measurements of TR-Ab, IMMULITE 2000 TSI assay for TSI, and enzyme linked immune sorbent assay (ELISA) for TSBAb.

Results

The baseline level of common biochemical analytes of individuals enrolled in the study were in the normal range. The distribution of TR-Ab in males was significantly higher than that in females ($P < 0.05$), while there was no statistical difference for TSB-Ab measurements between males and females. The healthy distribution of TRAb, TSI and TSBAb were established.

Conclusions

TRAb and TSBAb are dispersed in healthy individuals, while TSI cannot be quantified in healthy individuals due to methodological reasons. We suggest that TSBAb and TRAb health distribution be taken into account when diagnosing autoimmune thyroid disease.

doi: 10.1016/j.cca.2022.04.295

M006

Validation of serum methotrexate levels by tandem mass spectrometry

F. Sak [a], S. Abusoglu [a], D. Eryavuz Onmaz [a], G. Abusoglu [b], A. Unlu [a]

[a] *Department of Biochemistry, Selcuk University Faculty of Medicine, Konya, Turkey*
[b] *Department of Medical Laboratory Techniques, Vocational School of Health, Konya, Turkey*

Background-Aim

Methotrexate (MTX) is widely used for the treatment of different kinds of malignancy, psoriasis, rheumatological diseases, and the medical termination of pregnancy. The aim of this study was to validate a mass spectrometric method for determination of serum methotrexate levels.

Methods

This study was carried out between November 2019 and January 2020. MTX levels were measured on API 3200 (ABSCIEX) Systems via Phenomenex Luna C18 (3μm, 4.6 × 50mm) column. Briefly, 250 μL of sample or standard was taken and 60 μL of HCl with a pH of 3 was added. 100 μL of internal standard was added and then dissolved in 500 μL of methanol. The mixture was vortexed for 30 sec and centrifuged at 13.000 rpm for 15 minutes. After centrifugation, 200 μL of mixture were seperated from the supernatant and 40 μL was injected to mass system.

Results

Method was linear between 0.3-2500 ng/mL ($r2 = 0.992$). Recovery values ranged between 94-108%. CV values were 4.6 and 4.5% for 39 and 2500 ng/mL methotrexate concentrations.

Conclusions

This method is robust and provides chromatographic seperation to improve specificity in the therapeutic drug monitoring laboratory. The validated LC-MS/MS method can be successfully applied to the routine therapeutic drug monitoring of MTX in clinical laboratories.

doi: 10.1016/j.cca.2022.04.296

M007

Preparative purification of albumin: Current status and trends

N. Shurko, T. Danysh, S. Mylyashkevich, V. Novak

State Institution «Institute of Blood Pathology and Transfusion Medicine NAMS of Ukraine», Ukraine

Background-Aim

Background. Albumin is a nonglycosylated, single-chain polypeptide, a multifunctional protein in plasma, with an average concentration of 40 g $L-1$, comprising 585 amino acids in its mature form and molecular weight of 66.5 kDa. An albumin is a basic protein of organism and has numerous irreplaceable functions. Concentrates of albumin are utilized during operative interferences, in intensive therapy, at traumas and burns, diseases of buds and liver et al.

The synthetic dyes play an important role as affinity ligands in protein purification aspecialy of albumin. Dyes have the advantage in comparison to biological ligands from the perspective of cost, ease of immobilization, security, stability and adsorption capacity.

The aim of the work: optimization of a process obtaining albumin by the use dye-ligand affinity chromatography.

Methods

Synthesis of sorbents, dye-ligands affinity chromatography, methods for determining concentration of albumin, statistical data analyses.

Results

The ethyl alcohol fractionation method is used in industry for blood protein‘s fractionation, however the purity of plasma proteins is not sufficient enough. The chromatography considered the best choice for albumin and other protein‘s purification.

Dye-ligand chromatography developed as an important method for albumin purification because they offer many advantageous over other forms of affinity chromatography. The following substances were used in the work: macropore silica matrix based on Diasorb-aminopropyl 800/70, fraction 0.25–0.50 μm of pore sizes 1500 Å, kovalently modified by the following triazine and vinyl-sulfon active dyes: Cibacron Brilliant Yellow 3GP, Procion Blue HB, Procion Blue MXR, Procion Yellow HE3G, Procion Gelb M4R, Procion Red MX5B, Reactive Brown 10, Reactive Red 120, Reactive Green 5, Reactive Green 19, Active bright orange KH, Active violet 4K, Active bright blue KH, Active bright red 5SH, Active burgundy 4SG and Active purple 4GT

We selected to best match the purification of albumin: Diasorb-Active purple 4GT, Diasorb-Procion Gelb M4R, Diasorb-Procion Blue HB, Diasorb-Procion Blue MXR and Diasorb-Active bright blue KH. The Diasorb-Active violet 4K absorbs of albumin the lowest. The selected sorbents bound 74-86 % of albumin and preservation of up to 73.4 – 81.2 % of the initial concentration.

The elution of albumin was performed according to the following scheme: 1 M NaCl, 50 mm Tris-HCl buffer, pH 7.4; 0.25 M ∑-ACC, 25

% iPro, 50 mm Tris-HCl buffer, pH 7.4; 1 M NaCl, 25 % iPro, 50 mm Tris-HCl buffer, pH 7.4.

Conclusions

Sorbents were selected that can be used in the technology of purification of albumin. Established that sorption of albumin with sorbents was carried out by hydrophobic and ionic interaction.

doi: 10.1016/j.cca.2022.04.297

M008

AI-Based genome analysis for NIPT and liquid biopsy

C. Ki

GC Genome, South Korea

Background-Aim

The advancement of clinical genetics and genomics is accompanied by innovations in molecular technologies as well as data analysis technologies. For the molecular technologies, two key technologies made the greatest impact on the clinical genetics and genomics including the Sanger sequencing technology invented in the mid-1970s and polymerase chain reaction (PCR) technology developed in the mid-1980s. Based on these two technologies, the field of molecular microbiology and molecular diagnostics of genetic conditions has been able to make remarkable progress for about 20 years since 1990s and now clinical genomics field becomes the most rapidly evolving specialty in medicine.

Methods

Sanger sequencing has been monopolistic position for almost two decades since it was invented by Frederick Sanger in 1977. However, novel sequencing technologies called Next-Generation Sequencing (NGS) finally appeared in early 2000s and they are becoming a power to change the foundation of medical field in conjunction with the 4th Industrial revolution.

The evolution of high-throughput molecular technologies such as NGS have accelerated the accumulation of unprecedented amount of genomics data, which have changed traditions of genetic data analysis. In the Sanger sequencing era, genetic data were manually analyzed by trained doctors or technicians, which is impossible in the NGS era.

Results

Usually, genomic data produced by NGS technologies are processed by automated algorithms including read mapping, variant calling, annotation and interpretation. In addition, detection of genomic changes such as large deletions or duplications, genomic instabilities, translocations, and fusions are also analyzed by various algorithms. Nevertheless, these data are still checked and further analyzed by trained bioinformaticians or doctors. Artificial intelligence (AI) refers to 'the simulation of human intelligence in machines that are programmed to think like humans'. Advances in AI software such as deep learning algorithms has led to a big changes in the medical field including radiology and pathology. In clinical genomic analysis field, AI is still new but needs attention for better performance.

Conclusions

In this talk, I'll present recent updates on AI-based genome analysis especially emphasizing on the non-invasive prenatal testing (NIPT) and non-invasive cancer diagnosis using cell-free DNA (ctDNA) called liquid biopsy.

doi: 10.1016/j.cca.2022.04.298

M009

AI-based microbiome analysis

J.S. Song

GC Genome, Youngin, Gyeonggi-do, South Korea

Background-Aim

The human body harbors an enormous number of microbiota that play critical roles on human health and diseases. Numerous studies have revealed that dysbiosis of human microbiome in various parts of the body is associated with the pathogenesis of most of diseases in the body. These advancements in our understanding of human microbiome are due to the revolutions in sequencing technology with vastly increased amounts of metagenomic sequencing data and advanced computational methods from bioinformatics.

Machine learning is a method of data analysis that aimed to generate predictive models based on an underlying algorithm and a given dataset and is becoming to applied to biology ubiquitously. With decreasing sequencing costs and increasing of the size of datasets in microbiome studies, machine-learning methods have also been gradually applied to microbial studies for accurately predicting disease.

In this talk, I'll present applications of machine-learning analysis on microbiome data and the real experience of machine-learning analysis for predicting hypertension using gut microbiome data.

Methods

Not applicable.

Results

Not applicable.

Conclusions

Not applicable.

doi: 10.1016/j.cca.2022.04.299

M010

AI-based genome analysis for non-invasive prenatal testing (NIPT)

C. Ki

GC Genome, South Korea

Background-Aim

The advancement of clinical genetics and genomics is accompanied by innovations in molecular technologies as well as data analysis technologies. Since early 2000s, innovative sequencing technology called Next-Generation Sequencing (NGS) have been developed and are becoming a power to change the foundation of medical field in conjunction with the 4th Industrial revolution.

The evolution of high-throughput molecular technologies such as have accelerated the accumulation of unprecedented amount of genomics data, which have changed traditions of genetic data analysis. In the Sanger sequencing era, genetic data were manually analyzed by trained doctors or technicians, which is impossible in the NGS era.

Non-invasive prenatal testing (NIPT) is a method of estimating the risk that a fetus will born with certain genetic abnormalities, especially chromosomal aneuploidies such as Down syndrome (trisomy 21), Edward syndrome (trisomy 18), and Patau syndrome (trisomy 13). Traditionally, maternal serum testing and fetal ultrasonography have been used to screen fetal aneuploidies but NGS is replacing these traditional screening tools based on the low false-positive rate, high sensitivity and specificity.

Genomic data produced by NGS technologies are processed by automated algorithms including read mapping, variant calling, annotation and interpretation. In addition, detection of genomic changes such as large deletions or duplications, genomic instabilities, translocations, and fusions are also analyzed by various algorithms. Nevertheless, these data are still checked and further analyzed by trained bioinformaticians or doctors.

Artificial intelligence (AI) refers to 'the simulation of human intelligence in machines that are programmed to think like humans'. Advances in AI software such as deep learning algorithms has led to a big changes in the medical field including radiology and pathology. In clinical genomic analysis field, AI is still new but needs attention for better performance. In this talk, I'll present recent updates on the application of AI-based genome analysis for NIPT.

Methods

Not applicable.

Results

Not applicable.

Conclusions

Not applicable.

doi: 10.1016/j.cca.2022.04.300

M011

Advancements in digital health in monitoring of diseases

U. Dubey[b], A. Ranjan[a], H. Dubey[a]

[a] *All India Institute of Medical Sciences, New Delhi, India*
[b] *Dr. Akhilesh Das Gupta Institute of Technology &Management, New Delhi, India*

Background-Aim

Technological development in monitoring of a person's health is the need of time.

Methods

Digital health is presently in nascent stage, so I am trying to analyse Literature reviews.

Results

Wearable health technology, Fitness bands: It is used to monitor health. It has sensors which measures their heart rate, blood pressure and temperature, sleeping patterns etc.

Smart piezoelectric necklaces: It uses artificial Intelligence. It detects swallowing of medications. During swallowing its piezoelectric sensor converts neck movements into electrical signals for transmission in to a smart phone apparatus to be read.

Implantable and ingestible sensors: Advancements in microelectronics, data processing and wireless communication have resulted in to the devices which can be implanted under the skin or ingested. It allows continuous and unobtrusive monitoring of key vital signs, like heart rate. It can also alert patients and caregivers if a problem is detected. Wearable therapeutic devices: Optune system, wearable over head is used to treat glioblastoma. It delivers electric signals to stop cell division & cancer growth. Pain-relief in neuro-technology: Quell a band that is wrapped around calf uses electricity to block pain signals. Automated home-based monitoring: Pain can be managed with telecare. Scheduled telephone calls may be placed for necessary advice. Home blood tests: A remote monitoring device is under research to test a drop of blood at home to measure white cells count, hemoglobin.

Conclusions

Digital technology has created innovative models of healthcare delivery that empower people living with diseases and also for health conscious people.It has made life of health care workers easy in monitoring the recover progression of a patient. It is being widely accepted.

doi: 10.1016/j.cca.2022.04.301

M012

Filterless-filter: Centrifuge-free microfluidic cell separation technology

M. Kang[e], T. Shin[e], Y. Lee[e], S. Shin[e], S. Seungjung[e], C. Sungyoung[a], A. Seong Soo A[b], S. Jaewoo[d], S. In Bum[c], Y. Hoyoung[e]

[a] *Department Of Biomedical Engineering, Hanyang University, Seoul, Republic of Korea*
[b] *Department Of BionanoTechnology, Gachon University, Sungnamsi, Gyeonggido, Republic of Korea*
[c] *Department Of Laboratory Medicine, Kangwon National University College Of Medicine, Kangwondo, Republic of Korea*
[d] *Department Of Laboratory Medicine, Yonsei University College Of Medicine, Seoul, Republic of Korea*
[e] *R&D, Curiosis Inc., Seoul, Republic of Korea*

Background-Aim

Cell separation is the beginning and the end of every cell-based R&D and manufacturing. Cell separation is required in numerous applications, such as medium exchange for typical cell culture, white blood cell separation (buffy coat) for gDNA extraction or diagnosis, peripheral blood mono-nucleus cell (PBMC) isolation for cell therapy product development, and blood plasma separation for diagnostic applications.

Despite long history of biological researches and progresses, still the centrifugation is the most common and golden standard method for cell separation from medium or other cells with respect to the density differences. However, centrifugation occurs at high centrifugal force, hence the physical pressures can damage or stress the cells. Furthermore, majority of centrifugation is performed at the outside of clean benches, exposing cells to the potential dangers of contaminations and accidents.

Methods

Injection molded microfluidic device was designed and fabricated for cell separation. The microfluidic device contains slanted microgroove arrays which can induce the secondary flow. In order to verify the cell separation efficiency, cell lines (suspension: Jurkat, HL60, Raji, K562, adherent: NIH3T3, HeLa, MCF7) and whole blood were used. Specially designed syringe pump was used to operate microfluidic device.

Results

Filterless-filter technology applied microfluidic cell separation device separated cells from medium successfully. The separated cells were also concentrated while separation. The suspension cells were found to be concentrated 23.52 times and 21.37 times in low(~10^5 cells/ml) and high(~10^6 cells/ml) concentration cells, respectively. Enrichment of 24.55 times and 21.93 times was also observed in adherent cells at low(~10^5 cells/ml) and high(~10^6 cells/ml) concentrations, respectively. The cell recovery rate was 98.44%. White blood cells(WBC) could be separated and concentrated from whole blood by filterless-filter technology. The original ratio between RBC to WBC was 1,000:1 but the ratio was changed to 50:1 (RBC:WBC) after separation with 95% recovery.

Conclusions

Our microfluidic cell separation technology can be used for cell enrichment, white blood cell separation, and blood plasma separation using fluid dynamic phenomena. We have designed the micro-grooves engraved microchannel by migrating/directing cells toward one side of microchannel. The micro-grooves were strategically designed to target cell size, and curved grooves with respect to the main direction of microchannel. These microgrooves changed the aspects of bulk flow streamline, and the changed streamline induced the secondary flows perpendicular to the microchannel direction. These secondary flows separated the cells without physical filters. In conclusion, the filterless-filter technology is a fast, simple and versatile method for the cell separation and can be applied to all cell-based processes as an alternative to the centrifugation.

doi: 10.1016/j.cca.2022.04.302

M013

Loop-mediated isothermal amplification (LAMP): An advanced molecular point-of-care technique for the detection of mycobacterium tuberculosis

R. Elizabeth Thomas, S. Kv, B.T Edwin, A. Vs, J.K Paul

Agappe Diagnostics Ltd, India

Background-Aim

India having the largest number (26.9 Lakhs in 2019) of Tuberculosis cases in the world lacks an affordable, accurate, simple point-of-care (POC) diagnostic test applicable in resource-limited endemic areas.

Lack of an effective POC diagnostic assay is a critical barrier for effective treatment and control of TB.

We have developed an affordable, accurate, simple and rapid assay based on Loop mediated isothermal amplification (LAMP) for TB detection.

Methods

LAMP primer design – All 6 primers (FIP,BIP,F3,B3,LF,LB) specific to the target DNA were designed using primer explorer software.

The LAMP reaction was performed in a total volume of 25µl.

The reaction mix consist of dsDNA binding dye, 6 primers, dNTPs, buffer and enzyme.

Sample – Standard TB DNA control (Amplirun total MTB control)

The mixture was incubated at 65˚C for 30min and the real time amplification was detected using MISPA LUME Real time LAMP analyzer (Agappe Diagnostic Ltd).

Results

Current assay can detect as low as 5 copies/reaction of the target in the DNA sample.

Clinical performance need to be studied on sputum samples.

This assay may be used as POC method to screen large population for Tuberculosis in less TAT.

Conclusions

This assay may be used as POC method to screen large population for Tuberculosis in less TAT.

doi: 10.1016/j.cca.2022.04.303

M014

Processed plasma materials of fructosamine measurement from in vitro glycation

W. Duanginta[a], N. Apiratmateekul[b], W. Treebuphachatsakul[b]

[a] *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand*

[b] *Reference Material and Medical Laboratory Innovation Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok 65000, Thailand*

Background-Aim

Fructosamine is a glycated serum protein that represents average glycemia over 2 to 3 weeks. Fructosamine has been presented as an alternative glycemic control measure for diabetic individuals and has a good correlation with hemoglobin A1C (HbA1c) measurements. The fructosamine reference material in the proficiency testing (PT) program was limited. The objective of this study was to investigate the conditions for in vitro glycation of fructosamine in processed plasma material (PPM) produced according to ISO 17034-reference material producer.

Methods

The investigations were performed by incubating frozen plasma from a blood bank with 100, 200, and 300 mM of D-glucose in phosphate buffer (PBS) pH 7.4 at 37°C for 48 hours. Fructosamine production in PPMs was determined at random using an immunoassay method, and a test for homogeneity and stability based on ISO Guide 35.

Results

Using 200 mM D-glucose at 37°C for 48 hours was suitable for fructosamine concentrations of 820 μmol/L with a significant increase ($p < 0.05$). The increase in fructosamine was followed by a decrease in albumin and total protein.

Conclusions

The PPMs of fructosamine were stable in the refrigerator for 14 days, according to ISO Guide 35 may be the application to use as the reference sample for quantifying fructosamine in quality control and PT program.

doi: 10.1016/j.cca.2022.04.304

Clinica Chimica Acta 530 (2022) S8–S20

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Analytical Technology

M015

Establishing and verifying a very rapid inductively coupled plasma-mass spectrometry method to determine iodine concentrations in amniotic fluid, breast milk and cerebrospinal fluid

Y. Zou

Peking Union Medical College Hospital, China

Background-aim

Given that both maternal iodine insufficiency and excess can cause adverse effects such as poor cognitive performance, delayed physical development and even higher mortality in fetuses and infants, the determination of maternal iodine status is very important. In this study, we established and verified a very rapid inductively coupled plasma-mass spectrometry (ICP-MS) method for the determination of amniotic fluid iodine concentration (AFIC), breast milk iodine concentration (BMIC) and cerebrospinal fluid (CSF) iodine concentration (CSFIC).

Methods

Amniotic fluid, breast milk and CSF were collected from residual samples at Peking Union Medical College Hospital (PUMCH). The linearity, detection limit, precision, recovery, carryover and matrix effect of the ICP-MS method were thoroughly evaluated according to the EP-10-A2 evaluation protocol approved by the Clinical and Laboratory Standards Institute (CLSI) guidelines. Furthermore, we also evaluated the distribution of AFIC, BMIC and CSFIC in clinical patients from PUMCH.

Results

The correlation coefficient (r) was higher than 0.99 (0.995-1.000). The limit of detection (LOD) was 0.233 μg/L, and the limit of quantification (LOQ) was 0.778 μg/L. The repeatability was 1.5%-1.8%, 1.9%-4.0% and 1.8%-4.0% and the within-laboratory coefficient of variation (CV%) over the period of five days was 3.3%-9.2%, 7.2%-8.0% and 3.2%-7.8% for amniotic fluid, breast milk and CSF, respectively. The recovery rates ranged from 97.7% to 109.8%. Moreover, the median iodine concentrations in amniotic fluid, breast milk and CSF in clinical patients from PUMCH were 176.3 μg/L, 136.0 μg/L, and 81.8 μg/L, respectively.

Conclusions

Rapid, stable and accurate ICP-MS methods of iodine detection were established for amniotic fluid, breast milk and CSF in this study.

doi: 10.1016/j.cca.2022.04.306

M016

Development of a routine LC-MS/MS assay for simultaneous quantification of six beta-lactam antibiotics

P. Pettersson-Pablo, J. Oxelbark

Department of Laboratory Medicine, Örebro University Hospital, Örebro, Sweden

Background-aim

Therapeutic drug monitoring (TDM) is being increasingly recognized as an essential clinical tool in designing and evaluating pharmacological treatment in various conditions. For beta-lactam antibiotics, a standard treatment in many cases of sepsis, the time during which the tissue concentration of the antibiotic exceeds the minimum inhibitory concentration (MIC) of the pathogen is the main determinant of their antimicrobial effect. Recent studies have shown that there is a significant risk of insufficient treatment in critically ill patients. The aim of this project was to develop a beta-lactam multiassay capable of providing rapid and accurate TDM results for routine clinical use for the following antibiotics: Benzylpenicillin, Meropenem, Cefotaxime, Flucloxacillin, Piperacillin and Tazobactam.

Methods

A quick and efficient protein precipitation work-up for serum samples was developed, incorporating individual isotope labeled internal standards. A very quick UPLC-method, with a cycle time of 3.0 minutes, and using triple quadrupole MS-detection, affords results quickly over a broad assay range. The method was fully validated with regard to matrix effects, extraction efficiency, linearity, accuracy, precision, carry-over, and stability.

Results

Benzylpenicillin, Meropenem, Cefotaxime, Flucloxacillin, Piperacillin and Tazobactam were assayed in the range of 0,2-100 mg/L and Flucloxacillin 1-500 mg/L with acceptable linearity and good precision. Precision was better than 6% CV over the full assay range for each analyte. Extraction efficiency and accuracy were significantly improved by incorporating individual internal standards, giving rise to excellent results (extraction efficiency >96% for all analytes).

Beta-lactams are known to have poor stability. In our case, satisfactory stability was demonstrated when primary tube samples kept at room temperature were centrifuged within 2 hours from sampling, and then immediately aliquoted and frozen (-20°C).

Conclusions

The developed assay provides rapid and accurate measurements of antibiotic concentrations of six beta-lactam antibiotics in serum samples.

The assay performs with low CV%'s for low as well as high sample concentrations. Its robustness over a wide analytical range is well suited for use in a clinical setting and permits accurate determination of under as well as over treatment of the patient.

doi: 10.1016/j.cca.2022.04.307

M017

Validations of a new blood collection tube product for plasma glucose and hemoglobin A1C testing

W. Treebuphachatsakul [a], P. Chuaychoo [b], C. Khamtor [b], N. Sangla [b], K. Kongros [a], N. Apiratmateekul [a], R. Wiriyaprasit [b]

[a] *Department of Medical Technology,Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*
[b] *Reference Material and Medical Laboratory Innovation Research Unit, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*

Background-aim

Sodium fluoride (NaF) tubes are regularly used for blood specimen collection for plasma glucose determination. However, drawbacks of NaF occur with poor anticoagulant property and decreasing in plasma glucose at the first two hours after blood collection. The objective of this study was to compare the new blood collection tube, Innomed® tube that coated inside with NaF plus EDTA and an anti-glycolysis agent (D-mannose) for plasma glucose and hemoglobin A_{1C} (HbA_{1c}) testing.

Methods

Innomed® tube was produced with the process according to GMP and ISO 13485 for medical device production. Twenty two fresh blood specimens were collected from healthy and diabetic patients following CLSI Gp 34-A guideline. Glucose and HbA_{1c} data obtained from Innomed® tube was compared to those from reference tubes, NaF plus K_3EDTA and EDTA tubes.

Results

Bias of paired glucose data between Innomed® tube and reference tube at baseline was 2.15%.Plasma glucose from Innomed® tube and NaF plus K_3EDTA decreased 1.60 % and 2.50% respectively at 2 hours from base line. Plasma glucose obtained from Innomed® tube was not significantly difference ($P > 0.05$) from those of NaF-K_3EDTA tubes at 24 hours. HbA_{1c} in whole blood samples obtained from Innomed® tubes was not significant difference ($P > 0.05$) from those of EDTA tubes at 2 hours after blood draw with bias δ1.50%.

Conclusions

Innomed® tube could preserve glucose in plasma at least 24 hours and preserve glucose better than NaF plus K_3EDTA tubes at the first 2 hours after blood draw. Whole blood collected by Innomed® tube also could be used for HbA_{1c} testing within 2 hours after blood collection.

doi: 10.1016/j.cca.2022.04.308

M018

Prolactin and postpeg-prolactin level in psychiatric patients on antipsychotics treatment: Impact on the severity of hyperprolactinemia

N. Ruljancic [e], A. Bakliza [e], S. Vuk-Pisk [a,b], K. Matic [a], N. Geres [a], E. Ivezic [a], I. Filipcic [b,c,d]

[a] *Department of Integrative Psychiatry, Psychiatric Hospital "Sveti Ivan", Zagreb, Croatia*
[b] *Faculty of Dental Medicine and Health, "Josip Juraj Strossmayer" University of Osijek, Osijek, Croatia*
[c] *Faculty of Dental Medicine and Health, "Josip Juraj Strossmayer" University of Osijek, Osijek, Croatia*
[d] *School of Medicine, University of Zagreb, Zagreb, Croatia*
[e] *Department of Laboratory Diagnostics, Psychiatric Hospital "Sveti Ivan", Zagreb, Croatia*

Background-aim

Three stages of severity of hyperprolactinemia (HPRL) have been suggested, mild HPRL below 1000 mlU/L, moderate between 1000 and 2000 mlU/L and severe HPRL, above 2000 mlU/L. The strategy of treatment and differential diagnosis differs according to a prolactin (PRL) cutoff value shown in several guidance for the management of antipsychotics-induced HPRL.

The aim is to demonstrate the impact of PRL levels after macroprolactin precipitation with polyethylene glycol (PEG) on the severity of HPRL in the serum of patients on antipsychotic therapy.

Methods

98 female patients aged 32y (range 19y-47y) on antipsychotics therapy with a psychotic disorder and with HPRL were included. Total PRL level and PRL after PEG precipitation of macroprolactin (postPEG-PRL) was determined by chemiluminometric method on Beckman Coulter Acces2 analyser. The reference interval recommended by the manufacturer and verified is 71 - 568 mlU/L.

Results

Total PRL levels (median 1471 mlU/L, range 577-4192 mlU/L) and postPEG-PRL levels (median 1453 mlU/L, range 536-4360 mlU/L) were showed statistically significant positive correlation ($rho = 0.982$, $p < 0.0001$). The mean PRL recovery following PEG precipitation was 94, 41% (range 51%-118%). There were substantial agreement (kappa test = 0.859) between HPRL severity based on total PRL levels and post-PEG-PRL levels. Regardless of this, six patients were classified with severe HPRL according to total PRLlevel (>2000mlU/L) but according to postPEG-PRL levels (1000mlU/L -2000mlU/L) with moderate HPRL severity. Three patients were classified with moderate HPRL severity according to total PRL levels but according to postPEG-PRL levels (<1000mlU/L) were classified with mild HPRL severity.

Conclusions

Several guidance for the management of antipsychotics-induced HPRL recommended PRL measurement on pretreatment samples and in case of HPRL, screening for macroprolactin.

PostPEG-PRL level can also be helpful to determine HPRL severity which may affect further management of patients with antipsychotics-induced HPRL.

doi: 10.1016/j.cca.2022.04.309

M019

Predicting erythrocyte sedimentation rate from full blood count data

A. Chawe [b], D. Chisompola [a]

[a] *Ndola Teaching Hospital, Zambia*
[b] *St Francis Hospital, Zambia*

Background-aim

Erythrocyte sedimentation rate (ESR) is one of the most commonly used markers of inflammation in clinical practice. It is useful in aiding diagnosis, managing, and follow-up of certain inflammatory conditions and prognosis of noninflammatory conditions such as prostate cancer, stroke, and coronary artery disease.

The use of electronic information management system in laboratories has made it easy to collect huge data, therefore enabling opportunities to learn and identify patterns in data, for either prediction or classification

Methods

Regression, Decision trees, Random Forests, and Neural networks, Models were built to predict and to classify ESR. Root Mean squared error (RMSE) and Mean Absolute Error (MAE) were used as matrics for assessing the performance of models on ESR prediction as a continuous Variable.

When treated as a categorical, with three classes, i.e. Low, Medium, High, and as a two-class variable, with only Low and High, three Models were used, Neural networks, Random Forests and Decision trees respectively. Their performance was evaluated by their accuracy in predicting a class, i.e. how correctly they classify a case as being Low, Medium, or High, respectively.

Results

Regression model and Random forests, performed better than Decision trees when predicting ESR as a continuous variable, with Decision Trees having (RMSE = 34.18579, MAE = 25.62104). whereas Random Forests (RSME = 31.26080, MAE = 23.79673) and Linear Regression (RMSE = 32.16826, MAE = 24.80904).

Neural network performance was poor on the classification of three classes, Low, Medium, High, it achieved the lowest accuracies (0.452 and .750) on the 3 classes and 2 classes variables respectively. Random forests achieved the highest performance (0.682 and 0.770) on 3 classes and 2 classes respectively. Decision Trees gave (0.670 and 0.758) on 3 classes and 2 classes.

Conclusions

ESR is still being used for aiding diagnosis and for follow up. The ability to predict it accurately, will make it cheap, easy to perform and rapid.

The accuracy of the models in predicting and classifying ESR is remarkable considering only FBC data was used. Further analysis should include data, that's known to affect ESR.

Keywords
Prediction
Classification
Erythrocyte Sedimentation rate
Random Forests
Decision Trees
Linear regression
Machine Learning

doi: 10.1016/j.cca.2022.04.310

M020

LC-MS/MS assays in the medical laboratory: 15 Years of experience

D. Svinarov, L. Kasabova

Alexander University Hospital, Faculty of Medicine, Medical University of Sofia, Bulgaria

Background-aim

Triple quadrupole LC-MS/MS with its unique selectivity and sensitivity has become prominent tool to resolve analytical challenges in laboratory medicine. This study presents 15 years of experience with LC-MS/MS in our laboratory.

Methods

Assays for immunosuppressive drugs (IMS), anticonvulsants (AEDs), 25-hydroxy-vitamin D (25VD), individual steroid hormones and steroid profiling in whole blood (WB), plasma (PL), oral fluid (OF) and dried blood spots (DBS) were integrated in two analytical systems. In system 1, analytes were extracted from 100 µl of WB or PL with organic solvent for IMS and 25VD, and dilute and shoot phase separation protein precipitation, developed and employed for AEDs and mycophenolic acid. Isocratic separation was performed on a common C18 analytical column with easily interchangeable mobile phases of aqueous methanol with ammonium acetate and or formic acid. System 2 encompassed all steroid assays with common sample preparation for PL, OF and DBS and isocratic or gradient separation on a common C18 analytical column and mobile phases with ammonium fluoride. Electrospray positive and negative ionization, and selected reaction monitoring were used to follow the respective predominant transitions. Validation strategy was strictly adhered to industrial and clinical guidance.

Results

For each analyte, selectivity was assessed with 9÷12 individual sources of WB, PL, OF, or DBS with matrix effect in the range 87÷112%; extraction recoveries were 60÷93%; linearity was assured in the clinically relevant range with $R2 > 0.996$; accuracy, bias and precision were within ± 15% both within-run and between-runs; stability results being: freeze-thaw for three cycles of 24 h; post-preparative documented for 36÷72 h at 8oC, short-term at ambient temperature, proven for 6÷24 h in the dark and for 2÷6 h in daylight; stock solution and long term in WB, PL, OF - for 1÷4 months at -20oC. All of the above validation parameters being within the predefined acceptance criteria of ±15%.

Conclusions

With validation according to current industrial or clinical requirements, a throughput of 100÷200 samples per working day and immedi-

ate method switching, these unified systems provide convenience and optimal versatility for the LC-MS/MS medical laboratory.

doi: 10.1016/j.cca.2022.04.311

M021

3D printed-lactate amperometric biosensor for real-time noninvasive health monitoring in human sweat

P. Kuntawong, U. Kongintr, C. Promptmas

Department of Biomedical Engineering, Faculty of Engineering, Mahidol University, Thailand

Background-aim

Wearable sensors have developed as a major growth area for users in sport applications since they capacitate real-time and noninvasive monitoring towards maintaining a health status and performance. Currently research in this field has focused on mentioning in flexible skin sensing which durability, skin compatibility, and accurately are the main purpose. Lactate plays an important role in the clinical medicine and sports. It is significance to evaluate and diagnose the health status related to lactate acidosis and oxygen deficit situations.

Methods

In this work, we demonstrate lactate monitoring with conductive 3D-printed filament-based amperometric enzymatic flexible sensor modified with bovine serum albumin (BSA)/glutaraldehyde (GA) crosslinking to immobilize lactate oxidase, covering with polyvinyl chloride and then attached to a medical-grade adhesive sheet required for applying to human skin. Hydrogen peroxide is the end product of this reaction which then oxidizes on 3D-electrode surface and produces amperometric current proportional to amount of lactate present in human sweat. The sweat lactate were recorded via chronoamperometric method and the signal will real-time sending to mobile phone application via Near Field Communication (NFC) system.

Results

Conductive 3D printed-lactate amperometric biosensor was successfully performed consists of a 28 mm2 mediated lactate oxidase working electrode, silver ink reference electrode, and glassy carbon counter electrode. Lactate oxidase was effectively immobilized on conductive 3D printed electrode by the above fabrication method.

Conclusions

We have represented the first sampler of conductive 3D-printed electrode amperometric biosensor which allows real-time monitoring of sweat lactate. This novel wearable lactate biosensor, where is noninvasive and simple-to-operate, performs desirable properties under sports and fitness routines to track exercise intensity.

doi: 10.1016/j.cca.2022.04.312

M022

Performance evaluation of microslide and open channel on VITROS XT 7600 – Establish a clinical testing system for clinical chemistry in the event of a disaster-

S. Yokoyama [b], R. Kikuchi [b], H. Matsuyama [b], K. Ohashi [c], R. Watarai [b], G. Hayashi [c], Y. Numata [c], T. Hagiwara [c], T. Kobayashi [c], Y. Ando [b], T. Matsushita [a]

[a] *Department of Clinical laboratory, Nagoya University Hospital, Japan*
[b] *Department of Medical Technique, Nagoya University Hospital, Japan*
[c] *Ortho Clinical diagnostics, Japan*

Background-aim

Currently, general integrated analyzers that depend on water supply are commonly used for clinical chemistry tests at many medical facilities in Japan. Water supply suspension during a disaster event is a major concern for those facilities. Hence, we evaluated the performance and usability of Clinical Chemistry Microslides and Open Channel Reagents of Clinical Chemistry on VITROS XT 7600 Digital Chemistry System (VITROS), which is waterless system.

Methods

VITROS and LABOSPECT 008 (008) were used to evaluate its performance and usability by correlation analysis between 26 items of each microslide (VITROS: dry method) and 008 liquid reagent (wet method). Furthermore, the 008 liquid reagent was mounted on the open channel (wet method) of VITROS and 008 to evaluate the correlation between the two instruments by 19 items (wet method). This study was approved by the Nagoya University Hospital Ethical Committee (Identification number: 2010-1038).

Results

26 items evaluated using the VITROS dry method and the 008 wet method, 19 out of 26 items had a correlation coefficient of 0.990 and/or more. However, some estrangements were observed in albumin, amylase and CK-MB. Of the 19 items evaluated using the VITROS wet method, good results were obtained with a correlation coefficient of 0.996 and/or more with the 008 wet method except for inorganic phosphorus. The correlation between VITROS and 008 was generally good. The estrangements between the measured values of the albumin, amylase and CK-MB in the VITROS by dry method is presumed to be due to the difference in the measurement principle, substrate and reagent specificity.

Conclusions

It is considered possible to establish an inspection system using the VITROS wet method in a situation where there is suspended water supply during a disaster event.

doi: 10.1016/j.cca.2022.04.313

M023

UPLC-MS/MS method for simultaneous determination of 12 steroid hormones using modified commercially manufactured kit

J. Waś [b], M. Niedolistek [b], A. Prejbisz [a], A. Januszewicz [a], A. Lutyńska [b]

[a] *Department of Hypertension, National Institute of Cardiology, Warsaw, Poland*
[b] *Department of Medical Biology, National Institute of Cardiology, Warsaw, Poland*

Background-aim

The sensitive detection of a wide panel of adrenal steroid hormones [cortisone (E), aldosterone (A), cortisol (C), DHEAS, corticosterone (B), 11-deoxycortisol (11DC), androstenedione (AD), 11-deoxycortisone (11DE), testosterone (T), 17-hydroxyprogesterone (17OHP), dihydrotestosterone (DHT) and progesterone (P)] for diagnostics suitable for hypertension patients was implemented. Commonly used immunoassays in steroid detection are known by their lack of sensitivity, narrow dynamic range and problems with selectivity. Thus, the development of reliable multi-analyte LC-MS/MS method for steroid analysis in serum/plasma is extremely challenging. The objective of work covers the highlighting the crucial parameters of LC-MS/MS analysis that have influence on sensitivity, specificity and reproducibility of steroid panel detection.

Methods

We introduced the validated LC-MS/MS QTRAP 6500+ (Sciex) candidate reference measurement procedure for the quantification of 12 steroid hormones in human plasma. The validation included evaluation of accuracy using six levels of calibration standards and three levels of QC samples (low, medium, high). Certified analytical kit dedicated for steroid hormones (aldosterone, cortisol, cortisone, 11-deoxycortisol, corticosterone, 11-deoxycortisone, progesterone, 17-hydroxyprogesterone, androstenedione, DHEAS, testosterone, dihydrotestosterone) – MassChrom® (Chromsystems, Germany) was used for analyses.

Results

E, A, C, DHEAS, B, 11DC, AD, 11DE, T, 17OHP, DHT& P were eluted at retention times of: 3.51, 4.04, 4.20,5.25, 6.09, 6.35, 7.03, 7.40, 7.54, 7.82, 8.42, and 8.82, respectively. The calibration curves (n = 6) were collected. The method was found linear in the range of: E: 1.04-38.20 ng/mL, A: 0.03-2.88 ng/mL, C: 9.72-289.0 ng/mL, DHEAS: 115.0-5894.0 ng/mL, B: 0.51-47.60 ng/mL, 11DC: 0.09-15.0 ng/mL, AD: 0.19-14.0 ng/mL, 11DE: 0.05-2.81 ng/mL, T: 0.05-11.50 ng/mL, 17OHP: 0.10-21.60 ng/mL, DHT: 0.05-1.38 ng/mL and P: 0.16-24.60 ng/mL and the r2 consistently reached above 0.99 values. The curves were calculated by a weighted linear regression analysis with w = 1/x implemented for improving adjustment at low concentrations with correlation coefficients consistently reaching 0.99. The reproducibility was satisfactory in whole range tested with the RSD of 1.29-12.53 %.The accuracy of E, A, C, DHEAS, B, 11DC, AD, 11DE, T, 17OHP, DHT & P obtained was as follows: (-1.53; + 2.08%), (-12.17; +11.80%), (-9.28; +5.85%), (-4.41; +3.31%), (-3.34; + 2.40%), (-4.64; +4.21%), (-4.16; +7.04%), (-8.32; +14.32%) (-10.68; +5.65%), (-6.82; + 6.39%), (-7.14; +11.33%), (-7.39; +5.32%), respectively.

Conclusions

LC-MS/MS because of sensitivity, specificity, reproducibility and accuracy sustains a gold standard for determination of steroids in Cushing's syndrome, hyperaldosteronism, adrenogenital syndrome, hypogonadism and polycystic ovary syndrome testing.

doi: 10.1016/j.cca.2022.04.314

M024

Molecularly imprinted electrochemical sensor for the determination of homocysteine

U. Kongintr, B. Lertanantawong, P. Kuntawong, C. Promptmas

Department of Biomedical Engineering, Faculty of Engineering, Mahidol University, Thiland

Background-aim

A molecularly imprinted electrochemical sensor was developed for the detection of homocysteine (Hcy). Hcy serves as a marker of inflammation in cardiovascular disease.

Methods

Screen printed carbon electrode (SPCE) was modified with carbon nanotube and ionic liquid to enhance the imprinted polymer and then improve the sensitivity of the sensor. Characterisation of MIP was performed with Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) and the modified SPCE with Cyclic voltammetry (CV).

Results

CV and DPV demonstrated that the modified SPCE is responsive towards the Hcy molecule. The sensor showed a linear relationship with the concentration of Hcy in the range of 5 to 150 μM, with a limit of detection of 0.9016.

Conclusions

The utility of the sensor was proved by the determination of Hcy from blood plasma samples with good recovery.

doi: 10.1016/j.cca.2022.04.315

M025

Validation of direct method for cadmium quantification in urine

V. Vasilev [b], B. Pencheva [a], R. Mihaylov [b]

[a] *Clinical Laboratory "RAMUS", Sofia, Bulgaria*
[b] *Clinical Laboratory "RAMUS", Sofia, Bulgaria*

Background-aim

Cadmium is well known with its toxic effects on different organs and tissues in human body. During cadmium exposition damage occurs in skeletal muscles, renal, bone, cardio-vascular and other systems, liver failure develops, which leads to mortality. We aimed to validate direct method for cadmium quantification in urine samples.

Methods

Validation of direct electrotermic atomic absorption spectrometry (ETAAS) method for cadmium quantification in urine samples went through several methodic points: ashing temperature optimization, atomization of water standards and urine, calibration curves, evaluation of limit of detection (LD), low limit of quantification (LLOQ), intra- and inter-assay precision, accuracy, characteristic mass.

Results

Ashing temperature optimization was performed by matrix modifier contains 0.005 mg palladium nitrate and 0.003 mg magnesium nitrate, in 0.2% nitric acid, in order to effectively eliminate chemical interferences. With this modifier and pyrolytically covered tube with Lvov platform, were established maximal pyrolytic temperature of 1800°C and 650°C for ashing. For calibration curve were used a) liquid standards in five concentrations (0.5 – 2.5 mg/L) and b) standard additive method. Every standard was measured twice, and corrected against modifier. LD was evaluated by 22 times measured modifier; we established 0.002 μg/L. Intra-assay precision showed CV 4.2%; inter-assay repeatability – CV 4.3%. Accuracy was evaluated by recovery method and was compared with control material, traced to ICP-SFMS.

Conclusions

Evaluation of cadmium in urine gives the opportunity for non-invasive receipt of biological specimen. Validation of direct method for quantification of cadmium in urine presents a novelty in our clinical laboratory practice.

doi: 10.1016/j.cca.2022.04.316

M026

Performance characteristics of selected immunoassays for enantiomers of ketamine and hydroxynorketamine in urine samples

S. Yu-Ting [b], L. Huei-Ru [a]

[a] *Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan*
[b] *Institute of Medical Biotechnology, Tzu Chi University, Hualien, Taiwan*

Background-aim

Ketamine, an anesthetic agent, has become one of the most commonly used illicit drugs in the world. Recently, U.S. FDA has approved S-ketamine as antidepressant drug. Some researchers also found that enantiomers of ketamine and its metabolite, hydroxynorkteamine, has antidepressant effect. Immunoassays are commonly used to test for drugs of abuse. Medical use of S-ketamine or 2R,6R-hydroxynorketamine is expected to cause positive immunoassay result due in part to antibody cross reactivity. The cross reactivity of chiral ketamine and hyoxynorketamine in common commercial immunoassays for ketamine is required to be evaluated.

Methods

Four commercially available immunoassays for ketamine are evaluated for their effectiveness in serving as the preliminary test methodology for the analysis of racemic ketamine, R-ketamine, Sketamine, racemic hydroxynoektamine, 2R,6R-hydroxynorketamine, and 2S,2Shydroxynorketamine. Additionally, the cross-reactivity of immunoassay of ketamine and hydroxynorketamine is assessed with 2D molecular similarity calculations.

Results

The 2D molecular similarity analysis correlates well with racemic target analytes, indicating that this method could be helpful in predicting cross reactivity on immunoassays. The cross reactivity performance for enantiomers of ketamine and hydroxynorketamine between those four immunoassays for ketamine shows minor difference.

Conclusions

The cross-reactivity data of enantiomers of ketamine and hydroxynorketamine presented in this study could help toxicology laboratories and clinicians in interpreting unexpected results, particularly when enantiomers of ketamine and hydroxynorketamine are used in depression treatment.

doi: 10.1016/j.cca.2022.04.317

M027

Monoclonal immunoglobulin interference in Beckman Coulter AU5800

J. Han, W. Su, Q. Di, W. Ji, J. Zhang, Y. Wang, P. Li

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100730, China

Background-aim

Monoclonal immunoglobulin (M protein) is mainly secreted by a large number of plasma cells with abnormal clonogenic hyperplasia. M protein is commonly seen in monoclonal immunoglobulin proliferative diseases, such as multiple myeloma (MM), Waldenstrom macroglobulinemia (VM), heavy chain disease, light chain disease, etc. M protein has unique physical, chemical and immunological properties, and has been reported to interfere with many clinical tests. The purpose of this study was to evaluate the interference of monoclonal immunoglobulin

in Beckman Coulter chemistry analyzer AU5800, and to propose solutions for removing interference of M protein.

Methods

Comparison of 40 cases of different concentrations and types of M protein serum specimens and 6 cases of apparent healthy person serum specimens, quantitative detection of GGT, LDH, ALP, TBil, DBil, PA, Na, Ca, P, Glu, UA, Cr, BUN, HDL-C, hs-CRP respectively in Beckman Coulter AU5800 and Johnson & Johnson VITROS 5600. The deviation between the different detection systems were calculated. The reaction curves of M protein positive serum samples on Beckman Coulter AU5800 were observed to identify the abnormal response curves. For the samples with abnormal reaction curve, different methods were used to try to eliminate the interference.

Results

The quantitative results of TBil, DBil, Na, Ca, P, Glu, UA, Cr, BUN and HDL-C were all lower than the national inter-laboratory quality assessment (EQA) analysis standard in AU5800 and dry chemistry. For the ALP test, the dry chemical and enzymatic quantitative results of M protein positive serum samples were significantly higher than those of normal human serum samples. In LDH test, the quantitative results of dry chemical test methods of all samples were higher than those of enzymatic test methods, with methodological differences. The abnormal response curve are observed in P, UA, and PA, IgG, IgA and IgM. Dilution can eliminate interference to P. For PA dilution can also be used as the first choice to eliminate interference. For UA the best method is to change the detection system or precipitate M protein before detection.

Conclusions

M protein interference induced the response curve of Beckman Coulter AU5800 was abnormal or the result was biased, but it was related to the concentration and type of M protein. Dilution can reduce some interference, or change of the detection system to eliminate M protein interference.

doi: 10.1016/j.cca.2022.04.318

M028

Deceptive of glycated hemoglobin quantification in diabetic patient with hemoglobin variant

M. Mitchai

Medical Molecular Biology Center, Clinical pathology department, Khon Kaen Hospital, Ministry of Public Health, Thailand

Background-aim

Glycated hemoglobin (HbA1c) is widely used in the management of diabetes. Therefore, hemoglobin variants are the most common in Thailand and have impacted to determination. The reliability and accuracy for HbA1c detection have become very important. We evaluated the deceptive of HbA1C measurement in diabetic patient with Hb variants on routine HbA1c assay by comparing capillary electrophoresis principle.

Methods

The study was performed within diabetic patients, diabetic clinic, Khon Kaen Hosptial. Blood samples were collected after 8 hours fasting and analyzed HbA1c quantification using routine immunoturbidity (Roche c501), (Roche Diagnostics, Germany) and capillary electrophoresis (Sebia Capillarys 2 Flex Piercing), (Sebia, France). Fasting plasma glucose was determined by enzymatic method (Roche c501), (Roche Diagnostics, Germany). All specimens were analyzed hemoglobin typing using capillary electrophoresis (Sebia Capillarys 2 Flex Piercing), (Sebia, France)

Results

Among 798 samples, median values of fasting plasma glucose (FPG) was 126 (25-974) mg/dl and HbA1c was 8.1 (4.2-22.3) %. The prevalence of Hb variants in this population were Heterozygous HbE 29.8 % (n = 238), Homozygous HbE 1.93% (n = 15), Hb CS trait 1.54 % (n = 12) and CSEABart's 0.26 % (n = 2). HbA1c analyzed by immunoturbidity assay gave a significantly higher result, ranging from 4.2-22.3% (p-value < 0.001). HbA1c quantification by Capillary electrophoresis revealed abnormal peaks, comprising 0.58 % to 93.7% of total hemoglobin in Hb variant patients and demonstrated hemoglobin fractionation similar to Hb typing electropherogram. Samples from Heterozygous HbE patients with decrease hemoglobin A presented a significantly lower result (p-value < 0.001). Homozygous HbE patient without hemoglobin A showed undetectable HbA1c and a statistical significant (p-value < 0.001), while immunoturbidity assay provided the median values of HbA1c 8.3% (4.6% – 10.2 %).

Conclusions

HbA1c measurement from routine assay in patients with Hb variants showed erroneous result compared to CE principle. The CE system could detect hemoglobin variants together with HbA1c tested. The laboratories should be aware of the limitation of their methods with respect to interference from Hb variants found commonly in local population and suggest an alternative HbA1c quantification method.

doi: 10.1016/j.cca.2022.04.319

M029

Analysis of plasma anti-epileptic drugs using the XEVO TQD for clinical research

S. Balloch [a], L. Calton [a], G. Hammond [a], P. Park [b], C. Song [b]

[a] *Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow SK9 4AX, UK*

[b] *Waters Korea Limited, Room 905-7 CCMM Building, 101 Yeouigongwon-ro, Yeongdeungpo-gu, Seoul 07241, South Korea*

Background-aim

Waters has developed a method for the clinical research of the following 18 anti-epileptic drugs and metabolites in plasma; 10,11-dihydro-10-hydroxycarbamazepine, carbamazepine, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, phenobarbital, phenytoin, primidone, topiramate and zonisamide (1-100 μg/mL); oxcarbazepine and perampanel (0.1-10 μg/mL); tiagabine (0.01-1 μg/mL) and valproic acid (2-200 μg/mL).

Methods

Matrix matched calibrators were prepared using in-house stocks and pooled plasma. Samples (50 μL) were treated with internal standard in methanol. A water/methanol/ammonium acetate gradient was used

with a Waters CORTECS C8 column on a Waters ACQUITY UPLC I-Class FTN and Xevo TQD mass spectrometer utilizing polarity switching in a 5 minute run.

Results

No system carryover was observed following analysis of plasma samples containing the highest concentration calibrators.

Analytical sensitivity investigations indicated precise quantification (δ20% CV, δ15% bias) at concentrations equal to or lower than the lowest concentration calibrator.

Total precision and repeatability were assessed (3 pools, 5 replicates, 5 days; n = 25) and determined to be δ9.5% RSD.

Linearity experiments determined the method was linear for phenobarbital, topiramate and zonisamide and quadratic for the remainder of the panel.

Analytes eluted in regions free of major ion suppression or enhancement. Evaluation of matrix effects at low and high concentrations indicated compensation by the internal standard.

Addition of high concentrations of several endogenous materials did not affect quantification. Full chromatographic resolution of the metabolite carbamazepine epoxide from isobaric oxcarbazepine was established.

Serum external quality assurance samples (n = 10-30) for accuracy testing were analyzed, except for oxcarbazepine and retigabine (not included). All samples passed the supplied criteria, with mean deviations δ10.6% from assigned concentrations.

Conclusions

This method for clinical research demonstrates excellent precision and accuracy with minimal matrix effects and allows for the multiplexing of 18 antiepileptic drugs and metabolites in plasma.

For Research Use Only. Not for Use in Diagnostic Procedures.

doi: 10.1016/j.cca.2022.04.320

M030

Analysis of plasma aldosterone using the Xevo TQ-XS for clinical research

S. Balloch [a], L. Calton [a], D. Foley [a], G. Hammond [a], P. Park [b], C. Song [b]

[a] *Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow SK9 4AX, UK*

[b] *Waters Korea Limited, Room 905-7 CCMM Building, 101 Yeouigongwon-ro, Yeongdeungpo-gu, Seoul 07241, South Korea*

Background-aim

Aldosterone is a mineralocorticoid steroid hormone that plays a key role in the regulation of blood pressure. We developed a LC-MS/MS method for the measurement of plasma aldosterone for clinical research purposes. An analytically sensitive method was developed using a mixed-mode Solid Phase Extraction (SPE) 96-well plate. Automated extraction was employed, enabling high throughput of samples. Samples were injected onto an ACQUITY UPLC™ I-Class system with separation on a Waters™ CORTECS™ C18 2.7μm column with VanGuard™ precolumn. Detection was performed using a Waters Xevo™ TQ-XS mass spectrometer to help quantify very low physiological concentrations of aldosterone.

Methods

Aldosterone certified reference material was used to create calibrators in stripped pooled serum and QC material in pooled plasma. EQA samples from UK NEQAS were analysed to evaluate analytical method bias. Samples were precipitated, followed by dilution with water. SPE was carried out with a Waters Oasis™ MAX μElution 96 well plate. Automated extraction was performed using the Tecan Freedom Evo 100/4 Liquid Handler. Using an ACQUITY UPLC I-Class system, samples were injected onto a CORTECS C18 2.7μm 2.1 x 100 mm column with VanGuard™ precolumn using an ammonium fluoride(aq)/methanol gradient and quantified with a Waters Xevo TQ-XS mass spectrometer.

Results

The method demonstrated no significant carryover or matrix effects and was shown to be linear from 3 – 1500 pg/mL for aldosterone. Analytical sensitivity investigations indicate the analytical sensitivity of this method would allow precise quantification (< 20%) at 3pg/mL with a S/N (PtP) > 10. Coefficients of variation (CV) for total precision and repeatability on 5 occasions for 13pg/mL, 103pg/mL and 1057pg/mL QC material were all δ6.3% (n = 25) for aldosterone. Analysis of EQA samples (n = 15) demonstrated a mean bias of -3.2% compared to the EQA MS laboratory mean for the samples.

Conclusions

We have successfully quantified aldosterone in plasma using an automated SPE protocol with LC-MS/MS analysis, for clinical research purposes. The method demonstrates excellent sensitivity, linearity, precision and bias with minimal matrix effects.

For Research Use Only. Not for Use in Diagnostic Procedures.

doi: 10.1016/j.cca.2022.04.321

M031

A simple DNA strip test for human papilloma virus

P. Nakowong [a], T. Chaibun [d], P. Jearanaikoon [c], C. Promptmas [a], W. Buajeeb [b], B. Lertanantawong [a]

[a] *Department of Biomedical Engineering, Mahidol university, Nakhon Pathom, Thailand*

[b] *Department of Oral Medicine and Periodontology, Mahidol University, Nakhon Pathom, Thailand*

[c] *Faculty of Assoicated Medical Sciences, Khon Kaen university, Khon Kaen, Thailand*

[d] *Faculty of dentistry, Mahidol University, Nakhon Pathom, Thailand*

Background-aim

Cancer is the most common cause of death and tend to increase steadily, recently. This disease is caused by abnormal cells in the body which have rapid growth and spread to other parts of the body, causing the body to be unable to control. Oral cancer is the third highest cause of death in developing countries. The cancer is also a type of deadly cancer which occurred in the mouth and then mutations in genetic level can occur on the lips, tongue, gums, cheeks, palate, and to nearby organs, lymph nodes and bloodstream. One of the leading risks of this cancer is Human papilloma virus (HPV) accumulation, HPV16 and HPV18 are the most prevalent in the cancers. Currently, the diagnosis of oral cancer is done by biopsy to identify abnormalities of cells but the cutting process may cause injury to the area around the cut piece.

Methods

The HPV strip test is based on DNA hybridization and using gold nanoparticles on a labelling. The synthesized gold nanoparticles (AuNP) was conjugated to DNA probe by ligand exchange. The optimization and characterization of AuNP-DNA were studied. This strip test was used to detect HPV from collected sample. DNA was extracted from tissue samples, and HPV genotyping was performed using amplification process. Finally, the lateral flow test was performed.

Results

The results are performed with naked eyes on lateral flow detection. The accuracy of results was verified by competitive assay using an unequal mixture of HPV16 and HPV18 DNA. We evaluated a simple DNA strip test for Human papilloma virus in real samples.

Conclusions

A simple DNA strip test for Human papilloma virus was done on paper-based material using lateral flow assay where prior steps are needed such as DNA extraction and amplification. This strip test is for the visual detection of HPV genotypes 16 and 18 which is a major risk factor for oral carcinogenesis and high-risk types has been observed in pre-cancerous and cancerous tissues. This strip is rapid, visualize with the naked eyes, no require additional instrument, simple and reduce injury due to the sample collection process.

doi: 10.1016/j.cca.2022.04.322

M032

False-positive results of HSTNI determing

A. Klasić, D. Polak-Erceg, T. Bezek

Special Hospital for Medical Rehabilitation Krapinske Toplice, Croatia

Background-aim

Immunoassays use antibodies to detect an analyte of interest (antigen). Presence of interfering antibody (heterophilic antibody or anti-animal antibody) in a sample can cause false–positive or false–negative result in immunoassay. The aim was to prove that hsTnI 1779,0 ng/L in a 53-year-old female patient with chest pain is not a sign of acute miochard infarction, but rather an existing interfering antibody.

Methods

HsTnI was determined on Architect, Abbott (CMIA method), during 24 hours. It was observed that multiple hsTnI determinations did not follow the usual hsTnI dynamics characteristic for AIM. There was no linearity during sample dilution and no compatibility with the result obtained in ECLIA method, Elecsys, Roche.

Results

HsTnI was 1779,0 ng/L when patient came to the hospital. One hour later hsTnI was 1576,4 ng/L, three hours later hsTnI was 1668,0 ng/L and 24 hours later hsTnI was 1725,5 ng/L. Sample dilution 1:5; 1:10 and 1:20 showed that there was no linearity. TnT obtained using a different method was 0,007 g/L (this value is within referent interval).

Conclusions

Based on results obtained on different analysers via different methods we can exclude AIM, but at the same time suspect into existence of interfering antibody which causes false-positive results of hsTnI.

doi: 10.1016/j.cca.2022.04.323

M033

Performance of a new point-of-care analyser for blood gases and electrolytes

T.C. Aw [b], Q. Liu [b], F. Poey [b], R.X. Pang [b], S.P. Tan [b], C.S. Lau [a]

[a] *Department of Laboratory Medicine, Changi General Hospital, Singapore*
[b] *Department of Pathology, Sengkang General Hospital, Singapore*

Background-aim

Abbott Diagnostics (Point of Care) introduced an updated point of care testing (POCT) device (i-STAT Alinity) in 2017. As a new 1000 bed hospital in Singapore, we decided to introduce POCT to several urgent care locations in our hospital (emergency department, intensive care unit, operating theatre and catheterization laboratory).

Methods

We evaluated the analytical performance of the CG8+ cartridges [pH, pCO2, pO2, Na, K, ionized calcium (iCa), glucose (Glu) and haematocrit (Hct)] on the i-STAT Alinity analyser against our laboratory analysers: blood gases/iCa (Roche Cobas B221), electrolytes/Glu (Roche Cobas 6000) and Hct (Sysmex XN9000). We studied the performance of 18 i-STATs over 25 days. The performance for all instruments used in this study on the College of American Pathologists (CAP) external quality assurance program has been satisfactory. POCT results are available in two minutes. Results on the Alinity can be transmitted via wireless/docking station to a cloud-based server (infoHQ, Abbott) to our laboratory information system (Cerner) prior to the electronic medical record. Statistical analyses were performed using MedCalc Statistical Software version 19.1.6 (MedCalc Software Ltd, Ostend, Belgium).

Results

Precision (CV%) at two levels of controls was acceptable across all analytes (pH 0.07-0.12/0.08-0.11, pCO2 0.9-3.1/1.2-2.3, pO2 1.7-5.0/0.6-5.4, iCa 0.5-1.4/0.3-1.7, Na 0.2-0.6/0.3-0.7, K 0.1-1.0/0.3-0.8, Glu 0.3-0.8/1.0-2.8, Hct 0.9-3.0/0.4-1.0). Assays were linear for pH 6.55-7.94, pCO2 19-91mmHg, pO2 55-436mmHg, iCa 0.32-2.25mmol/L, Na 101-175mmol/L, K 2.3-7.8mmol/L, Glu 1.5-33.4mmol/L, HcT 11.5-67.0%. There was close agreement between the i-STAT and our laboratory analysers ($r = 0.950$-0.999 across all assays) with the mean difference between assays being acceptable. For ease of management, only one type of cartridge (CG8+) is deployed in our hospital. Approximately 700 accredited i-STAT users utilize an average of 700 cartridges per month.

Conclusions

The performance of the CG8+ cartridges on the i-STAT Alinity is good, within the manufacturer's claims, comparable to our main laboratory analysers and fit for operational use. Users are satisfied with the i-STAT's ease of use.

doi: 10.1016/j.cca.2022.04.324

M034

Comparison of two methodologies for the quantification of ions in bile

P. Argente Del Castillo Rodríguez, C. Gómez Cobo, J.A. Delgado Rodríguez, M.A. Ballesteros Vizoso, J.M. Bauça

Hospital Universitario Son Espases, Spain

Background-aim

Bile is a yellow-green body fluid produced by the liver. Analysis of its composition can help us discover possible causes of litiasis.

There are different methodologies for the quantification of ions in body fluids. The techniques most frequently used in the clinical laboratory are potentiometric techniques. There are two types: indirect potentiometry (IP), which involves a pre-analytical dilution and assumes a non-water fraction of 7% and is used in biochemical analyzers and direct potentiometry (DP) that uses undiluted whole blood samples and is used in gasometers.

The objective of the present study was to compare the bile ion results obtained by two different techniques common in the clinical laboratory.

Methods

Seventy-three bile samples were analyzed. In all of them, Na+, K+ and Cl- were measured, with prior informed consent of the patients. They were analyzed by IP [Architect platform (Abbott Diagnostics, US)] and DP (GEM Premier 4000, Instrumentation Laboratory, US). In addition, proteins in bile were measured by the urine protein (benzethonium chloride) technique to evaluate possible interference. The values obtained were compared by means of the t-student parametric test and a linear Pearson regression. An alpha error of 5% was fixed.

Results

The protein concentration in 93% of the bile samples was undetectable (<0.068 g/L). Chloride was non-quantifiable in 25 samples. The correlation between both methods was bad for the 3 ions ($r < 0.975$). There are significant differences for the three electrolytes analyzed in both methodologies ($p < 0.001$). For sodium and for potassium, a mixed error is observed (values 2.87 and 1.46 times higher in the case of IP, respectively). For chloride, a constant systematic error was observed (7.44 mmol/L lower in the case of IP).

Conclusions

It has been proven that there are significant differences between both methodologies. The higher value of sodium and potassium in the samples analyzed by PI could be due to the low concentration of proteins in the samples analyzed for bile. The phenomenon of pseudohypernatremia in cases of hypolipemia and hypoproteinemia is well known, which emphasizes the importance of evaluating lipemia and proteinemia as possible interferences in the quantification of ions by IP. Furthermore, the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) has recommended PD as the method of choice for measuring electrolytes in critically ill patients. The consequences of misdiagnosing these patients can be severe. In order to determine which sodium and potassium result is better, an analysis with the reference method (ICP: Inductively Coupled Plasma Mass Spectrometry) should be performed.

doi: 10.1016/j.cca.2022.04.325

M035

Performance evaluation of the maglumi immunoassay analyzer in procalcitonin levels with two immunoassays

Y.S. Je, M.K. Lee, H.J. Kim, C.K. Kim, H.S. Lim, K. Lee

Department of Laboratory Medicine, Seoul Clinical Laboratories, South Korea

Background-aim

Procalcitonin (PCT) is now being recognized as useful tools for monitoring infection and inflammation. We evaluated MAGLUMI immunoassay analyzer with two commercially available procalcitonin immunoassays to evaluate the analytical performance and compare the correlation among them.

Methods

Procalcitonin was evaluated in 129 samples using a MAGLUMI PCT assay (SNIBE), Elecsys BRAHMS PCT assay (Roche), VIDAS BRAHMS PCT assay and Krypto BRAHMS PCT assay. We evaluated 42 low level samples (<0.5 ng/mL), 42 level samples between 0.5-2 ng/mL, 25 level samples between 2-5 ng/mL, 10 samples between 5-10 ng/mL, 6 samples between 10-30 ng/mL, 4 samples between 30-200 ng/mL The precision and linearity as well as the method of measurement were compared. Additionally, we evaluated the carryover rates between specimens.

Results

The total precision (% CV) of the MAGLUMI PCT assay in measuring low and high level controls (level 1, 2) was 6.46% and 3.80%. A Good correlation was observed between MAGLUMI PCT assay and Elecsys BRAHMS PCT assay ($r = 0.980$), VIDAS BRAHMS PCT assay ($r = 0.939$) and Krypto BRAHMS PCT assay ($r = 0.907$). Measured PCT concentrations showed good linearity ($R2 = 0.9962$, 0.04-100.00 ng/mL). Carryover effect was 0.78%.

Conclusions

The MAGLUMI immunoassay analyzer showed adequate performance and considered suitable for assessment of procalcitonin status in clinical routine.

doi: 10.1016/j.cca.2022.04.326

M036

Comparison of the analytical performance of the maglumi immunoassay analyzer in measuring 25-OH vitamin d with two immunoassays and liquid chromatography-mass spectrometry

Y.S. Je, M.K. Lee, H.J. Kim, C.K. Kim, H.S. Lim, K. Lee

Department of Laboratory Medicine, Seoul Clinical Laboratories, South Korea

Background-aim

Vitamin D plays important roles in the functioning of various systems of body. 25-OH-Vitamin D concentrations are currently used in clinical practice to assess Vitamin D status. We evaluated MAGLUMI immunoassay analyzer with two commercially available Vitamin D immunoassays and Liquid Chromatography-Mass Spectrometry (LC-MS) to assess the analytical performance and compare the correlation and accuracy among them.

Methods

Vitamin D was measured in 126 samples using the MAGLUMI 25-OH Vitamin D assay (SNIBE), Elecsys Vitamin D assay (Roche), ADVIA Centaur Vitamin D total assay (Siemens) and QTRAP 5500 Mass Spectrometer. We evaluated 5 samples (<10 ng/mL), 48 samples (10-29 ng/mL), 67 samples (29-100ng/mL) and 6 high level samples (>100 ng/mL). The precision and linearity as well as the method of measurement were compared.

Results

Correlations among the evaluated assays showed strong positive linear relationships (correlations among MAGLUMI and Elecsys, MAGLUMI and ADVIA, MAGLUMI and QTRAP 5500: r = 0.943, r = 0.982, r = 0.941, respectively). The total precision (% CV) of the MAGLUMI Vitamin D assay in measuring low and high level controls (level 1, 2) was 4.50% and 4.38%. In the linearity test, R2 was 0.9976 for Vitamin D (6.2-148.3 ng/mL).

Conclusions

The MAGLUMI immunoassay analyzer showed good performance and appeared to be suitable for routine analysis of Vitamin D status.

doi: 10.1016/j.cca.2022.04.327

M037

A robust liquid chromatography-tandem mass spectrometry method to simultaneously measure nicotinamide adenine dinucleotide, nicotinamide mononucleotide, 8-OXO-7,8-dihydroguanosine, and 8-OXO-7,8-Dihydro-2′-deoxyguanosine in serum and urine

Z. Yutong

Department of Laboratory Medicine, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing 100730, China

Background-aim

To establish and validate a robust liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous measurement of nicotinamide adenine dinucleotide (NAD+), nicotinamide mononucleotide (NMN), 8-oxo-7,8-dihydroguanosine (8-oGsn), and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-odGsn) in serum and urine samples for evaluation of the status of human oxidative stress.

Methods

A Waters TQ-XS triple quadrupole MS/MS system coupled with an ACQUITY Primer HSS T3 column were used for evaluations. According to CLSI C62-A and EP-15 guidelines, the clinical performance of the assay was validated. Furthermore, matched serum and urine samples from 22 apparently healthy patients, 20 patients with atherosclerosis, and 18 patients with dementia were evaluated based on the robust method.

Results

The recovery rates of serum NAD+, urine NAD+, serum NMN, urine NMN, serum 8-oGsn, urine 8-oGsn, serum 8-odGsn, and urine 8-odGsn were 95.3–111.1%, 88.0–110.3%, 98.4–108.9%, 88.5–108.6%, 88.8–112.4%, 102.4–114.1%, 88.5–107.7%, and 94.9–102.6%, respectively. In addition, the LC-MS/MS method was deemed robust based on the results of inter-assay and total coefficient variation, matrix effects, and carryover. The limits of quantification were 100, 50, 5, and 5 pg/mL for NAD+, NMN, 8-oGsn, and 8-odGsn, respectively; these values were adequate for accurate measurement of these molecules in human serum and urine. Lower levels of NAD+ and NMN and higher levels of 8-oGsn and 8-odGsn were related to higher oxidative stress in patients with dementia compared with those in healthy individuals.

Conclusions

A robust LC-MS/MS method for the simultaneous measurement of NAD+, NMN, 8-oGsn, and 8-odGsn in serum and urine was established and validated.

doi: 10.1016/j.cca.2022.04.328

M038

High-throughput analysis of underivatized methylmalonic acid and total homocysteine in serum and urine by LC-MS/MS assay

X. Ma

Department of Laboratory Medicine, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100730, China

Background-aim

Total homocysteine (tHcy) and methylmalonic acid (MMA) can be used for the evaluation of the status of Vitamin B12 (VB12). Yet, few analytical methods have been described to simultaneously quantify tHcy and MMA, due to high analytical requirements, such as sensitivity at nanomolar level, separation performance for isomers, and retention properties for polar compounds. This research aims to develop an accurate and rapid LC-MS/MS method for the simultaneous quantification of tHcy and MMA in serum and urine.

Methods

In this study, underivatization methods were employed for sample pretreatment. In addition, the utilization of Primer HSS T3 column enables both the retention of the polar compound tHcy and the separation of MMA and its endogenous interference succinic acid (SA). A complete methodological validation of the method was performed according to the guidelines of CLSI-C62A. 148 matched serum and urine samples were collected and analyzed using the method.

Results

The average recovery for serum tHcy was 103.3%, urine tHcy-106.7%, serum MMA-96.8%, and urine MMA-103.0%. The inter-assay CV for serum tHcy was 1.5-2.1%, urine tHcy was 0.8-1.5%, serum MMA was 3.3-7.7%, and urine MMA was 2.5-4.6%. The intra-assay CV for serum tHcy was 8.2-8.5%, urine tHcy was 2.3-6.0%, serum MMA was 7.5-13.6%, and urine MMA was 5.9-7.5%. The matrix effect for serum tHcy was 89.1–104.0%, urine tHcy was 94.3-103.3%, serum MMA was 88.9-109.3%, and urine MMA was 93.5-101.3%. The carryover was determined to be less than 0.5%. Standards and samples were stable in −20 °C for at least two months. The limits of quantification (LOQ) were 5ng/mL for MMA, and 100ng/mL for tHcy, which is ade-

quate to cover potential MMA and tHcy concentrations in human serum and urine.

Conclusions

In this study, a high-throughput LC-MS/MS method was developed and validated for the simultaneous quantification of tHcy and MMA in human serum and urine. The MMA and tHcy levels of 148 subjects were measured by this method. The distribution data suggests that the levels of serum tHcy and serum MMA has significant difference between VB12 normal and deficient groups. Serum MMA and serum tHcy levels are inversely correlated with VB12 status.

doi: 10.1016/j.cca.2022.04.329

M039

Verification of serum protein electrophoresis on the cappilarys 2 flex piercing automaton

I. Elmezgueldi, B. Mouhoub, O. Nassiri, S. Belmahi, S. Farih, A. Amrani, A. Idrissi, E. Sebbar, M. Choukri

Faculty of Medecine and Pharmacy of Oujda, Morocco

Background-aim

The verification/validation of analytical equipment and methods is both part of this reasoning and an indispensable condition for their use and is one of the priorities of the medical biologist. The aim of our study is to verify the electrophoresis of serum proteins on the Cappilarys 2 Flex Piercing automaton.

Methods

The evaluation methodology concerned the scope A which is based on the recommendations of the Valtec protocol of the French Society of Clinical Biology, as well as those of the SH-GTA O4 protocol of the COFRAC. We studied the repeatability on normal and pathological serum samples, and the reproducibility on normal and pathological internal quality control samples.

Results

The values of the coefficient of variation of repeatability and reproducibility obtained by our study are overall satisfactory and are in accordance with the requirements issued by the supplier and those issued by RICOS. In addition, these results are consistent with those of other similar studies.

Conclusions

This type of study will provide a solid basis for the realization of an accreditation procedure for the tests used in our laboratory. For any laboratory wishing to be accredited according to the ISO 15189 standard, the validation/verification of methods is a determining criterion. It is an essential step to be taken before the implementation of the newly acquired equipment.

doi: 10.1016/j.cca.2022.04.330

M040

Precision, linearity, and comparability of TPSA and TCL on COBAS pure integrated solutions under routine-like conditions

H. Gu [a], J. Hong [a], S. Chun [a], M. Kim [c], A. Sofianidis [b], K. Klopprogge [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*
[b] *Roche Diagnostics GmbH, Mannheim, Germany*
[c] *Roche Diagnostics Korea, Seoul, Republic of Korea*

Background-aim

The novel cobas® pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a serum work area laboratory analyzer, comprising two analytical units: a clinical chemistry unit including ion selective electrodes (cobas c 303) and an immunochemistry unit (cobas e 402). We assessed precision, linearity, and comparability of tPSA and TCL assays on cobas pure integrated solutions at one site.

Methods

A multicenter study was conducted at five sites (Switzerland, Germany, Republic of Korea; Sep–Dec 2020). At one site (Republic of Korea), tPSA and TCL repeatability and intermediate precision on cobas pure were assessed using quality control (QC) material. Linearity was tested using five concentrations over the respective assay measuring range. Deming regression analyses were conducted for method comparisons between cobas pure and Abbott Alinity/Beckman Coulter UniCel DxI 800 (tPSA), and cobas pure and Siemens Vista Dimension/the laboratory's liquid chromatography–mass spectrometry (LCMS) method (TCL).

Results

tPSA repeatability coefficients of variation (CVs) were 1.0% and 1.1% and intermediate precision CVs were 1.1% and 1.2% for two respective QC materials. TCL repeatability CVs were between 1.0% and 1.6% and intermediate precision CVs between 3.1% and 3.8% for three respective QC materials. Over the measuring range (0.17–92.05 ng/mL), tPSA was judged linear (systematic error: 0.02 ng/mL [1.7%]; acceptance criteria: <0.16 ng/mL [12.5%]). Over the measuring range (0.91–27.43 ng/mL), TCL was judged linear (systematic error: 0.04 ng/mL [2.6%]; acceptance criteria: 0.06 ng/mL [4.0%]). For tPSA, Deming regression slopes were 1.09 between cobas pure and Abbott Alinity/Beckman Coulter UniCel DxI 800 (y-intercepts: -0.23 ng/mL and -0.20 ng/mL, respectively), and for TCL were 0.97 and 1.11 between cobas pure and Siemens Vista Dimension/LCMS method (y-intercepts: 1.80 ng/mL and 0.51 ng/mL, respectively).

Conclusions

The results demonstrate that cobas pure integrated solutions shows very good precision for tPSA and TCL using QC material. Both assays are linear on cobas pure integrated solutions and comparable tPSA results were observed with Abbott Alinity and Beckman Coulter UniCel DxI 800, as well as TCL results with Siemens Vista Dimension and the LCMS method.

doi: 10.1016/j.cca.2022.04.331

M041

Comparability of selected assays on COBAS pure integrated solutions under routine-like conditions

J. Hong [a], H. Gu [a], S. Chun [a], M. Kim [c], A. Sofianidis [b], K. Klopprogge [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*
[b] *Roche Diagnostics GmbH, Mannheim, Germany*
[c] *Roche Diagnostics Korea, Seoul, Republic of Korea*

Background-aim

The novel cobas® pure integrated solutions system (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) is a serum work area laboratory analyzer, comprising two analytical units: a clinical chemistry (CC) unit including ion selective electrodes (ISE) (cobas c 303) and an immunochemistry (IC) unit (cobas e 402). In a multicenter study, we assessed the comparability of the cobas pure integrated solutions system versus respective routine analyzers at four sites under routine-like conditions. Here, we report the comparability to routine non-Roche analyzers from one site in Seoul, Republic of Korea.

Methods

The study was conducted at five sites in Switzerland, Germany, and the Republic of Korea, from Sep to Dec 2020. At four sites, method comparison experiments using routine leftover samples evaluated comparability of the cobas pure integrated solutions system with respective routine analyzers. At three sites, the routine analyzers were Roche (cobas INTEGRA 400 plus, cobas e 411, cobas pro, and cobas 8000), and at one site (Seoul) were non-Roche analyzers (Beckman Coulter AU5822 Clinical Chemistry Analyzer, Abbott Alinity I, and Siemens ADVIA Centaur). There were 20 method comparisons vs. non-Roche methods. In total, 20 selected analytes covering CC (ALB, ALP, AST, CA, CHOL, CREA, CRP, GLUC, MG, PHOS, TP, UA, UREA), ISE (Cl, K, Na), and IC (CEA, TSH, FOL, Vit. B12) were assessed. Passing/Bablok regression analyses were carried out: slopes, intercepts, and correlations for method comparisons were calculated.

Results

Nearly 7000 result pairs were included in the analysis. For ISE, the bias at the medical decision point between cobas pure integrated solutions and Beckman Coulter AU5822 varied between 1% deviation (Cl, Na) and 4% (K). CC assay bias varied from 0% (CA) to 22% deviation (CRP). The IC method comparison regression analysis yielded slopes from 0.99 (CEA) to 1.28 (FOL).

Conclusions

The results of this study demonstrate that the cobas pure integrated solutions system is comparable to Beckman Coulter AU5822 Clinical Chemistry Analyzer ISE and CC results. CEA and TSH method comparisons to Abbott Alinity I and Vit. B12 and FOL comparisons to Siemens ADVIA Centaur have shown that differences in methods and reference ranges must be observed for comparison.

doi: 10.1016/j.cca.2022.04.332

M042

Simultaneous detection of respiratory infectious diseases using immunoprecipitation and liquid chromatography-tandem mass spectrometry

Y. Song, R. Gibson, S. Samra

Thermo Fisher Scientific, San Jose, CA, United States

Background-aim

With recent emergences in new infectious diseases and their variants, there is a need to develop a faster and more specific analytical tool to detect different respiratory infectious diseases such as SARS-CoV-2 or influenza viruses. Not only their symptoms are similar at early stages, but also, they are both enveloped viruses with several common biological properties, often leading to challenges in disease identification.

Among different viral components, nucleocapsid protein or nucleoprotein (NP) is highly conserved, less post-translational modifications possessed, and mostly specific for each infectious disease virus types. Therefore, targeting NP could be more advantageous to the method development, achieving much simpler and robust method with minimal subsequent modifications.

This study describes a targeted approach for simultaneous detection of NPs from different respiratory infectious diseases using immunoprecipitation (IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Multiple viruses, SARS-CoV-2, influenza virus A and B types, respiratory syncytial virus, and human coronavirus (HCoV-229E), were selected to show that this method can distinguish different disease viruses.

Methods

Sample collected via nasopharyngeal swabs in viral transport media was directly subjected to IP using Thermo Scientific™ Pierce™ MS-Compatible IP Kit (Streptavidin). The IP purified samples were then digested using SMART Digest™ Trypsin Kits and analyzed by Thermo Scientific™ Vanquish™ MD HPLC system hyphenated to Thermo Scientific™ TSQ Altis MD mass spectrometer. Data processing was performed using TraceFinder™ LDT software 1.0.

Results

Combining IP and LC-MS/MS resulted in a highly targeted approach with the high sensitivity and specificity. The method detected sub tens to hundreds amol of peptides on LC column. Also, it simplified the overall sample preparation process eliminating prior protein precipitation and post sample clean-up. Since the NPs mostly remain unchanged or less modified regardless of variants, the method doesn't need tremendous alterations once established.

Conclusions

This targeted approach can be applied to other enveloped viruses' detection. Automated IP method is available with KingFisher system so it could lead to a faster turn-around time and higher throughput of the method.

doi: 10.1016/j.cca.2022.04.333

Clinica Chimica Acta 530 (2022) S21–S24

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Atherosclerosis

M280

The relationship of dyslipidemia and the clinical manifestations of atherosclerosis in the elderly

V. Sujayeva *, I. Karpova, O. Koshlataya, O. Spirina, A. Kravtchenko

Republican Scientific and Practical Centre Cardiology, Belarus

Background-aim

To assess the functional state of the cardiovascular system and lipid metabolism in elderly patients and to identify the effect of dyslipidemia on the clinical manifestations of atherosclerosis.

Methods

96 patients aged 70.6 (66.0; 73.0) years were examined, 33 (33.3%) were men, 64 (66.7%) - women. Hypertension I-II stage was present in 87 (91%), myocardial infarction - 8 (8%), angina pectoris - 23 (24%), PCI (6) (7%), heart failure NYHA Class 1-2 - in 78 (81%). Patients have not previously received statins. We performed ultrasound analysis of carotid arteries and echocardiography (Eco) and endothelial function using the Vivid-9 system. We studied endothelium-dependent relaxation according to the method of D. Celemajer et al, 1992). Computed tomography angiography of the coronary arteries (CTCA) and coronary calcium index were performed on a dual-energy, 384-slice CT scanner of the premium class Siemens Somatom Force from General Electric Medical Systems (Germany). We also performed standart biochemical blood test with determination of the lipid spectrum, determination of apolipoprotein A-1 and apolipoprotein B in blood serum - by immunoturbidimetric method on a biochemical analyzer “ARCHITECTPLUS: 4000”, USA.

Results

According tocarotid arteries ultrasound data, 96.9% of patients were diagnosed significant (> 50%) stenoses of the carotid arteries. A positive ($p < 0.05$) correlation was found between the degree of their stenosis, on the one hand, and the coronary calcium index and the coefficient of atherogenicity of the lipid spectrum, on the other. The coronary calcium index was higher in smokers, patients with diabetes mellitus and in alcoholambuising. Atherosclerosis of coronary arteries according to CTCA was detected in 78.5% of patients. Coronary artery calcification according to CTCA was observed in 75% of patients. According to Eco aortic valve calcification was found in 67.9%, mitral valve - 75%. Disregulation of the vasomotor function of the endothelium occurred in 64.6% of patients, while a third of patients showed pathological vasoconstriction.

Conclusions

The high prevalence of clinical / subclinical manifestations of atherosclerosis in elderly people, which were interrelated with indicators of the blood lipid spectrum, dictates the need for statin therapy for both primary and secondary prevention.

doi: 10.1016/j.cca.2022.04.335

M281

High-sensitivity C - reactive protein: A novel and promising marker of coronary heart disease in obese population

S.R. Duwal *

Central Diagnostic Laboratory and Research Center, Nepal

Background-aim

C-reactive protein (CRP), an acute phase reactant that reflects low-grade systemic inflammation, produced in the liver in response to stimulus by inflammatory cytokines. Inflammation is thought to play a major role in the pathophysiological mechanisms of CVD and minor elevations in its levels are considered to be a strong, independent predictor of cardiovascular events.

Aim: To check High-Sensitivity C - reactive protein as Novel and Promising Marker of Coronary Heart Disease in obese population.

Methods

After consent, Anthropometric measurement was done according to WHO guidelines. Weight and height of candidate was taken in upright standing position without shoes. BMI , Waist circumference, hip circumference, wrist circumference was measured. Baseline venous blood sample was collected aseptically according to WHO guidelines with various inclusion and exclusion criteria. Processing was done in central diagnostic laboratory and research center. Hs-CRP was measured in serum by using enzyme linked immunosorbent assay using microwell ELISA diagnostic system.

Results

Out of 100 patients 43.3% were female and 56.7% were male. Mean + SD of Age, BMI, Waist circumference, Hipcircumference, Waisthipratio, hsCRP were 44.93 + 10.92, 29.41 + 2.98, 100.97 + 7.0,105.94 + 6.5, 0.94 + 0.1 and 42.86 + 3.5 respectively. Pearson correlation of

hs-CRP and anthropometric indices of BMI, Waist circumference, Hipcircumference, Waisthipratio, hs-CRP, R value were 0.252, 0.050, 0.23,-00.16 and P value were 0.016, 0.642, 0.025, and 0.124 respectively.

Conclusions

hs-CRP has prognostic utility in patients with acute coronary syndromes and is a strong independent predictor of future coronary events. According to our present findings increase in BMI and Hipcircumference were directly associated with increase in hs-CRP, which represents the increased cardiovascular susceptibility in the obese population.

doi: 10.1016/j.cca.2022.04.336

M282

Immature platelet fraction and mean platelet volume in differentiating acute coronary syndrome types

E. Indriastuti [b,*], Y. Hernaningsih [b], Y. Nadar Indrasari [b], A. Andrianto [a]

[a] *Department of Cardiology and Vascular Medicine, Airlangga University, Indonesia*
[b] *Department of Clinical Pathology, Airlangga University, Indonesia*

Background-aim

Acute coronary syndrome (ACS) is classified in to ST-elevation myocardial infarction (STEMI), non-ST elevation myocardial infarction (NSTEMI) and unstable angina (UA). Platelets play important roles in ACS pathogenesis. Immature platelet fraction (IPF) is a parameter that reflects proportion of immature platelets compared to total platelets. Mean platelet volume (MPV) parameter reflects mean platelet size. The IPF and MPV parameters can predict platelet activation. This study aimed to analyze the differences of IPF and MPV among ACS patients.

Methods

This study was an observational analytical cross sectional study conducted in the Dr. Soetomo Hospital during May-September 2019. The subjects consisted of 30-STEMI, 25-NSTEMI and 24-UA patients. The EDTA-samples were measured for IPF and MPV using Sysmex XN-1000. The differences of IPF and MPV among STEMI, NSTEMI, and UA patients were analyzed using Kruskall-Wallis and Mann-Whitney test.

Results

The IPF was significantly higher in STEMI patients than NSTEMI and UA patients with the p value of 0.013 and <0.001, respectively. The IPF values of NSTEMI patients were higher than UA patients ($p = 0{,}004$). The MPV was significantly higher in STEMI than UA patients ($p < 0{,}001$). The MPV was also significantly higher in NSTEMI than UA patients ($p = 0{,}002$). However, there was no significant difference of MPV between STEMI and NSTEMI ($p = 0{,}078$).

Conclusions

The IPF was significantly different among each types of ACS patients gave an opportunity using this parameter to differentiate the ACS types. The MPV was significantly higher in myocard infark patients compare to UA patients that also gave a possibility using this parameter in differentiating both conditions.

doi: 10.1016/j.cca.2022.04.337

M283

Profiling of triglycerides in lipid droplets of monocytes derived macrophage after stimulation with fatty acids and human lipoproteins

R. Shrestha [b,*], Z. Chen [b], T. Sakurai [b], H. Chiba [a], S. Hui [b]

[a] *Department of Nutrition, Sapporo University of Health Sciences, Japan*
[b] *Faculty of Health Sciences, Hokkaido University, Japan*

Background-aim

Uptake of oxidized lipoproteins by macrophages results in the formation of lipid droplets (LD)-laden macrophages or foam cells, which play a key role in the pathogenesis of atherosclerosis. Despite the importance of such lipid-laden macrophages in atherogenesis, very less is known about the molecular composition of LD and its triglycerides profiling. Therefore, the purpose of this study is to analyze the chemical composition of LD in the human monocytes derived macrophage.

Methods

Peripheral blood monocytes derived macrophage are treated with fatty acids (FA), native and oxidized lipoproteins of various degrees (VLDL, IDL, LDL, and HDL). The LD induced after such treatment were aspirated from the cells using 3D mobile manipulator visualized under a bright-field microscope and subjected to the LTQ Orbitrap analysis equipped with nano-electrospray ionization source.

Results

Treatment with FA, native and oxidized lipoproteins induced the formation of LD in the human macrophage which can be aspirated and used for the mass spectrometric analysis of lipids by solvent-tips extraction. On the basis of calculated elemental composition and fragmentation pattern in the mass spectra, we identified several molecular species of triglycerides (TG) and its hydroperoxides (TGOOH) in the cells treated with oxidized lipoproteins. The relative amount of TGOOH in LD depends on the degree of oxidation of lipoproteins.

Conclusions

The molecular composition of LD in human macrophages was determined. We identified and characterized several molecular species of TG and its hydroperoxides in the LD of macrophage. The existence of hydroperoxides in LD is possibly involved in atherogenesis.

doi: 10.1016/j.cca.2022.04.338

M284

The impact of risk factors on biochemical and lipid parameters among youngs

D. Pap [b,*], V. Canic [a]

[a] *Municipal Institute for Lung diseases and Tuberculosis, Belgrade, Serbia*
[b] *Students Health Protection Institute Novi Sad, Department for laboratory diagnostics, Serbia*

Background-aim

Individual lifestyle and habits have great impact on general health. The term “risk factors” is used to describe genetic, physical and chemical

characteristics, as well as lifestyle factors that predict an increased risk for cardiovascular disease (CVD).

The objective of this study was to perform a continuous analysis of the impact of risk factors on biochemical parameter and lipid status in a population of students at the University of Novi Sad, as a continuous research from 2016 to 2019.

Methods

240 students of the University of Novi Sad took part in the study, 117 males and 123 females. These 240 participants were divided into two groups, the control group and the risk group. The criteria for the selection of participants for the control group were BMI < 25 kg/m^2 and the waist circumference (WC) lower than 94 cm for males and 80 cm for females.

The criteria for the risk group were an increased BMI and/or the waist circumference over these values.

Biochemical, hematological and lipid status analyses were performed in both groups.

Results

The obtained results indicated that there was no statistically significant difference in the mean values for all the examined lipid parameters in the survey groups. The values of lipid status were fairly uniform in both student groups and were within normal limits, although there was an increasing trend in the parameter values among students in the risk group. By comparing hematological and biochemical parameters which were monitored in both groups, it was found that there were statistically significant differences in certain parameter values: Hgb/whole blood, fibrinogen, serum creatinine and uric acid level, the activity of the enzymes ALT and GGT. All hematological and biochemical parameters were higher in the risk group, but still within normal limits. A further comparison of other biochemical parameters showed that there was an insignificant difference between two groups in the level of SE, the concentration of fibrinogen in the blood plasma, the concentration of glucose, urea and total serum protein.

By comparing the lipid status of the total number of new participants with the status of the both groups in the preceding research, it was determined that only the HDL- cholesterol value was significantly higher in the new group of respondents. By comparing the values of the parameters of lipid status between the control group of this and the previous study, an insignificant increase in the value of the Chol, LDL -ch, IA and FR was found.

Conclusions

After the continuous analysis of the impact of risk factors on biochemical parameters and lipid status of the respondents, it was concluded that the student population of the University of Novi Sad is at risk for cardiovascular disease later in life. All this indicates the importance of early diagnosis of all precursors of atherosclerosis, early prevention and modification of all risk factors.

doi: 10.1016/j.cca.2022.04.339

M286

Determinants of scavenger receptor expression levels in human macrophages

M. Ralefatane [a,*], E. Cave [a], N. Crowther [b]

[a] *Department of Chemical Pathology, University of the Witwatersrand, South Africa*

[b] *Department of Chemical Pathology, University of the Witwatersrand and National Health Laboratory Services, South Africa*

Background-aim

In 2015, an estimated 17.5 million people died from cardiovascular diseases (CVDs), 7.4 million of these deaths were due to coronary heart disease and 6.7 million were due to stroke. Atherosclerosis is the major cause of stroke, coronary heart disease and myocardial infarction in western society. Atherosclerosis involves the accumulation of oxidized low-density lipoprotein (OxLDL)-laden foam cells (FC) within the blood vessel intima. Macrophages are transformed to FCs by the uptake of OxLDL, a process mediated by scavenger receptors (ScR), which include MSR-1, CD36 and CD68. Atherosclerosis differs in prevalence across ethnic groups, being less common in African than Asian-Indian or European populations. Therefore, our aim was to measure ScR gene expression from macrophages isolated from these three ethnic groups.

Methods

Ten participants were recruited from each ethnic group (African, European and Indian). Anthropometry and fasting serum lipid and glucose levels were measured. Monocytes were isolated from whole blood and converted to macrophages using standard cell culture procedures. Macrophage RNA was isolated and reverse transcribed using a RevertAid first strand cDNA synthesis kit. Relative gene expression was calculated using the ⊗⊗Ct relative quantification method with ®-actin used as the normalization control. Multivariable regression analysis was performed to identify the determinants of macrophage ScR expression.

Results

Gene expression of the CD36 ScR correlated with age (® = 0.33, p = 0.02), LDL (® = 0.29, p = 0.03) and CD68 expression (® = 0.57, p < 0.001). Expression of CD68 correlated with triglycerides (® = 0.45, p = 0.005) and European ethnicity (® = 0.56, p < 0.001). Expression levels of MSR-1 correlated positively with LDL (® = 0.27, p = 0.01) and negatively with HDL (® = -0.33, p = 0.003) and African ethnicity (® = -0.73, p < 0.001).

Conclusions

These data demonstrate that serum lipids may modulate foam cell formation via effects on macrophage ScR gene expression. Furthermore, ethnic differences in atherosclerotic plaque formation may be mediated through differential CD68 and MSR-1 ScR expression levels within macrophages.

doi: 10.1016/j.cca.2022.04.341

M287

Comparing the in vitro level of foam cell formation in young and older adults

M. Ralefatane [a,*], E. Cave [a], N. Crowther [b]

[a] *Department of Chemical Pathology, University of the Witwatersrand, South Africa*

[b] *Department of Chemical Pathology, University of the Witwatersrand and National Health Laboratory Services, South Africa*

Background-aim

Atherosclerosis is characterized by cholesterol accumulation within the walls of arteries, a process driven by the focal accumulation of oxidised-LDL (OxLDL)-laden foam cells (FC). Atherosclerotic disease is more prevalent in older subjects (35-69 years); however, there is no data comparing FC formation across different age groups. Therefore, the aim of this study was to compare in vitro FC formation between young and older adults.

Methods

Whole blood was obtained from healthy young (<25 years, n=11) and older (ε40 years, n=11) subjects. Monocytes were extracted from buffy coats by gradient centrifugation using Histopaque, and cultured overnight. The monocytes adhered to the culture plate and non-adherent lymphocytes were removed through washing with tissue culture medium. Monocytes were differentiated into macrophages by incubation in media containing 100 ng/ml of macrophage colony-stimulating factor (MCSF). Formation of FCs was induced through exposure of macrophages to copper-oxidized human low-density lipoprotein (OxLDL) for 48 hours. The level of FC formation was determined by measuring intra-cellular lipid accumulation (ICLA) using the lipid-specific dye Oil red O, and was expressed as a percentage of that in cells not exposed to OxLDL.

Results

The ICLA was significantly higher in the older (151.1 ± 27.9%) than the younger (100 ± 22.1%; p=0.0001) group. A multivariate linear regression model suggested that age (®=0.62, p=0.002) is the main predictor of macrophage ICLA.

Conclusions

This study shows that older subjects have a higher level of in vitro FC formation than younger participants. This may partially explain the increased prevalence of atherosclerotic disease observed with aging.

doi: 10.1016/j.cca.2022.04.342

M288

Effects of monacolin K, and B1, C and K2 vitamins -Containing nutraceutical on cholesterol homeostasis re-establishment and CVD risk reduction in hypercholesterolemic subjects

T. Gojkovic *, S. Vladimirov, J. Kotur-Stevuljevic, N. Bogavac-Stanojevic, A. Zeljkovic, J. Vekic, T. Antonic, V. Spasojevic-Kalimanovska

Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia

Background-aim

Hypercholesterolemia is caused by cholesterol homeostasis disruption, and it greatly contributes to the pathogenesis and progression of cardiovascular diseases. The efficiency of cholesterol homeostasis maintenance can be assessed by measuring serum concentrations of non-cholesterol sterols (NCSs) which serve as cholesterol synthesis and absorption surrogate markers. Monacolin K, isolated from red yeast rice, influences cholesterol synthesis by inhibiting HMG-CoA reductase activity, and reduces serum total cholesterol (TC) concentration.

Methods

This longitudinal study included 30 hypercholesterolemic patients, with systematic coronary risk estimation (SCORE) values <10%, who received 3-months-long treatment with ARTEROprotect® FORTE, a nutraceutical mixture containing monacolin K, and C, B1 and K2 vitamins, produced by Abela pharm, Beograd, Serbia. Serum NCSs were quantified by HPLC-MS/MS method.

Results

TC, LDL-cholesterol (LDL-C), and triglycerides (TG) concentrations (p<0.001), as well as SCORE values (p<0.001, p<0.01, respectively) were lowered by the treatment. Concentrations of cholesterol synthesis markers were decreased (p<0.001), whereas levels of cholesterol absorption markers remained unchanged after the treatment. Cholesterol synthesis markers reduction correlated positively with reductions in lipid status parameters.

Conclusions

These results suggest that treatment with monacolin K containing nutraceutical favorably influences lipid status parameters and atherogenic indexes by acting on cholesterol synthesis.

doi: 10.1016/j.cca.2022.04.343

Clinica Chimica Acta 530 (2022) S25–S28

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Autoimmune Disease,Allergy

M289

Evaluation of two automated immunoassays for the detection of anti-nuclear antibody

S. Yoon, H. Moon, M. Park, H. Kim, H. Chung, M. Hur, Y. Yun

Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Republic of Korea

Background-aim

Although indirect immunofluorescence assay (IIFA) is still considered the gold standard for anti-nuclear antibody (ANA) detection, it is a labor-intensive and subjective task. Recently, two fully automated immunoassays for ANA screening were developed, which were EliA CTD Screen (Thermo Fisher Scientific, Freiburg, Germany) and QUANTA Flash CTD Screen Plus (Inova Diagnostics, San Diego, USA). We evaluated the rate and profile of positive samples using these assays in the rheumatology clinic and the general examination as the baseline data. We also compared the clinical performance of these assays with traditional IIFA to diagnose ANA-associated rheumatic disease (AARD).

Methods

In total, 406 (200 rheumatology clinic and 206 general examination) serum samples were included in this study. We evaluated the positive rates of EliA and QUANTA Flash and tested positive samples in any assays by the extractable nuclear antigen (ENA) assay using EUROLINE ANA Profile 3 (EUROIMMUN, Lubeck, Germany). We evaluated the concordance and agreement between assays. We compared the clinical performance for diagnosing AARD using receiver operating characteristic curves and calculated the adjusted cut-off value. Statistical analyses were performed using MedCalc Statistical Software (version 17.2; MedCalc Software, Ostend, Belgium).

Results

The positive rates of EliA and QUANTA Flash were 48.5% and 52.0%, respectively, in the rheumatology clinic samples, and 4.9% and 8.3%, respectively, in the general examination samples. The concordance and agreement between EliA and QUANTA Flash were 92.6% and strong (= 0.817), respectively. Those between EliA and IIFA were 79.0% and weak (= 0.582), respectively, and those between QUANTA Flash and IIFA were 80.5% and moderate (= 0.607), respectively. In IIFA+/EliA- and IIFA+/QUANTA Flash-samples, 28.1% and 22.2% were ENA antibody positive, respectively. In IIFA-/EliA or QUANTA Flash+ samples, 50.0% were ENA antibody positive and all (100.0%) were SS-A (Ro) positive. EliA, QUANTA Flash, and IIFA showed good performance for diagnosing AARD (AUC, 0.917, 0.911, and 0.862, respectively), and the adjusted cut-off values of EliA and QUANTA Flash (>0.56 ratio and >9.7 CU, respectively) could improve their performances.

Conclusions

The fully automated immunoassays showed high concordance and strong agreement among each other, with comparable or superior clinical performance compared to IIFA for diagnosing AARD. These assays could be efficient options in clinical immunology laboratories with high-volume tests in combination with IIFA. Clinical cut-off values could be adjusted according to the purpose of these assays or the laboratory workflow in each laboratory.

doi: 10.1016/j.cca.2022.04.345

M290

Dynamics of cytomorphological changes in acantholytic cells during treatment of acantholytic pemphigus

I.A. Kruglova[d], K.I. Krasnova[a], O.V. Utkin[c], S.V. Zinov'Ev[b]

[a] *Nizhny Novgorod Branch of the Federal State Budgetary Institution "State Scientific Center of Dermatovenerology and Cosmetology", Russia*
[b] *Federal Budgetary Educational Institution "Privolzhsky Research Medical University", Russia*
[c] *Federal Budgetary Institution of Science " Nizhny Novgorod Research Institute of Epidemiology and Microbiology. Academician I. N. Blokhina", Russia*
[d] *State Budgetary Healthcare Institution of the Nizhny Novgorod Region "City Hospital 35", Nizhny Novgorod, Russia*

Background-aim

One of the criteria for making a diagnosis of acantholytic pemphigus (AP) is the detection of Tcank cells (acantholytic) in smears-prints from the bottom of fresh erosions, using the cytological investigation (CI). The absence of Tcank cells in smears is one of the conditions for the cancellation of therapy based on the use of high doses of glucocorticosteroid drugs, which, in addition to the therapeutic effect, have a side effect on the patient's body.

Goal. Identify cytomorphological features of acantholytic cells (AC) in various treatments for acantholytic pemphigus

Methods

The dynamics of the CI smears-prints from the bottom of fresh erosions in patients with a vulgar form of AP in comparison with the clinical manifestations of the disease and the treatment.

70 cytological preparations were evaluated (from patients with vulgar pemphigus – 62. of these, 44 drugs are used in the treatment of glucocorticosteroids (GCS), and 18 in the treatment of GCS + cytostatics). Other siteproperty obtained from patients with supraliminally dermatoses). The coloring of the preparations was performed using the Romanovsky method. The visualization was performed using the "Zeiss Primo Star" microscope (Carl Zeiss, Germany), using an x1000 magnification.

Results

When analyzing the cytological material, the following features of AC were revealed: before the start of therapy, a large number of AC prevailed, the nuclear-cytoplasmic ratio was shifted towards the nucleus, uneven staining of the cytoplasm multi-core AC cells were located in clusters of 4-15 cells. Background of the drug - a different combination of elements of inflammation

On the 7th day of GCS, there was a decrease in the number and size of AC, the location in clusters, with the appearance of individual separately lying AC. On the 14th day-a decrease in the number of AC, the shift of the nuclear-cytoplasmic ratio towards the cytoplasm, a decrease in the number of multi - core cells, the location-separately.

Therapy with GCS and cytostatics for 7-day - reducing the number of AC, the nuclear-cytoplasmic ratio is shifted to the nucleus, increased numbers of multinucleated cells, located in clusters. On the 14th day, there was a decrease in the number of AC, a shift in the nuclear-cytoplasmic ratio towards the cytoplasm, a decrease in multi-core cells, and a disjointed location.

In patients with dermatoses supraliminally changes in AK was not observed

The study noted 2.6% of non-informative material.

Conclusions

The use of CI with morphological assessment of changes in AC in AP is directly dependent on the therapy and can be one of the possible criteria for evaluating its effectiveness, as well as the basis for optimizing the treatment regimen.

doi: 10.1016/j.cca.2022.04.346

M291

A survey on antinuclear antibodies testing in Korea

K. Kim [a], I. Jeong [b], H. Moon [c]

[a] *Department of Laboratory Medicine, Dong-A University College of Medicine, Busan, South Korea*
[b] *Department of Laboratory Medicine, Dong-A University College of Medicine, Busan, South Korea*
[c] *Department of Laboratory Medicine, Konkuk University Medical Center and Konkuk University School of Medicine, Seoul, South Korea*

Background-aim

Anti-nuclear antibodies (ANA) detected by indirect immunofluorescence assay (IIFA) on HEp-2 cells are important for the diagnosis of systemic rheumatic diseases. ANA is an entry criterion on 2019 classification criteria for systemic lupus erythematosus (SLE). The computer-aided immunofluorescence microscopy (CAIFM) and International Consensus on ANA staining Patterns (ICAP) are introduced in ANA interpretation. The purpose of this study was to investigate the status of ANA testing in Korea.

Methods

E-mail survey was done on July 2019. The 15 questions and a comment column were emailed to 49 clinical pathologists working on 42 tertiary referral hospitals and 7 reference laboratories. The 37 pathologists were replied (reply rate: 75.5%) Statistical analysis was performed using the answers of 36 institutions because one hospital did not perform an ANA test.

Results

All laboratories responded were tested for ANA by IIFA on Hep-2 cells. 21 laboratories (58%) used 1:40 as the screening dilution titer, and the titer of final dilution varied. Ten of 12 laboratories using CAIFM were used for negative/positive determination, and 59% of 12 laboratories were used for reading the fluorescence pattern. 33 clinical pathologists (92%) responded as they knew about ICAP. 33 laboratories (92%) responded they do not use Anti-Cell (AC) code. The intention to use AC code was answered in 16 laboratories (48%).

Conclusions

The laboratories used 1:40 dilution screening titer need to be considered to diagnose SLE. This survey will help standardize ANA reporting and establish Korean ANA reporting guidelines.

doi: 10.1016/j.cca.2022.04.347

M292

Association of vitamin-d deficiency with anti-thyroid peroxidase antibodies positive hypothyroidism – An experience of single tertiary health care system in central

J. Palanchoki [a], B.K. Yadav [b], V.k. Sharma [b], E.T. Tuladhar [b], M. Raut [b], A. Bhattarai [b]

[a] *Coomunity Medicine, Kathmandu University Medical School, Kavre, Nepal*
[b] *Department of Biochemistry, Maharajgunj Medical Campus, TU Teaching Hospital, Kathmandu, Nepal*

Background-aim

Vitamin-D {25(OH)D} has been found to be associated with several diseases in human for long time and its deficiency is currently considered as one of the global health problem. The role of vitamin D plays a significant role in several mechanisms and some recent research paper emphasized its significant role in immunomodulation. Some studies demonstrated that vitamin D plays a significant role in reducing autoimmune thyroiditis (AIT), however, the results are not conclusive. This study is designed to find the association of Vitamin-D deficiency with anti-thyroid peroxidase (Anti-TPO) antibodies positive hypothyroidism Nepalese patients.

Methods

Total Ninety-five over thypothyroidism patients visiting Tribhuvan University Teaching Hospital (TUTH) and not on Vitamin D supplementation were enrolled in this cross sectional study. The anti-TPO and Vitamin D were assayed by fully automated Chemiluminescence Immunoassay (CLIA), Maglumi 1000 (Snibe Diagnostic) and Vitros 3600 Immunodiagnostic system, respectively. Study populations were devided into two groups with anti-TPO antibodies positive and anti-

TPO antibodies negative. Serum Vitamin D were compared in two groups. Statistical analysis was done by Statistical Package for the Social Sciences (SPSS) version 21.

Results

Serum Vitamin D was significantly lower in anti-TPO antibodies positive patients than in anti-TPO antibody negative patients ((13.6 ± 2.8 ng/ml verses 26.8 ± 2.3 ng/ml, p < 0.001). This study also found inverse correlation between Vitamin D and anti-TPO antibody (r = -0.43, p = 0.01).

Conclusions

Our study demonstrated that anti-TPO antibody positive hypothyroidisim patients have more significant Vitamin D deficiency. Therefore, this study recommends the screening of vitamin D level among hypothyroid autoimmune thyroiditis patients.

doi: 10.1016/j.cca.2022.04.348

M293

Systemic lupus erythematosus - Proinflammatory and antiinflammatory cytokines

L. Zvezdanovic, D. Markovic, V. Cosic, V. Stojiljkovic, T. Cvetkovic

Center of Medical and Clinical Biochemistry

Background-aim

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases. Numerous factors can influence the onset of SLE and development of some clinical disease manifestations with various organ involvements and occurrence of characteristic symptoms and disease signs. This paper studies the balance between proinflammatory and antiinflammatory cytokines in serum of patients with SLE.

Methods

Complete biochemical and immunologic laboratory processing of the biomaterial, enabled classification of SLE patients (n = 55), into the following groups: patients with cutaneous disease manifestation, S-SLE; patients with neurolupus, N-SLE; patients with joint changes, J-SLE; patients with blood vessel changes-vasculitis,V-SLE. Twenty healthy volunteers, comprised the control group. Concentration of proinflammatory and antiinflammatory cytokines was determined by ELISA tests.

Results

The increase was at its highest in patients with neurolupus (P < 0,001) and joint disease (P < 0,01), while cutaneous and vascular forms were of lesser significance (P < 0,05). Comparing the groups, we noticed significant TNF-⟨ increase in joint and neurolupus related to vascular SLE (P < 0,05). Interleukin-4, demonstrated statistically significant increase in neurolupus patients 11,96 ± 2,91pg/ml and vascular lupus 10,93 ± 1,77pg/ml compared to control values 8,97 ± 1,90 pg/ml for (P < 0,05). The increase of the IL-10 concentration is of statistical significance in neurolupus patients 16,25 ± 4,31pg/ml and in vascular disease 15,23 ± 2,18 pg/ml compared to controls 5,13 ± 1,51, for (P < 0,01) and skin disease 12,87 ± 2,28 pg/ml, with somewhat lower significance of (P < 0,05). Interleukin-13, showed the increase concentration in the whole SLE group 4.27 ± 1.41pg/ml related to controls 2.17 ± 0.67pg/ml at the level of significance of (P < 0,05).

Conclusions

The results of this paper indicate that TNF-⟨ can be of special importance in the N-SLE pathology. TNF-⟨ released from inflammatory cells acts synergistically in the circulation, inducing peripheral vasodilatation. Increased IL-10 can be associated with neuropsychiatric menifestations of the disease. Inhibitors of cytokine production are being extensively studied as potential therapeutics in SLE.

doi: 10.1016/j.cca.2022.04.349

M294

Non celiac gluten sensitivity-diagnostic and therapeutic challenges

D. Markovic, L. Zvezdanovic

Center of Medical and Clinical Biochemistry

Background-aim

Non celiac gluten sensitivity (NCGS) is a gluten-mediated disease which has, from recently, pays great attention due to an increasing number of a patients with hypersensitivity to gluten-containing food.

Methods

Panel of 15 experts met in London in 2011 and developed a new classification of gluten-related disorders According to the pathophysiological mechanisms, all of these disorders are divided into three major groups: autoimmune (celiac disease, dermatitis herpetiformis and gluten ataxia), allergic (wheat allergy, wheat-dependent anaphylaxis, baker's asthma and contact dermatitis), immune and non-allergic (non-cellular gluten sensitivity). NCGS is a condition in which ingestion of gluten-containing food leads to immunological, morphological and symptomatic manifestations, in persons with excluded the existence of celiac disease or wheat allergy. The first patients with NCGS symptoms were described in the 1978s by Ellis and Linaker.

Results

Epidemiological data vary from study to study, so the prevalence ranges from 0.6 to 6%. The pathogenesis is heterogeneous and still not well-known. The dominant role has an innate immune response, along with other factors, such as intestinal inflammation with changes in intestinal microbiota and permeability. It is characterized by gastrointestinal and extra-intestinal symptoms that occur shortly after consuming gluten-containig food, and withdraw after the beginning of use of a gluten-free diet.

Conclusions

A lack of biomarkers is still the main limiting factor in clinical studies, which makes it difficult to differentiate NCGS from other gluten related disorders. Future studies should define guidelines and provide answers to questions related to pathogenesis, diagnosis and therapeutic approach to NCGS.

doi: 10.1016/j.cca.2022.04.350

M295

Computer-aided immunofluorescence microscopy in antinuclear antibody testing; are we there yet?

F. Wathigo, I. Abdulhafedh

The Nairobi Hospital, Kenya

Background-aim

Indirect immunofluorescence microscopy using Human epithelial cell line-2 (HEp-2) or acceptable derivatives such as HEp-2010 cells, is regarded as the gold standard for screening for antinuclear antibodies (ANA). Conventional manual microscopy is labour intensive and often lacks proper standardization. As a result, several automated systems have been developed that generate results based on software-driven pattern recognition systems. In this study, we evaluated the performance of a fully automated Indirect Immunofluorescence system (Europattern suite, Euroimmun) in detection of ANA, compared to visual interpretation by a skilled observer as the reference standard.

Methods

A total of 357 patient samples were analyzed by the automated system. Results generated by the software-driven pattern recognition system were compared against visual interpretation made by the skilled observer; the latter regarded as the reference standard.

Results

A total of 377 patterns, comprising 185 positive and 192 negative patterns, were reported from the 357 samples. The software (Europattern) had an overall sensitivity of 85% and a specificity of 67% in discriminating positive and negative results. The software correctly identified the exact ANA pattern in 58% of the results. In addition the software was able to correctly estimate the titer to within one-dilution in 73% of the results. The majority (67%) of the false negative results arose from samples with low titers (δ1: 320).

Conclusions

Results obtained by the software were in good agreement with the visual interpretation by the observer. However, the software proved less accurate for mixed patterns and the less common patterns such as mitotic and dense fine speckled. Development of more sophisticated software and hardware will see Artificial Intelligence play a bigger role in interpretation of ANA results. In the meantime, visual interpretation by a skilled observer remains the mainstay as the automated systems evolve.

doi: 10.1016/j.cca.2022.04.351

M296

Severe autoimmune hemolytic anemia associated with mycoplasma pneumoniae - A case report

I. Lukić[a], T. Rolić[a], S. Mandić[a], M. Lukić[b], V. Horvat[a], V. Šerić[a]

[a] *Department for Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Osijek, Josipa Huttlera 4, HR-31000 Osijek, Croatia*
[b] *Institute of Clinical Laboratory Diagnostics, Osijek University Hospital, Josipa Huttlera 4, HR-31000 Osijek, Croatia*

Background-aim

We report a case of severe autoimmune hemolytic anemia (AIHA) associated with cold agglutinins following Mycoplasma pneumoniae infection. We find the importance of reporting this case in giving prominence to the spectrum of multiple organ partaking in Mycoplasma pneumoniae infection.

Methods

A 53-year-old Caucasian man was presented to the Emergency Department of our hospital with symptoms of chest pressure, shortness of breath, fever, fatigue and dark urine of 7 days duration. Patient's clinical presentation and laboratory parameters were consistent with hemolysis and a working diagnosis of AIHA was made. Diagnosis of AIHA is based on detection of autoantibodies by direct antiglobulin test (DAT) showing a pattern of antibodies and complement C3d and presence of cold agglutinins.

Results

Initial investigation showed a very low erythrocyte count 0.44x1012/L, hemoglobin level of 78 g/L and a spuriously low hematocrit 0.050 L/L. RBC indices were spuriously increased, especially MCHC 1560 g/L, MCV 113.6 fL. A peripheral blood smear revealed leukocytosis and prominent erythrocyte clusters. These findings suggested the presence of cold agglutinins in the patient sample. After incubating the tube at 370C for two hours, the CBC results seemed not to be corrected. Lactate dehydrogenase (LDH) and total bilirubin were, as expected, above the reference range. Other blood laboratory tests, such as liver profile and coagulation parameters, were within normal limits.

To confirm the diagnosis of AIHA, further screening was done with mono specific DAT and indirect antiglobulin test (IAT). Direct antiglobulin test showed predominately anti C3c and anti C3d antibodies, but anti IgA, anti IgM and anti IgG antibodies were also present. IAT was also strongly positive. The above findings indicated AIHA of cold type.

Conclusions

Lymphoproliferative and autoimmune disorders, as well as infections such as Mycoplasma pneumoniae can be associated with the production of cold agglutinins. Nevertheless, severe hemolysis is exceptional in cases with Mycoplasma pneumoniae infection. As stated in the literature, the induction of cold agglutinins may be triggered by the formation of mycoplasma-receptor complexes.

doi: 10.1016/j.cca.2022.04.352

Clinica Chimica Acta 530 (2022) S29

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Biobanking

M043

Serum, plasma and DNA biobanking project from Korean National Health and Nutrition Survey

K.H. Roh [a], S. Choi [b]

[a] *Center of Molecular Diagnostics, Seegene Medical Foundation, Seoul, South Korea*

[b] *Department of Research and Development, Seegene Medical Foundation, Seoul, South Korea*

Background-aim

Korean National Health and Nutrition Survey (KNHNS) were performed by government fund from the Ministry of Health and Welfare. The KNHNS is a cross-sectional health survey with actual laboratory test with which nationally representative sample of the Korean population from 1998. This continuous nationwide projects were done by Center of Disease Control and Prevention (CDC) in Korea. With this project, bio banking is another government project which keep serum, plasma and DNA for research. 2019 is the first year of 8th KNHNS project.

Methods

The Seegene Medical Foundation (SMF) is an independent laboratory which has a nationwide network and provides specimens collection, transportation, and testing in Korea. Every week trained interviewers conducted interview and phlebotomists collected blood specimens for laboratory tests from preselected persons in 4 different regions using national survey tool. These specimens were transported to SMF main laboratory in Seoul. Serum separation tube (SST) and EDTA whole blood tube (EDTA) were transported with temperature monitored storage box. Each 300 uL serum and plasma aliquots were prepared and also did nucleic acid extraction from EDTA whole blood tube. For quality control purpose, DNA purity and integrity, bacterial contamination were evaluated. Finally, each aliquot tubes were temperately stored in SMF and move to national bio bank.

Results

From January 2019 to December 2019, informed consents were received from 4,403 persons by interviewer and blood samples were collected. DNA purity and integrity were both acceptable and no significant bacterial contamination were found. Usual genomic DNA was recovered over 100 ug and separated into 3 different tubes.

Conclusions

We conduct this bio banking project in Korea after 2008. These high quality bio specimens are very important resource for research and can represent general population in Korea. National funded bio banking project is essential for studying of immunization rate, antibody positive rate or molecular testing for infectious disease and emerging disease.

doi: 10.1016/j.cca.2022.04.354

Clinica Chimica Acta 530 (2022) S30–S33

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Bioinformatics, Data Management

M044

Development of machine learning model for diagnostic disease prediction based on laboratory tests

D.J. Park [a], M. Park [b], Y. Kim [d], Y.H. Park [c]

[a] *Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, The Catholic University of Korea, ST. Vincent's Hospital, Republic of Korea*
[c] *Division of Hematology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[d] *Finance Fishery Manufacture Industrial Mathematics Center on Big Data, Republic of Korea*

Background-aim

Machine learning is a specialization of computer science closely related to pattern recognition, data science, data mining and artificial intelligence. The use of deep learning and machine learning in medical field is increasing, mainly visual, audio and language data fields. However, DNN (deep neural network) is not well used in medical structured data, and few studies have been conducted. Our purpose is to build new optimal ensemble model of blending a DNN model and two machine learning models in disease prediction using laboratory test features (parameters). And we analyze the differences between DNN and machine learning models in predictive performances. Finally, we reveal the feature importance relating each disease.

Methods

We curated data sets and selected 86 attributes (different laboratory test features) based on value counts, clinical importance related features and missing values. We finally collected sample data sets on 5145 cases including 326,640 (73.82%, 326,640/442,470) laboratory test results. We classified total 39 specific diseases based on ICD-10 codes. These datasets were used to construct boosting machine learning models of LightGBM and XGBoost and a DNN model using Tensor flow. We created ensemble model of a DNN and two machine learning models.

Results

The optimal ensemble model achieved 81% F1-score and 92% prediction accuracy in five most likely diseases. Deep learning and machine learning models showed differences in predictive power and predictive disease class pattern. We used confusion matrix for predictive models. In addition, we analyzed the feature importance using the SHAP value method. The parameters showing high predictive power in our models were CRP, LDH, r-GTP, platelet, ALT and creatinine. We identified and found out the correlation between each parameter and each disease in this study.

Conclusions

Our new machine learning model (DNN and boosting models) achieved a high efficiency of disease prediction in classifying diseases, using purely laboratory test parameters, without using a patient's medical history, physical examination, or radiology data. And we calculated the impact of feature importance related specific disease. Our study will be of great help in predicting and diagnosing diseases.

doi: 10.1016/j.cca.2022.04.356

M045

The importance of detecting vitamin D in newborns

D.C. Liao, W.W. Chen, H.P. Liao

Clinical pathology Department, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Background-aim

Vitamin D is a group of fat-soluble vitamins that can be obtained by ingesting food or self-synthesized by ultraviolet rays from sunlight strike the skin. Its function is to maintain calcium and phosphorus balance and bone health. The basic requirements of vitamin D vary from region to region, mainly because the degree of sunlight exposure varies, and the best indicator for measuring vitamin D concentration is 25-OH VITD in serum. This concentration reflects the vitamin D content in blood after UV exposure and dietary intake.Bone diseases caused by insufficient or deficient vitamin D are rickets, osteomalacia, and osteoporosis, and the vitamin D content in breast milk is usually very low.

Methods

We used the ARCHITECT 25-hydroxy Vitamin D reagent to measure the vitamin D content in the serum of 34 newborns and then classified them according to common standards for vitamin concentration in the body.

doi:10.1016/j.cca.2022.04.356

Results

11 were severely deficiency (<10ng/mL) and 22 were deficiency or insufficiency (10- 30 ng/mL), only one was optimal (> 30 ng/mL), and the results showed that almost all newborns were deficient in vitamin D.

Conclusions

The American Institute of Endocrinology recommends that infants aged 0 to 1 years maintain a daily vitamin D intake of 400-1000 IU to achieve a serum concentration of more than 30 ng/mL. Most of the human body's vitamin D is produced by exposure to nature sunlight. Newborns may be restricted and cannot synthesize vitamin D in this way. Although most newborns are deficient in vitamin D, testing the vitamin D concentration can help diagnose whether their vitamin D intakes needs to be increased. To achieve a blood concentration of more than 30 ng/mL.

doi: 10.1016/j.cca.2022.04.357

M046

Comparative analyses of the gene expression data obtained from microarray to obtain correlation of OSMF to OSCC

S. Ukey, P. Sharma

Department of Biochemistry, AIIMS, Jodhpur, India

Background-aim

Head and neck cancer is one of the most commonly diagnosed malignancies worldwide. Oral squqmous cell carcinoma(OSCC) is frequently diagnosed in advanced stages when metastases to regional lymph nodes are already present. Even with combination of surgery, chemotherapy and radiotherapy these patients have a high risk of recurrence. Thus, an important goal would be the identification of biomarkers for early detection. During the development of OSCC clinical disease characteristics are driven by genomic changes in the oral mucosa caused by either environmentally induced genomic changes or intrinsic genetic or epigenetic alterations. Malignant transformation in OSMF is seen due to Chronic tobacco, alcohol abuse and Human Papilloma Virus (HPV) infection. These factors affect cell cycle regulation, hypoxia, processes leading to DNA double strand breaks, senescence and many other pathways related to carcinogenesis. It has been proposed that oral cancers arising in oral sub- mucosa fibrosis(OSMF) constitute a clinico-pathologically distinct disease, believed to arise from differential mechanisms of carcinogenesis. Our aim is to correlate genetic and epigenetic alteration between OSMF and OSCC to obtain differential expression profile of genes involved in carcinogenesis, miRNA profiles and methylation profiles to obtain a clear picture for progression from OSMF to OSCC.

Methods

DATASETS- Microarray data for gene expression, miRNA, methylation profile in OSMF and OSCC obtained from NCBI- Gene expression Omnibus(GEO)

Analyses using R Studios

Results

The expression profile of certain genes like socs1, bax ,bid and few more are lower in fibrosis compared to carcinoma which may indicate the lesser oncogenic charcter of OSMF which may lead to carcinoma with increase in expression of these antiapoptotic genes. Similarly the markers such as miRNA were also analysed to obtain preliminary markers. Let-7-e was highly expressed in carcinoma compared to fibrosis and canbe used as marker for early diagnosis for carcinoma at fibrosis stage only. Methylation microarray data analyses is still in progress.

Conclusions

Analyses obtained from microarray of gene expression for fibribrosis and carcinoma gives differential expression of certain antiapoptotic genes which indicates the progressive nature of disease from fibrosis to carcinoma and miRNA expression profile differences may be useful for the early stage diagnosis of the disease.

doi: 10.1016/j.cca.2022.04.358

M047

Smartphone photomicrocraphy bridge between analogue and digital pathology – Indian experience

S. Patwardhan, M. Patwardhan

Dr. Patwardhan's Pathology Laboratory, Ulhasnagar, Mumbai, India

Background-aim

This presentation describes experience of pathologists with limited resources who have utilized affordable smartphone technologies to get experience of digital photomicrography in practice and teaching.

Methods

The solution we discovered was to exploit potential of smartphones and technologies by using microscope adaptor to get digital images.This adaptor "Smart Micro-vision" designed and patented by pathologist is affordable, easily available, stable, versatile, easy to install and use. It can connect any smartphone or tablet to any binocular microscope. Several Social network groups (each over 250 participants) administered by dedicated senior pathologists are key resources. Images on smartphone are shared regularly on social media- whats app group to discuss interesting histopathology, hematology and clinical pathology cases for diagnostic decision support and peer review. Teaching pathologists have used same modality for undergraduate and postgraduate teaching. Smartphone app for morphometry is developed with collaboration of I T engineers.

Results

This strategy has helped hundreds of pathologists to update their knowledge and skills without attending classes. They are comfortable to view, understand, discuss digital images and give opinion. They have archived digital images for documentation and self study. Undergraduate and postgraduate teachers and students from medical colleges have started using these technologies effectively.

Conclusions

Our experience in last over 3 years proves that smartphone technologies can take pathologists from analogue to digital era: pathologists including those from analogue era (trained before 2000) know advan-

tages of digital images and no longer have techno-phobia for digital technology. They are comfortable viewing digital images on electronic screen and opine. They are ready to accept digital pathology in their practice as and when it is available.

doi: 10.1016/j.cca.2022.04.359

M048

Clinical characteristic of severe asthma patients with JAK-STAT pathway activation

I.W. Masfufa, K.F. Chung, I. Adcock

National Heart and Lung Institute, Imperial College London, London, United Kingdom

Background-aim

Asthma is an inflammatory disease affected the lung passages. JAK-STAT pathway is found to be one of the potential pathway for the development of anti-inflammatory drugs for severe asthma condition. The main objective of this study is to analyze which clinical characteristics of severe asthma patients who are highly enriched for JAK-STAT gene signatures. These informations will be benefit for selecting group of patients for clinical trial of targeted treatment to prevent failure.

Methods

Gene Set Variation Analysis (GSVA) was used to analyse the gene signatures of severe asthma patients data gathered from The U-BIOPRED Project. The top/bottom 20% data of highly and lowly enriched adult severe asthma patients from the Enrichment Scores (ES) were used for group comparison analysis.

Results

The BMI value from highly enriched patients in JAK-STAT signature was in obese range with more than half used oral corticosteroid ($P = 0.001$) and underwent higher exacerbation with maximum 4 times in the past one year ($P = 0.047$). The group comparison result suggested that severe asthma patients with JAK-STAT activation showed less eosinophilic (5.95%), more neutrophilic (41.2%), and more mixed granulocytes (47.1%). Most pro-inflammatory signal via Janus Kinase (JAK) proteins binding to cytokine receptors result in Signal Transducer and Activator of Transcription (STAT) proteins activation. STATs work as transcription factor. These proteins have important role in the differentiation and activation of lymphocyte; IL-12 induces the activation of STAT4 which promoting the differentiation of Th1; STAT6, which promoting the differentiation of Th2, is activated by IL-4; and differentiation of STAT3-dependent Th17 which is stimulated by IL-6, IL-21, and IL-23. These mechanism provides evidence that increased Th1 response and Th17 cells promote neutrophilic inflammation in asthma .

Conclusions

The clinical characteristic of severe asthma patients who are highly enriched in JAK-STAT signatures are higher BMI value (within overweight & obese range), more patients used oral corticosteroid, frequent exacerbations, and involved neutrophilic airway inflammation.

doi: 10.1016/j.cca.2022.04.360

M049

Classification of pancreatic cancer stadium using recurrent neural network (RNN) model algorithm

R. Fajar, N.I. Kurniastuti

Department of Computational Biology, Yogyakarta State University, Indonesia

Background-aim

One way to detect the presence of pancreatic cancer is by examining it using Computed tomography (CT) scan. After an pancreatic cancer is detected, classification is done to determine the stage of cancer. In this study, we used the RNN model for the classification of Pancreatic cancer stages. This study aimed to explain the procedure and the accuracy of the Elman tissue RNN modeling in pancreatic cancer stage classification from the CT scan.

Methods

The process carried out is to convert the image of reed green blue (rgb) to a grayscale image on the CT scan data. After that the image was extracted with Gray Level Cooccurance Matrix which was designed using Graphical User Interface with Matlab. There are 14 features, namely energy, contrast, correlation, Sum of Square, Inverse Different Moment, sum average, sum variance, sum entropy, entropy, differential variance, differential entropy, maximum probability, homogeneity, and dissimilarity. The feature is used as input, which is then divided into training data and testing data. After that, Elman network RNN modeling was carried out with data normalization, best model design and data denormalization. The best model design was done by finding the number of hidden neurons and eliminating network inputs using the backpropagation algorithm. The best network structure obtained is 9 input neurons, 1 hidden neuron with the activation function sigmoid bipolar in the hidden layer, and linear function (purelin) at the output layer.

Results

The results of the best model training data and testing data were measured using sensitivity, specificity, and accuracy. So that from 74 training data obtained 92% accuracy rate, 96% sensitivity level as a reliable indicator when the results show pancreatic cancer, and 79% level of specificity as a good indicator when the results show normal pancreatic. While in 18 data testing showed 94% accuracy, 100% level of sensitivity and 80% level of specificity.

Conclusions

The conclusion in this study can be said that good classification results are obtained.

doi: 10.1016/j.cca.2022.04.361

M050

Verification of a portable device for the traceability of blood samples in real time developed at the Mohammed VI University Hospital of Oujda

E. Sebbar [a], M. Rehali [b], A. Azghar [a], I. Elmezgueldi [a], B. Mouhoub [a], M. Choukri [a]

[a] *Faculty of Medecine and Pharmacy of Oujda, Morocco*
[b] *IT Department, Mohammed VI University Hospital of Oujda, Morocco*

Background-aim

The objective of this study is the verification of a portable device for the traceability and the management of information concerning blood samples in real time, developed in the biochemistry laboratory of CHU Mohammed VI of Oujda. This device allows the traceability of : Date and exact time of the sampling, identity of the patient, identity of the collector, conformity of the sampling tube to the requested analysis, volume of the blood sampled, order of the tubes. This device allows the control of the pre-analytical phase, in particular the sampling stage in accordance with the requirements of the accreditation standard for medical laboratories ISO 15189 version 2012.

Methods

Our device was verified by a qualified biologist, we tested the device in 100 blood samples taken in our laboratory. The biologist checked the functionality of the device in the real conditions of the sampling, in addition he checked the non-conformities of the samples detected by the device and the accuracy of these detections. In addition, the non-conformity rate of the samples after the use of the device was compared with the basic average non-conformity rate of the samples in our laboratory. In addition, both the collector and the sampled patients completed questionnaires regarding satisfaction with the use of this device, specifying positive and negative points.

Results

The results of the use of this device were satisfactory, it allows a better traceability, in addition the rate of non-conformity of the samples after the use of the device was significantly lower than the basic average of the rates of non-conformity of samples in our laboratory. Both the collector and the patients sampled were generally satisfied with the use of this device.

Conclusions

The reliability of laboratory results does not only depend on the analysis technique, but also on the preanalytical phase and particularly on the sampling, which underlines the interest of using our device.

doi: 10.1016/j.cca.2022.04.362

M051

The variant call format normalization is essential for the accuracy of variant nomenclature

K. Park [a], J. Park [b], E. You [c], H. Kim [a], C. Park [a], Y. Kim [a]

[a] *Department of Laboratory Medicine & Genetics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Republic of Korea*
[b] *Department of Laboratory Medicine & Genetics, Samsung Medical Center, Republic of Korea*
[c] *Department of Laboratory Medicine, Inje University Pusan Paik Hospital, Republic of Korea*

Background-aim

The variant call format (VCF) is a current standard way for storing information about genetic variants. Accurate nomenclature of variants is an essential element for genetic diagnosis, while variant description from the VCF is not unique and standardized. Recent survey from Korea has indicated that more than half of the clinical laboratories have not performed VCF normalization procedure in their bioinformatics pipeline for the routine analysis. We aimed to evaluate the effect of variant normalization on variant nomenclature using two tools.

Methods

A Binary Alignment Map (BAM, GNG-21-04) file and variant information (n = 30) were provided from the Korean Association of External Quality Assessment Service. Variant annotations were done using Torrent Suite (5.10) and snpEff. The vt and GATK LeftAlignAndTrimVarinats were used to evaluate the performance of the VCF normalization.

Results

The descriptions of un-normalized variants (40%, 12/30) were considerably different from the true nomenclature, especially in indel variants. Simple application of VCF normalization procedure could provide an accurate nomenclature in most of the variants, regardless of the normalization tools (83%, 10/12).

Conclusions

The accuracy of variant nomenclature was considerably improved through normalization. The results by using vt and GATK LeftAlignAndTrimVarinats were comparable. The VCF normalization has not necessarily generated completely correct nomenclature but contribute to providing an accurate annotation of variants. This study suggested that VCF normalization should introduce into bioinformatics pipeline of clinical laboratories to ensure more reliable annotations of variants.

doi: 10.1016/j.cca.2022.04.363

Clinica Chimica Acta 530 (2022) S34–S41

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Biomarker Discovery

M052

Evaluation of extracellular fatty acid synthase and copeptin in obesity induced insulin resistance and metabolic syndrome

S. Eltabakh [a], M. Zeidan [a], H. Maged [a], M. Zikry [b], M. Khedr [a]

[a] *Alexandria University, Faculty of Medicine, Medical Biochemistry Department, Egypt*
[b] *Alexandria University, Faculty of Medicine, Internal Medicine Department, Egypt*

Background-aim

The incidence of obesity and its related insulin resistance is increasing dramatically in all societies of the world, with important pathological consequences such as type 2 diabetes mellitus (T2DM) and cardiovascular disease. T2DM is increasing at an alarming rate in both developed and developing countries, associated with a combined health and economic burden world wide.

The objective of this study was to evaluate the possibility of whether circulating extracellular fatty acid synthase (FASN) and COPEPTIN could be used as novel biomarkers associated with measures of insulin resistance and presence of metabolic syndrome in obese individuals.

Methods

The study was carried out on three different groups of subjects:

G1: Twenty obese adult individuals with metabolic syndrome.

G2: Twenty age-matched obese adults without metabolic syndrome.

G3 (control G): Twenty age-matched non obese healthy adult volunteers.

Both group (1) and (2) were chosen to be obese (Body mass index (BMI) is greater than 30 kg/m^2).

Age of subjects in all groups: 20-40 years.

All were subjected to: complete physical examination, assessment of criteria of metabolic syndrome according to the National Heart, Lung, and Blood Institute (NHLBI) and the American heart association (AHA), fasting plasma glucose (FPG), fasting insulin, lipid profile, assessment of insulin resistance by HOMA-IR and measurement of plasma copeptin and FASN levels using ELISA technique.

Results

Both the plasma copeptin and FASN levels were significantly increased in G1 than other two groups (the median of copeptin levels was 38.30 ng/ml for G1 vs 25.05 and 23.00 ng/ml for G2 and G3 respectively, $p < 0.001$.While that of FASN levels was 19.95 ng/ml for G1 vs 7.7 and 7.9 ng/ml for G2 and G 3 respectively, $p < 0.001$). Evident positive correlations were found between both plasma copeptin and FASN levels with FPG,TG,WC and BMI and negative correlation with HDL level ($p < 0.05$ for HOMA-IR and FPG ; $p < 0.01$ for TG ,WC, BMI and HDL), The area under the receiver-operating characteristic curve (AUC) for copeptin was 0.79 (95 % confidence interval, CI: 0.67 to 0.89).The AUC for FASN was 0.92(95 % confidence interval ,CI: 0.84 to 0.99) but was not significantly different from 0.86 (95 % CI:0.75 to 0.96) for HOMA-IR.

Conclusions

Our results suggest that circulating FASN and COPEPTIN could be used as novel biomarkers of obesity-induced insulin resistance that provide diagnostic advantage to measures of metabolic syndrome. To confirm these results, further multicenter prospective studies on a large scale of cases are needed.

doi: 10.1016/j.cca.2022.04.365

M053

Alteration of endocrine hormone profiles in different spectrum of TB disease

Y.T. Dabi [c], A. Edao [a], S. Kinde [a], L. Wassie [b]

[a] *Addis Ababa University, Ethiopia*
[b] *Armauer Hansen Research Institute, Ethiopia*
[c] *Wollega University, Ethiopia*

Background-aim

Hormones often act as immuno-modulators. Immune and endocrine interaction during infectious diseases may determine the failure or success of the immune response. This is particularly true for an infection like tuberculosis, in which pathogen and immune system coexist in a continuous interaction. The aim of this study was to assess selected endocrine hormones profiles in different spectrum of TB disease from repository plasma samples at the Armauer Hansen Research Institute (AHRI) laboratory.

Methods

A total of 226 plasma samples, collected from pulmonary and extra pulmonary TB patients, close household contacts of PTB patients and leprosy patients, were retrieved randomly from AHRI biorepository and evaluated for DHEA, cortisol, testosterone, estradiol, growth hormone and leptin hormones concentration using ELISA.

Results

Plasma cortisol level was significantly higher in PTB, TBLN and leprosy patients compared to both LTBI uninfected groups and infected groups. The levels of DHEA and leptin were significantly low in PTB patients compared to LTBI uninfected groups. Similarly, levels of leptin was significantly lower in TBLN and leprosy patients compared to LTBI uninfected groups. On the other hand, plasma levels of DHEA, estradiol, testosterone and leptin significantly increased in PTB patients following treatment, whereas the concentration of cortisol and human growth hormone declined significantly after treatment.

Conclusions

This alteration of hormones during TB disease and upon treatment suggests that hormones influence the immune response to MTB and therefore the course of the disease outcome.

doi: 10.1016/j.cca.2022.04.366

M054

Immune response patterns among early smear converter and non-converter dots initiated tuberculosis patients in selected health facilities in Addis Ababa, Ethiopia

D. Alemu Tareke

Department of Medical Laboratory, Arba Minch Health Science College, Arba Minch, Ethiopia

Background-aim

TB remains one of the deadly public health threats globally. Smear conversion considered as one of the surrogate markers for treatment outcomes that are also dependent on the host immune system. We aimed to test if smear conversion rate correlates with the strength of host immunity as tested in vitro.

Methods

Health facility-based, case-control study on 60 tuberculosis patients (30 cases and 30 controls) was conducted in selected health facilities in Addis Ababa from January –July 2018. After written informed consent obtained about 10 ml of venous blood samples were collected and TB antigen-specific immune responses were measured using enzyme-linked immunosorbent assay (ELISA). Data was entered using Epi-data version 3.1software and analyzed by SPSS ver. 25.0 and GraphPad ver.8.1.2. Logistic regression and non-parametric statistical tests were used to determine the mean difference among groups. A p-value <0.05 was considered statistically significant.

Results

We have found a substantial difference in the mean cytokine concentrations (in pg/ml) of IFN-© (7,121 vs. 909 and 6,917 vs. 800), TNF-⟨ (1,361.37 vs. 167.45 and 1,198.8 vs. 167.6) and IL-2 (407.34 vs.53.36 and 434.2 vs. 46.5) between smear converters and non-smear converters when exposed for PPD and ESAT-6, M. tuberculosis antigens, respectively.

Conclusions

Those with a history of prior TB treatment were more likely to have unconverted smear status (63.3 times higher). Pro-inflammatory responses were generally superior among early smear converters compared to non- converters. Strong correlation observed between cytokine levels and early smear conversion status. Multifunction T cells (as illustrated by a combination of IFN-©, TNF-⟨, and IL-2 responses) can be a potential host biomarker as correlate to early smear conversion

doi: 10.1016/j.cca.2022.04.367

M055

Rapid liquid chromatography–tandem mass spectrometry method for the simultaneous determination of pristanic acid, phytanic acid, and very long chain fatty acid levels

D. Wang

Peking Union Medical College Hospital, China

Background-aim

Very long chain fatty acids (VLCFAs), pristanic (Pri), and phytanic (Phy) are critical for the evaluation of patients with possible peroxisomal disorders due to single-enzyme defects affecting peroxisomal metabolism. The aim of this study was to establish and validate the method for the simultaneous analysis of serum levels of VLCFAs, Phy, and Pri. Furthermore, we compared the consistency of C22:0, C24:0, and C26:0 between this method and our previously established LC-MS/MS method.

Methods

Resideual serum were obtained from Peking Union Medical Colege Hospital. Passing Babloke and Bland Altman were used to analysis the consistency of different methods.

Results

The LC-MS/MS total run time was 8 min. Ammonium acetate was used in mobile phases A and B to completely separate Pri. There was good repeatability and within-laboratory coefficient variations (CVs) for VLCFAs, Phy, and Pri and the matrix effect and recovery were within $100 \pm 10\%$. The correlation coefficients of linearity of VLCFAs, Phy and Pri were > 0.99. The Passing–Bablok regression coefficients were 0.994, 0.997, and 0.997 for C22:0, C24:0, and C26:0, respectively. The slopes and intercept of regression were 0.99 and -1.08, 1.10 and -0.29, and 0.99 and -0.20 for C22:0, C24:0, and C26:0, respectively. All the 95%

CI of intercepts and slopes were contained between 0 and 1, which indicates a good relationship between the two methods. Bland–Altman plots showed that the mean biases were 1.6, -0.5, and -0.14, respectively.

Conclusions

Thus, this LC-MS/MS method simultaneously detects C22:0, C24:0, C26:0, Phy, and Pri, and can be used in routine clinical measurements for the diagnosis of peroxisomal disease.

doi: 10.1016/j.cca.2022.04.368

M056

ADRB3, ROCK2 and GEF levels in overactive bladder patients

E. Firat [a], Z. Aybek [b], H. Aybek [a]

[a] *Pamukkale Universty, Department of Medical Biochemistry, Turkey*
[b] *Pamukkale Universty, Department of Urology, Turkey*

Background-aim

Overactive Bladder (OAB) occurs due to decreased function of the adrenergic pathway or increased function of cholinergic pathway in voiding physiology. In this study, our aim was to evaluate the changes in the levels of ADRB3 which is the receptor protein of adrenergic pathway in bladder; and ROCK2, GEF proteins in the cholinergic pathway and to investigate the diagnostic potential of these proteins in OAB.

Methods

This study included 60 patients with an OAB diagnosis and a healthy control group. All patients completed a validated OAB-V8 questionnaire. The serum levels of ADRB3, ROCK2, and GEF were examined by ELISA. ROC curves were generated to evaluate the diagnostic qualification of the protein levels for OAB diagnosis.

Results

The levels of ROCK2 was significantly increased in OAB patients but no correlation was determined between the levels of ADRB3, ROCK2 and GEF with OAB symptom score. ROCK2 provided the highest diagnostic accuracy alone (AUC: 0.651) with 84.9% sensitivity in ROC analysis. The combination of ROCK2 + GEF were seen to be good indicator (AUC: 0.755). The AUC for the ADRB3 + ROCK2 + GEF combination was 0.752 with 64.2%, sensitivity and 88.2% specificity. ADRB3 + ROCK2 combination was also sensitive for diagnosis (AUC: 0.70) with 69% sensitivity and 70.4% specificity.

Conclusions

These results suggest that alteration in ROCK2 levels and its combination with ADRB3 and GEF levels can be used as an auxiliary parameter to diagnose OAB syndrome and could shed light on pathophysiology.

doi: 10.1016/j.cca.2022.04.369

M057

Various types of GC-MS-based steroid metabolome profiling in the diagnosis of adrenocortical cancer

E. Malevanaia, Z. Shafigullina, L. Velikanova, N. Vorokhobina, E. Strelnikova

North-Western State Medical University Named After I.I. Mechnikov, Saint-Petersburg, Russia

Background-aim

The aim of the study was determination of different biochemical sings of adrenocortical cancer (ACC) in patients with adrenal tumors by investigation of urine steroid profiling (USP) with gas chromatography-mass spectrometry (GC-MS) method.

Methods

We examined 43 patients with ACC (more than 3 scores on the Weiss scale), 110 patients with adrenocortical adenomas (ACA) without malignant potential (MP), 28 healthy donors. USP was studied by GC-MS on Shimadzu GC-MS QP-2010 Ultra, 68 steroids were identified.

Results

Main biomarkers of ACC were identified: tetrahydro-11-deoxycortisol (THS), etiocholanolone (Et), dehydroepiandrosterone (DHEA) and its metabolites [androstendiol-17® (dA2-17®), 16®-OH-DHEA, androstentriol (dA3), 16-oxo-dA2], 17-OH-pregnanolone (17P), pregnandiol (P2), pregnantriol (P3), 11-oxo-P3, pregnendiol (dP2), pregnentriol-3⟨ (dP3-3⟨), 16dP2-3⟨. Sensitivity and specificity of these steroids for differential diagnosis of ACC and ACA were > 95% (AUC > 0.97). Additional ACC biomarkers were non-classical 5-ene-pregnenes: 16-OH-pregnenolone (16dP), 21dP, 21dP2, 11dP3, dP3-3®, 16dP2-3®, which were not detected in patients with ACA without MP. Patients with ACC had features of 5®-reductase activity increase, decrease of 11®-hydroxysteroiddehydrogenase (type II) activity, 11®- hydroxylase activity (79.1% of ACC), 21-hydroxylase activity (19.5% of ACC). It was revealed 4 types of steroidal patterns in patients with ACC. Common ACC signs were increase of urinary excretion (UE) of P2, P3, 5-ene-pregnenes (dP2, dP3, 16-OH-dP2) and presence of non-classical 5-ene-pregnenes. Urinary excretion of THS (>1000 μg/24 h), androstendione metabolites, DHEA (>2000 μg/24 h) and its metabolites (1 type) was also increased in 28 patients with ACC (65.1%). It was noticed gain in UE of THS (>2000 μg/24 h) and etiocholanolone in 6 ACC patients (13.9%), whereas urinary excretion of DHEA and its metabolites didn't differ from healthy donors (2 type). Increasing UE of DHEA (>14000 μg/24 h) and its metabolites, androsterone and etiocholanolone was observed in 5 patients with ACC (11.6%), while UE of THS didn't differ from healthy donors (3 type). There was no difference between UE of androgens and THS in 4 ACC patients and healthy donors.

Conclusions

Urine steroid metabolomics is a powerful innovative approach for early and differential diagnosis of adrenal tumors. It allows to distinguish malignant and benign tumors and determine malignant potential in early studies of diseases. It was revealed 4 types of USP by GC-MS in patients with ACC. It was observed alteration in activity of 5®-reduc-

tase, 11®-hydroxysteroiddehydrogenase (type II), 11®- hydroxylase and 21-hydroxylase in ACC patients.

doi: 10.1016/j.cca.2022.04.370

M058

The study on serum protein spectrum expression changes for patients with gastrointestinal disease after acupuncture

g. Yanhong

Department of Clinical Biochemistry, Chinese PLA General Hospital, Beijing 100853, China

Background-aim

Acupuncture therapy can make people produce a tingling sensation through the needle to stimulate acupoints of the human body. It has the function of dredging channels and collaterals and warming spleen and stomach for dispelling cold. At present, although the efficacy of acupuncture at Zusanliin the treatment of gastrointestinal diseases has been widely recognized, relevant basic research is still insufficient and mechanismsareunclear.In order to promote the innovation of traditional Chinese medicine in our country anddeeply elucidate the regulatory mechanism of acupunctureat Zusanliin gastrointestinal diseases, the current study selectedgastrointestinal diseases as subject and Zusanli et al. aseffectivestimulatingacupoints, intending to analyze the changes in serum biochemical indexes before and after acupuncture; on this basis, changes in their protein expressions were analyzed by biomass spectrometry.

Methods

Serum protein expression in 7 patients with gastrointestinal diseases before and after acupuncture were detected by MALDI-TOF mass spectrometer. Blood biochemical indexes in 7 patients with gastrointestinal diseases before and after acupuncture were detected by 7600DDP.

Results

Compared with patients with gastrointestinal disease before acupuncture treatment, the triglyceride (TG) level of patients with gastrointestinal diseases after acupuncture treatment decreased. There were 2 differential protein peaks (2625 (m/z), 2742 (m/z) between patients with gastrointestinal disease before acupuncture treatment group and after acupuncture treatment group. There were 4 differential protein peaks (2767(m/z), 2742(m/z), 2754(m/z),2568(m/z)) between patients with gastrointestinal disease before acupuncture treatment group and the control group; we establish two-dimensional analysis with 2767 (m/z) (x axis) and 2742 (m/z) (Y axis) and found that acupuncture treatment has obvious effect on patients with gastrointestinal disease , it is close to the normal.

Conclusions

2742 (m/z) and 2767 (m/z) play an important role in acupuncture treatment mechanism of patients with gastrointestinal disease.

doi: 10.1016/j.cca.2022.04.371

M059

Evaluation of 10 serum biomarkers in vancomycin-induced nephrotoxicity

K. Sangmi, P. Hyung-Doo, L. Soo-Youn

Departments of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

Background-aim

Vancomycin induced nephrotoxicity(VIN) is associated with a higher mortality rate. Since serum creatinine(SCr), the most commonly used biomarker to evaluate kidney function, has limitations for accurate detection of VIN, several potential biomarkers have been proposed. Here, we evaluated multiple serum biomarkers to explore their usefulness in the diagnosis of VIN.

Methods

We measured the serum levels of 10 biomarkers in patients who were given vancomycin therapy with or without VIN and healthy controls and analyzed the association between the biomarker levels and the development of nephrotoxicity. Initially, we measured serum levels of 10 biomarkers (IL-18, TNF-R1, CXCL10, osteopontin (OPN), TFF3, TIM-1, clusterin, cystatin C, RBP4 and NGAL) using a Luminex assay in 57 patients (20 with VIN, 17 without VIN, 20 healthy controls). Then, based on the result of Luminex assay dataset, six biomarkers (CXCL10, OPN, TFF3, cystatin C, RBP4 and NGAL) were selected and validated using enzyme-linked immunosorbent assay (ELISA) in 74 patients (28 with VIN, 23 without VIN, 23 healthy controls). Demographic, clinical and lab data were assessed. VIN was defined according to the consensus statement of the ASHP, IDSA, and SIDP.

Results

In the Luminex assay, the median serum level of IL-18, TNF-R1, CXCL10, osteopontin, TFF3, cystatin C and NGAL was significantly higher in the VIN patients than in the other two groups. In the ELISA assay of the six selected biomarkers, median levels of CXCL10 (509.2 vs 127.2 pg/mL), osteopontin (9502.8 vs 1345.4 pg/mL), TFF3 (27,542 vs 6,633 pg/mL), cystatin C (2,235 vs 774 ng/mL) and NGAL (157.5 vs 60.3 ng/mL) were significantly higher in VIN patients than in other two groups ($p < 0.05$). However, the concentration of RBP4 (30.36 vs 47.81 pg/mL) showed no significant difference. The ROC analysis of the six biomarkers in differentiating VIN patients and controls yielded AUCs ranging from 0.84 to 0.99, except for RBP4 (0.65).

Conclusions

The serum concentrations of CXCL10, osteopontin, TFF3, cystatin C and NGAL were significantly increased in VIN patients, suggesting that these markers have potential applicability in the diagnosis of VIN.

doi: 10.1016/j.cca.2022.04.372

M060

Evaluation of the cutoff for major AFP qualitative reagents in Korea

J.K. Lee [a], D. Jang [c], Y. Kim [c], M. Suh [c], T.S. Kim [e], W.I. Lee [b], J. Song [d], H. Park [a]

[a] Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea
[b] Kyung Hee University Hospital at Gangdong, Seoul, South Korea
[c] National Cancer Center, Goyang, South Korea
[d] Seoul National University College of Medicine, Seoul, South Korea
[e] Seoul National University Hospital, Seoul, South Korea

Background-aim

Serum alpha-fetoprotein (AFP) is useful for evaluating the diagnosis and treatment response of liver cancer. We evaluated the appropriateness of the cutoff value in various AFP reagents, which were widely used in Korea in 2016. The AFP qualitative reagent kits included Humasis AFP Card (Humasis, Anyang, Korea), Asan Easy Test AFP (Asan pharmaceutical, Seoul, Korea), HiSens AFP card (Genematrix Bio, Seongnam, Korea), Genedia AFP Rapid II (GCMS, Yongin, Korea), BioTracer (NanoEntek, Seoul, Korea) and SD bioline AFP (Alere, Yongin, Korea).

Methods

Five AFP levels were set using commercialized quality control (QC) materials (BioRad, Hercules, USA). Quantitative tests for each QC materials were performed three times per day for three days (nine times repeat test) by Centaur XP (Siemens Healthineers, Munich, Germany). For the six kinds of qualitative AFP reagents, 20 times repeat tests were done with five levels of QC materials to ensure consistent results are achieved. Cutoff value was 20 ng/mL in Humasis, Asan pharmaceutical, NonoEntek, and Alere, and it was 10 ng/mL in Genematrix Bio and GCMS.

Results

The concentration (mean ± SD) of the commercialized quality control materials was as follows: 9.68 ± 1.03 ng/mL (level 1), 37.08 ± 2.48 ng/mL (level 2), 71.84 ± 3.20 ng/mL (level 3), 136.53 ± 7.33 ng/mL (level 4), and 275.51 ± 12.15 ng/mL (level 5). Humasis AFP card showed all negative results on repeated tests with level 2 QC. Asan pharmaceutical reagents were all negative at all QC levels, it suggests that false negative rate was very high. GCMS reagents were all positive at all QC levels, it suggests that false positive rate was very high. In the Genematrix Bio, NanoEntek and Alere reagents, inconsistent results were found in Level 2 or Level 3 QC materials when the cutoff values were considered.

Conclusions

It is necessary to match the cutoff criteria for AFP qualitative reagents. Based on cutoff, reagents performance needs to be managed so that appropriate AFP measurement results appear consistent.

doi: 10.1016/j.cca.2022.04.373

M061

Urine matrix metalloproteinases (MMPS): Potential marker for bony fusion following spinal instrumentation

R. Rani [b], D. Agrawal [a]

[a] Neurosurgery, JPN Trauma Center, All India Institute of Medical Sciences, New Delhi, India
[b] Stem Cell and translational Neuroscience Laboratory, Neurosurgery, JPN Trauma Center, All India Institute of Medical Sciences, New Delhi, India

Background-aim

Background: The process of bony fusion is characterized by the production of a new organic matrix, known as osteoid and its subsequent mineralization, thus bridging the gap between the bone grafts (bridging callus). However, till date, clinically validated method to assess spinal bony fusion is not available. Specific metalloproteinases have been shown to be essential to processes of both angiogenesis and mineralized cartilage resorption and bone remodeling at different phases of fracture healing.

Aim: The aim of this study is to determine the potential of using simple urine based assay of the activity of two MMPs (MMP9 & MMP13) as a means of assessing the biological progression of bony fusion following spinal instrumentation.

Methods

Methodology: In this prospective study, patients with dorsolumbar injury undergoing instrumentation from one unit in neurosurgery over a 6 month period were enrolled for the study. Baseline demographics and ASIA Score were recorded in all cases. Urine samples were obtained from patients immediately within 72 hours after injury. The other samples were collected in similar manner at 1 month and 6 months after injury. All urine samples were stored into dry ice pellet tubes at −80° C. ELISA was performed to detect MMP9 & MMP13 level in urine samples. MMP-9 and MMP-13 estimation were done by ELISA at the baseline level and the 1 month follow up. CT of the spine was done at one month and at 6months of follow up.

Results

Results: Our study included a total number of 37 spinal cord injury subjects. 30 (81.08%) were male and 7 (18.92%) were female. The mean age of the subjects was 35.7 years (range 18-60 years). We were able to manage one month and six month follow-up for 24 patients and 10 patients respectively.

Of the 24 patients with one month of follow up, only one patient showed some evidence of fusion. 10 patients were evaluable at the six month follow up and 4 patients did not show any radiological evidence of fusion and 6 patients had radiological evidence of fusion.

The levels of urinary MMP9 ($p = 0.01$) and MMP13 ($p = 0.04$) were significantly increased at six months in the patients with fusion vis-à-vis these who did not have fusion.

Conclusions

Conclusion: The study suggests that urinary levels of MMP9 and MMP13 have potential as metabolic markers to monitor the progression of fracture healing at six months.

doi: 10.1016/j.cca.2022.04.374

M062: Biomarker Discovery

Identification of biomarkers in patients with colorectal cancer in Kazakh population

L. Akhmaltdinova, V. Sirota, I. Kadyrova, V. Zhumaliyeva, D. Babenko, O. Avdienko, M. Maratkyzy

Karaganda Medical University, Kazakhstan

Background-aim

More than a million people suffer from colon cancer and about 500 thousands of deaths from this disease are registered annually in the world. Kazkhstan remains in high prevalence of oncological diseases among CIS countries, and Karaganda area is one of the leading in Kazakhstan in this respect. Despite the existing screening programs for colorectal cancer, the development of new markers does not stop, including serum biomarker panels for early diagnosis, and in patients with an identified disease, panels for assessing the prognosis, metastasis and invasiveness of the process.

To study the role of serum biomarkers in the diagnosis of colorectal cancer and assess their role in invasive tumor growth in the Kazakh population.

Methods

We performed cohort study in Kazakh patients. Determination of serum biomarkers was carried out by 24 Milliplex Map (Millipor) Human Circulation Biomarker Kit. PD-L1 was determined using Human ProcartaPlex™ Kit (by Termo Fisher), oncomarker CA 72-4 was identified using ELISA kit by Xema (Russia). Statistic processing included Kruskal-Wallis and Mann-Whitney analysis. Significance level was taken as $p \leq 0.05$. Informed consent was obtained for the examination of patients. Personal privacy rights were observed.

Results

Null and alternative hypothesis include absence or presence of biomarkers change respectively. A group of patients first time diagnosed with colorectal cancer (n = 215) took part in the investigation. The average age: (Me [Q1;Q3]) 66.6 years old [62;72]; 45% men and 55% women. A standard clinical examination and questioning to reveal the risk factors were performed. Biomarker blood test was done prior to surgery. Control group included 53 relatively healthy participants aged Me [Q1;Q3]47 [43;57] without ancestors of first or second family line having oncology of any localisation. Comparison group was presented by 55 patients 59,5 [53,3;65] of age with inflammatory intestine diseases (Crohn's disease, intestinal polyps and gastrointestinal polyposis). Experimental group consists of 215 patients aged 18-70 years old with a diagnosis of colorectal cancer (adenocarcinoma), excluding the hereditary form of CRC. After which the patients were divided into subgroups: with the presence of invasive growth (n = 69) and with its absence (n = 153).

In none of the cases did the biomarkers studied reveal significant differences in the content between the group of healthy individuals and those with non-cancerous bowel diseases. Classical markers CA 19-9 M12,0[5,46;18,70], p < ,0001 and CA 125 6,43[2,4; 13,47], p = 0,0008 show an increase in the level specific for invasive growth, significant compared with all other groups . CA 15-3 10,57 [4,97; 29,4], p = 0,039 also maximally increases in the group of CRC with invasive growth, however, significance is achieved only in comparison with the comparison group. The classic CEA marker 2477,6 [1166,8; 8153,7],p < ,0001 and HE 4 6658 [4563;8856], p < 0,0001 rises equally in both groups in CRC, with no difference between the groups. Markers such as CYFRA21-1 1416,14 [906,1; 2213,68], p < 0,001 and OPN 6121 [3970; 11536], p = 0,001 in the group with invasive growth of CRC significantly differ both from the control group and from the comparison group, however, they do not allow to distinguish between the group with invasive and non-invasive growth of tumor growth . An increase in the serum level of the programmed cell death ligand -1 in the group with invasive CRC reaches significance with the control group (but not the comparison group), but it also almost does not differ within the group with CRC. VEGF 115,33 [90,6; 156,0], p = 0,0016 behaves atypically in our study, which grows significantly in the group with non-invasive growth, then in the group with invasive growths it is almost identical to non-oncological groups. The serum level of sFASL 57,63 [44,17; 86,86], p = 0,0001 (but not sFAS) behaves similarly, reaching a maximum in the group with non-invasive tumor growth.

Conclusions

Studies of biomarkers at different stages of tumor growth allow us to identify new mechanisms of tumor growth, use new data for diagnosis, study of treatment tactics and prognosis in particular for colorectal cancer. We found a relationship between the presence of invasion and rates of biomarkers in the Kazakh population.

doi: 10.1016/j.cca.2022.04.375

M063

Evaluation of plasma copeptin as a novel biomarker of obesity-induced insulin resistance and metabolic syndrome

S. Eltabakh [b], M. Zeidan [b], H. Maged [b], M. Zikry [a], M. Khedr [b]

[a] *Internal Medicine, Alexandria University, Alexandria, Egypt*
[b] *Medical Biochemistry, Alexandria University, Alexandria, Egypt*

Background-aim

The objective of the study was to evaluate whether circulating Copeptin could be used as a novel biomarker associated with measures of insulin resistance and presence of metabolic syndrome in obese individuals.

Methods

The study was carried out on three groups: G1: Twenty obese individuals with metabolic syndrome, G2: Twenty age matched obese adults without metabolic syndrome, and G3: Control group: Twenty age matched none obese healthy adults volunteers. Both group one and two to be obese with body mass index (BMI) is greater than 30 kg/m^2. All groups were subjected to: complete physical examination, assessment of metabolic syndrome criteria according to the National Heart, Lung and Blood Institute (NHLBI) and the American Heart Association (AHA), fasting plasma glucose (FPG), fasting insulin, lipid profile, assessment of insulin resistance by HOMA-IR and measurement of plasma Copeptin levels using ELISA technique.

Results

The results of plasma Copeptin levels were significantly increased in G1 than the other two groups (median 38.30 ng/ml for G1 vs 25.05 and 23.00 in G2 and G3 respectively, P < 0.001). Evident Positive correlation were found between plasma Copeptin levels and HOMA-IR , FPG , TG , WC , BMI and HDL level (P < 0.05 for HOMA-IR and FPG ; P < 0.01for TG , WC , BMI and HDL .The area under the receiver-operating characteristics curve (AUC) for Copeptin was 0.79 (95% confident interval , CI : 0.67 to 0.89).

Conclusions

Our results suggest that circulating Copeptin could be used as a possible biomarker of obesity induced insulin resistance that provide diagnostic advantage to measures of metabolic syndrome. Further

prospective multi center studies on a large scale of cases are recommended.

doi: 10.1016/j.cca.2022.04.376

M064

Hyperhomocysteinemia is associated with deep vein thrombosis

M. Boudaya [b], S. Fendri [b], R. Ben Salah [a], K. Jamoussi [b], Z. Bahloul [a]

[a] *CHU Hedi Chaker, Internal Medecine Departement, Sfax, Tunisia*
[b] *CHU Hedi Chaker, Laboratory of Biochemistry, Sfax, Tunisia*

Background-aim

Deep vein thrombosis is a public health problem for which the etiological research is an essential step in the management. Various studies have shown an association between hyperhomocysteinemia and venous thrombosis. We propose during our work to study the relationship between deep vein thrombosis and homocysteine.

Methods

This was an observational case-control study comparing 47 patients admitted to the internal medicine department aged less than 60 years for the management of deep vein thrombosis confirmed by radiological examination, with negative etiological investigation, with 47 healthy controls. These two groups were matched in age, sex and body mass index. The homocysteine assay was performed by an enzymatic technique.

Results

The mean age of patients and controls was 40.8 ± 10.5 years with extremes of 18 and 59 years. The two groups consisted of 27 men (57.5%) and 20 women (42.5%) with a sex ratio of 1.35. Homocysteine was significantly ($p < 0.001$) higher in patients (17.42 ± 8.5 μmol / L) compared to controls (9.41 ± 3.1 μmol / L), with a prevalence of hyperhomocysteinemia (defined by a homocysteine level > 15 μmol / L) of 61.7% in the patients against 4% in the controls. It was found that hyperhomocysteinemie was significantly correlated with the occurrence of deep vein thrombosis with an odds ratio of 3.54 with a 95% confidence interval [1.76 - 16.4] compared to the control group.

Conclusions

Homocysteine is an intermediate sulfur amino acid in the metabolism of methionine and cysteine. The increase of this metabolite should always be alarming pushing to further investigations and to preventing from thromboembolic complications.

doi: 10.1016/j.cca.2022.04.377

M065: Biomarker Discovery

Dosage of iohexol in serum and urine by HPLC-UV for direct measurement of glomerular filtration rate: matrix effect, stability and development of the method

S. El Assri [a], E. Sebbar [a], Y. Bentata [b], M. Choukri [a]

[a] *Central Laboratory, Mohammed VI University Hospital, Oujda, Morocco*
[b] *Nephrology and Kidney Transplantation Unit, University Hospital Mohammed, Oujda, Morocco*

Background-aim

The determination of glomerular filtration rate (GFR) is essential for the exploration of renal function. The most commonly used marker in clinical practice is creatinine clearance. However, it has limitations in some populations (obese patients) where the precision of GFR measurement is required. Contrast agents such as iohexol could be an excellent alternative as a marker of choice for DFG since he is safe and his clearance could replace that of inulin (reference marker).

Methods

The objective of our work is the evaluation of the stability, matrix effect and selectivity of the determination of iohexol in serum and urine.

Results

The method is linear for both matrices ($r2 > 0.99$) and the recovery are higher than 98.05%. in practice, no interference was detected. The results of the matrix effect showed a clinically acceptable variation in most concentration levels except for 100 g/ml where there was a slightly significant variation ($p < 0.05$). Analytes were considered stable under most storage conditions except in urine where stability is significantly decreased after 3 freeze-thaw cycles ($p < 0.01$) and after freezing to -20 °C for 2 months ($p < 0.001$).

Conclusions

According to these results, this method is simple, specific, linear and precise, which allows its application to the direct measurement of the DFG after method validation.

doi: 10.1016/j.cca.2022.04.378

M066

COV^{2}MS: A tool for simultaneous longitudinal epidemiological monitoring of a variety of pathogens

B. Van Puyvelde [b], K. Van Uytfanghe [a], M. Dhaenens [b]

[a] *Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium*
[b] *ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium*

Background-aim

A lesson learned already in the early phase of the COVID-19 pandemic is the need for diagnostic tools that target different biomolecules, using orthogonal experimental setups and fit-for-purpose specification of detection, in addition to the well accepted reverse transcription polymerase chain reaction (RT-PCR).

Methods

The Cov-MS effort developed an isotope dilution (based on QconCAT technology) - liquid chromatography mass spectrometry (LC-MS) method that allows accurate, high throughput measurement of SARS-CoV-2 nucleocapsid (NCAP) protein. It uses Stable Isotope Standards and Capture by Anti-Peptide Antibodies (SISCAPA) technology to enrich and quantify proteotypic peptides of the NCAP protein from trypsin-digested samples from COVID-19 patients. The method is for a bigger part automatable (in terms of the sample preparation, digestion, peptide enrichment and LC-MS measurements).

Results

The Cov^{2}MS assay is compatible with most matrices including nasopharyngeal swabs, saliva and blood plasma, with a sensitivity into the attomole range thanks to the peptide enrichment. The latter also reduces dependency upon LC and allows shortening of LC run time, resulting in the analysis of up to 500 samples per day per MS instrument. There is a strong positive correlation between the SISCAPA antigen assay and qPCR detection up to a Cycle threshold (Ct) of 30. Importantly, peptide enrichment allowed detection of NCAP protein in a pooled sample containing a single PCR positive patient mixed with 31 PCR negative samples, without loss in sensitivity. Finally, we also demonstrated that it is feasible to rapidly adapt the method for the incorporation of ever emerging variants of concern (VoC), and even other types of respiratory viruses (e.g. Influenza A and B).

Conclusions

In conclusion, since the Cov^{2}MS assay is insensitive to pooling and easily multiplexed, it can provide longitudinal epidemiological monitoring of large numbers of pathogens within a population and can be applied as an early warning system.

doi: 10.1016/j.cca.2022.04.379

Clinica Chimica Acta 530 (2022) S42–S48

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Bone Metabolism

M297

Hypophosphatasia in paedriatic population: an experience from a tertiary care center of Pakistan

T.H. Bukhari, I. Siddiqui, A.H. Khan

Aga Khan University Hospital, Karachi, Pakistan

Background-aim

Little attention has been paid to hypophosphatasia or low serum alkaline phosphatase (ALP) levels. Our aim was to determine the frequency of low serum ALP in pediatric population being tested for ALP in our laboratory.

Methods

A retrospective laboratory based study was conducted over a period of ten years Serum ALP of all children (18 years) being tested at clinical laboratory of AKUH from January 2007 to December 2016 were extracted from laboratory information system along with their demographics. The data were double checked by two data entry operators in EpiData (version 3.2) and data entry errors were removed. Duplication values like matched and mismatched were also checked by XLSTAT software. Clean data then was converted into SPSS (version 21). Frequencies and percentages were calculated for all study categorical values. Quantitative variable was calculated in terms of mean and standard deviation i.e. age and ALP. Cot off < 100 U/L was taken for low serum ALP.

Results

In ten years a total of 180,000 children were tested for serum ALP out of which low ALP values were seen in 21886 children (12.1%). Out of the total children with low ALP 66.3ssess (n = 14532) were females and 33.5% (n = 7351) were males. Children were further stratified into four age groups and lo ALP was found in 1% (n = 1789) in age <5 years, 0.60 % (n = 1152) in age group 5-10 years, 2% (n = 3677) in age group 11-14 years and 8.3% (n = 15268) in age group 15-18 years.

Conclusions

The frequency of low ALP was noted in 12.1% of the children. Patients with low ALP require further clinical, biochemical and radiological assessment to rule out hypophosphatasia.

doi: 10.1016/j.cca.2022.04.381

M298

Vitamin D deficiency a possible precursor to sarcopenia in young adult females: A cross sectional study from Karachi, Pakistan

L. Jafri [a], G. Naureen [d,e], S. Brennan-Olsen [d,e], D. Scott [b,c], A. Habib [a]

[a] *Dept. of Pathology and Lab Medicine, Aga Khan University, Karachi, Pakistan*
[b] *The University of Melbourne and Western Health, VIC, Australia*
[c] *School of Clinical Sciences at Monash Health, Monash University, Clayton, VIC, Australia*
[d] *The University of Melbourne, VIC, Australia*
[e] *The University of Melbourne and Western Health, VIC, Australia*

Background-aim

Vitamin D deficiency is highly prevalent among young women and men living in Karachi, Pakistan.

Low serum 25 hydroxyvitamin D (25(OH)D) levels are associated with low muscle mass and strength3 in older and young people. No data available on relationships between vitamin D status and muscle mass in young Pakistani adults.We aimed to investigate associations between the severity of vitamin D deficiency with muscle mass in younger females and males from Karachi, Pakistan.

Methods

Cross-sectional measures of muscle mass using bioelectrical impedance analysis (BIA) were ascertained from medical students enrolled at Aga Khan University Hospital, Karachi (2014). Serum 25 hydroxyvitamin D [25(OH)D] was determined and categorized as sufficient (ε50nmol/L), mild deficiency (30–49 nmol/L), and moderate to severe deficiency (<29nmol/L). Potential confounders included socio-demo-

graphic and anthropometric parameters, and lifestyle behaviours. Due to sex-interactions, sex-stratified generalized linear models with gamma distribution were used to assess associations between vitamin D and the separate outcomes of absolute muscle mass (kg), muscle mass/height (kg/m^2) and muscle mass/body mass index (BMI) [$kg/(kg/m^2)$].

Results

In our sample of 101 participants (58% females; median age 20, range 18-24 years), moderate to severe vitamin D deficiency was highly prevalent in females (47.5%), whereas greater proportion of males (76.2%) had mild vitamin D deficiency. In age-adjusted, sex-stratified models, absolute muscle mass (kg) was significantly lower in females with mild (-0.08, 95% CI: -0.127, -0.034) and moderate to severe (-0.067, 95%CI: -0.115, -0.019) vitamin D deficiency, compared to those with sufficient vitamin D levels. No further relationships were observed for females, and no associations between vitamin D and muscle mass were seen for males. Potential confounders did not influence associations.

Conclusions

The relationship of moderate to severe vitamin D deficiency in females with low absolute muscle mass (kg) suggests that serum 25(OH)D levels <29 nmol/L may be a contributing factor to low absolute muscle mass in females. Improving vitamin D levels in young adult females would improve muscle mass, and potentially reduce the likelihood of earlier onset sarcopenia in later life.

doi: 10.1016/j.cca.2022.04.382

M299

A correlation between receptor activator of nuclear factor-kb ligand (RANKL) and osteoprotegerin (OPG) with bone mineral density (BMD)

F.A. Aulia [b], S.U. Lestari [a], L. Anniwati [b], S.W. Mudjanarko [c], F.R. Marpaung [b]

[a] *Department of Biomedic Faculty of Medicine, Universitas Wijaya Kusuma Surabaya, Indonesia*
[b] *Department of Clinical Pathology Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Hospital Surabaya, Indonesia*
[c] *Department of Internal Medicine Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Hospital Surabaya, Indonesia*

Background-aim

Osteoporosis is a disease characterized by low bone mass, decreased bone tissue, and disruption of bone microarchitecture. The diagnosis of osteoporosis so far has been based on fracture manifestations after minimal trauma or by detecting low BMD. Measurement of RANKL and OPG levels has opened the discourse of a more specific picture of osteoblast and osteoclast regulation. The purpose of this study was to determine the correlation between RANKL and OPG levels with BMD.

Methods

A total of 58 postmenopausal females from 13 elderly in Integrated Community Health Care Surabaya and Sidoarjo were enrolled. Data were collected by recording age, the onset of menarche, onset of menopause, and BMI (Body Mass Index). BMD of the lumbar and proximal femur vertebrae were evaluated using Hologic® Discovery™ QDR™ Dual-energy X-ray Absorptiometry (DEXA). Serum RANKL and OPG levels were measured using sandwich ELISA from Elabscience®. RANKL/OPG ratio was obtained from the ratio between measured RANKL and OPG levels in serum.

Results

The Pearson's correlation test of this study showed no significant relationship between BMD and RANKL levels (lumbar: $p=0.203$; hip: $p=0.283$). The insignificant result was also shown in the correlation between BMD and OPG levels (lumbar: $p=0.412$; hip: $p=0.617$). The correlation test results showed a significant relationship between lumbar BMD and RANKL/OPG ratio only in the osteopenia group ($p=0.001$).

Conclusions

The RANKL/OPG ratio was significantly correlated only with osteopenia-BMD in postmenopausal females. Therefore, it could be used as supporting data in osteoporosis screening.

doi: 10.1016/j.cca.2022.04.383

M300

LC-MS/MS analysis indicates a narrower reference interval of free 25(OH)D in elderly population: An indication for free hormone hypothesis

D. Wang

Peking Union Medical College Hospital, China

Background-aim

Vitamin D deficiency is a global health problem in all age groups, especially in the elderly population. Serum 25(OH)D has been the biomarker of choice to access vitamin D nutrition status. However, the free hormone hypothesis proposes that free vitamin D might be a more reliable marker of vitamin D nutrition status. Thus, the aims of this study were to (1) evaluate the distribution of free 25(OH)D and establish reference interval of free 25(OH)D in elderly individuals, and (2) to access the association between free 25(OH)D and total 25(OH)D, 1,25(OH)2D3, 24,25(OH)2D3, calcium (Ca), alkaline phosphatase (ALP), and phosphorus (P) in elderly population.

Methods

A total of 312 healthy elderly individuals were enrolled in this study and clinical residual serum samples were collected. We measured free 25(OH)D, total 25(OH)D, 24,25(OH)2D, and 1,25(OH)2D by LC-MS/MS. Other biochemical analytes were measured by automatic analyzers.

Results

Our results showed that with an increase in the levels of total 25(OH)D, the levels of 25(OH)D3, 1,25(OH)2D, 24,25(OH)D, and free 25(OH)D too increased, whereas the levels of 25(OH)D/24,25(OH)2D decreased. Further, we observed that the level of free 25(OH)D showed significant positive correlation with the total 25(OH)D ($r=0.226$, $p<0.001$), 25(OH)D ($r=0.221$, $p<0.001$), and 24,25(OH)2D ($r=0.231$, $p<0.001$) but showed a negative correlation with 25(OH)D/24,25(OH)2D ($r=-0.185$, $p<0.01$). Moreover, the total 25(OH)D, 25(OH)D3, 24,25(OH)2D, and 25(OH)D/24,25(OH)2D were positively correlated with 1,25

(OH)2D. However, we did not observe any significant correlation between free 25(OH)D and 1,25(OH)2D even though free 25(OH)D was positively correlated with Cr ($r = 0.227$, $p < 0.001$).

Conclusions

Our results showed a narrower reference interval for free 25(OH)D (2.65 pg/ml, range: 1.10-5.53 pg/ml) than reported by direct measurement techniques and indicated that it could be a better biomarker to access bone-related health in elderly individuals.

doi: 10.1016/j.cca.2022.04.384

M301

Bone metabolism disorders in patients with chronic heart failure before and after heart transplantation

V. Sujayeva, O. Koshlataya, O. Spirina, A. Vavilava

Republican Scientific and Practical Center "Cardiology", Belarus

Background-aim

Heart transplantation (HT) is common method of treatment of end-stage heart failure. Prevention of rejection requires life-long immunosuppressive therapy,which causes a number of side effects included bones metabolism disorders (from osteopenia and up to bones fractures). However, the pathogenesis of above mentioned disorders is still not fully understood

Methods

We examined 70 patients aged 53.6 ± 9.6 years, 7 (10 %) of them were female. Dilated cardiomyopathy (DCMP) was the cause of HF in 23 (33 %), ischemic CMP – in 29 (41%), other CMP - in 18 (26%) of 70 patients. In 54 of 70 pts HT was performed in terms of 1 to 28 (average 10.6 ± 8.1) months before, another 16 pts were put in to the "waiting list" of HT. 41 (59%) of 70 pts had hypertension, 13 (19%) had diabetes melitus, and 25 (36%) had chronic kidney disease (CKD). We performed in all pts transthoracic echocardiography(TTEchoCG), daily monitoring of electrocardiogram (DMECG), 6-minute walking test, dual-energy x-ray absorptiometry (DXRA), morphometry, FRAX questionnaire, Minnesota Living with Heart Failure Questionnaire (MLHFQ) for objectification of the functional status of the circulatory system, detection of disorders of bone metabolism, control of efficacy and safety of drug and physical aspects of rehabilitation in all included in the study. Laboratory diagnostics included: blood sample test, biochemical blood analysis with determination of lipid spectrum and glucose, interleukin-6 (IL-6), C-reactive protein (CRP), determination of calcium-phosphorus metabolism (total and ionized calcium, creatinine, phosphorus), determination of bone metabolism (alkaline phosphatase, osteocalcin, ®-crossLaps).

Results

According to DXRA data, 78% of patients with HF before HT had ostopenia, osteoporosis was observed in 22% of the 16 pts, asymptomatic vertebral fractures were diagnosed in 11% of patients according to morphometry. Before surgery, all individuals with impaired bone metabolism had a low fracture risk calculated using the FRAX tool and averaged 2.1 ± 1.2 points. After HT, osteopenia was detected in 73% of 54 pts, osteoporosis - in 19%, asymptomatic vertebral fractures - in 16%. According to the FRAX scale, only 8% of 54 pts after HT had a moderate risk of fractures, and it was low in all those who had asymptomatic vertebral fractures according to morphometry. All fractures in patients before and after HT developed in persons who did not have osteoporosis, in 83% of cases - against a background of minor osteopenia in 1-2 segments, in 13% of cases – with normal values of bone mineral density (BMD) in all analyzed areas. All posttransplant fractures were 2 times more likely to develop within 1 year after surgery.

Conclusions

Patients with heart failure before and after heart transplantation need a comprehensive assessment of metabolic disorders of bone tissue.

doi: 10.1016/j.cca.2022.04.385

M302

Evaluation of bone turnover markers in girls with idiopathic central precocious puberty

J. Zhang, Y. Jiang

Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Key Laboratory of Birth Defects and Related Diseases of Women and Children (Sichuan University), Ministry of Education, China

Background-aim

Bone turnover markers (BTMs) are correlated with growth rate during normal puberty. The objective of this study was to investigate the changes in BTMs in girls with idiopathic central precocious puberty (ICPP).

Methods

Forty-five girls diagnosed with ICPP and 42 normal puberty control girls were enrolled. Serum levels of vitamin D (VD), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), total procollagen type 1 N-terminal propeptide (P1NP), ®-C-terminal telopeptide of type 1 collagen (®-CTX), and N-terminal midfragment of osteocalcin (N-MID) were measured.

Results

The average values of VD, IGF-1 and IGFBP-3 were 19.30(16.90-22.20) ng/mL, 322.00(207.50-443.00) ng/mL, 5.55(4.81-6.49) g/mL, respectively in the ICPP group. Compared with normal group, VD was significantly decreased ($p < 0.01$), IGF-1 was remarkably increased ($p < 0.01$) while IGFBP-3 between these two groups showed no significant difference ($p > 0.05$). Serum P1NP and N-MID levels in the ICPP group were significantly higher than those in the normal group (1050.00 [920.10-1151.50] ng/mL vs. 703.80 [535.30-908.93)] ng/mL, $p < 0.01$ and 81.37 [69.57-98.84] ng/mL vs. 69.41 [52.91-84.86] ng/mL, $p < 0.01$), while ®-CTX levels did not differ between the two groups ($p > 0.05$). The Spearman correlation analysis revealed that P1NP and N-MID were correlation with hight, weight, VD, and IGF-1 ($p < 0.05$). The univariate regression analyses revealed that the odds ratio (95% CI) values for P1NP, ®-CTX and N-MID were 0.993 (0.990–0.996; $p < 0.001$), 1.000(0.999–1.001; $p = 0.636$), and 0.955 (0.930–0.980; $p < 0.001$), respectively.

Conclusions

The bone formation level in ICPP girls was higher than that in normal girls. No obvious differences in bone resorption marker was found between the two groups. The results of correlation analysis hinted that P1NP and N-MID were found to be an independent factor for prediction of ICPP in girls.

doi: 10.1016/j.cca.2022.04.386

M303

Whole body bone scintigraphy vs. serum level of bone metabolism markers in prostate cancer patients

V. Horvat[a], N. Milicevic[b], I. Lukic[a], T. Rolic[a], S. Mandic[a], D. Mandic[a], V. Seric[a]

[a] *Department for Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Osijek, Osijek, China*
[b] *Department of Anaesthesiology, Reanimatology and Intensive Care, Faculty of Medicine, University of Osijek, Osijek, China*

Background-aim

Bone scintigraphy is a commonly used method for bone metastasis detection that affects the quality of life and indicates a poor prognosis and correlates with mortality in prostate (PCa) cancer patients, so metastasis presence or absence is one of the most important factors affecting the clinical approach. Although bone scintigraphy is the gold standard for monitoring bone metastasis, it is relatively expensive diagnostic tool and depends on the osteoblastic response. Aim of this study was to assess diagnostic accuracy of bone formation markers: total amino-terminal procollagen propeptides of type I collagen (TP1NP) and osteocalcin (OC) and bone resorption marker cross-linked C-terminal telopeptides of type I collagen (®-CTX) for early detection of bone metastasis.

Methods

This ongoing prospective study so far includes 36 male patients, 17 (median age 69 years, range 53-88) with clinically localized PCa (Gleason score (GS) δ7) and negative bone scintigraphy (SCI-) as a control group and 19 (median age 70 years, range 54-84) with bone metastasis (GS >7) confirmed by whole body bone scintigraphy (SCI+). Blood samples were drawn before treatments, collected at 8AM and centrifuged for 10 minutes at 3500 rpm. Samples were frozen at -20C until analysis. TP1NP, OC and ®-CTX were measured by the electrochemilunescence immunnoassay (ECLIA, COBAS e601 Roche Diagnostics Ltd.). Statistical analysis was performed using MedCalc.

Results

Our results show statistically significant difference in serum TP1NP, OC and ®-CTX level between bone SCI- PCa patients and SCI+ PCa patients. Median for TP1NP in SCI- group was 42.7 μg/l and 140.7 μg/l in SCI+ group (P = 0,045); for OC in SCI- 17.9 μg/l and 30.8 μg/l in SCI+ group (P = 0,015) and median for ®-CTX in SCI- group was 0.269 μg/l and 0.697 μg/l in SCI+ group (P = 0,003).

Conclusions

Although whole body bone scintigraphy is still considered to be the current standard in diagnosing bone metastasis, our preliminary results show that both bone resorption (®-CTX) and bone formation (TP1NP, OC) markers as non-invasive, easy-to-determine, not expensive tests, measured in only one serum sample might be a helpful tool for early bone metastasis detection and prompt therapy initiation.

doi: 10.1016/j.cca.2022.04.387

M304

The impact of obesity on bone turnover markers in children

W. Zhou, Y. Jiang

Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, China

Background-aim

Obesity is known to cause metabolic disturbances and the evidence supports a complex relationship between adiposity and bone health in obese individuals, and the risk is under-recognized for children. To date, there remains little study about the impact of obesity on skeletal development. Our aim was to investigate the association between serum bone turn markers concentration and obesity-associated dysmetabolism.

Methods

Subjects comprised 26 obese children and adolescents. The same number of age-matched, healthy controls were recruited. Body mass index (BMI), markers of bone turnover aminoterminal propeptide of type I procollagen (PINP), ®-cross-linked C-telopeptide of type I collagen (®-CTX), and molecular fragment of osteocalcin N terminal (N-MID) and glucose metabolism were measured. Thyroid function and vitamin D levels were investigated in both groups. In obese subjects the serum insulin-like growth factor 1 (IGF-1) and insulin resistance (HOMA-IR) were performed.

Results

The serum level of vitamin D (30.1 ± 5.6 VS 24,5 ± 3.8 ng/mL; p = 0.01) and markers of bone turnover P1NP(979.9 ± 324,5 VS 534.9 ± 139.3 n /mL; p < 0.01), ®-CTX (1614.1 ± 358.4 VS 1243.5 ± 372.3 pg./mL; p = 0.01) and N-MID (74.3 ± 22.7 VS 48.7 ± 17,8 ng/mL; p < 0.01) were significantly higher in the healthy than those in the obesity. Vitamin D (Spearman's rho = -0.451; p = 0.02) and BMI (Spearman's rho = 0.430; p = 0.03) were associated with P1NP and HOMA-IR (Spearman's rho = -0.457; p = 0.02) were associated with N-MID.

Conclusions

It seems there is not a linear relationship between adiposity and skeletal development during childhood. The excess fat appears to limit the effect on skeletal development. The higher HOMA-IR might negatively impact on the normal process of skeletal mineral apposition.

doi: 10.1016/j.cca.2022.04.388

M305

Machine learning in diagnosing osteoporosis risk has shown a correlation with chronic infectious diseases and allergies

V. Mudrov[c], M. Jovicic[a], S. Kazakov[b]

[a] *Belgrad University, Belgrade, Serbia*
[b] *Russian Medical Academy of Continuing Professional Education of Ministry of Health, Moscow, Russia Burdenko MAIN Military Clinical Hospital of Ministry of Defense, Moscow, Russia*
[c] *Russian Medical Academy of Continuing Professional Education of Ministry of Health, Moscow, 9-th Medico-Diagnostic Center of the Ministry of Defense, Moscow, Russia*

Background-aim

Modern laboratory diagnostics requires the use of artificial intelligence, the characteristic feature of which is not a direct solution to a problem, but training in the process of applying solutions to many similar problems. This problem is solved by "machine learning" by creating models that predict the unknown, based on known data. A multifactorial disease such as osteoporosis is caused by a variety of causes, inherited with high frequency. For today, there is no single laboratory test for bone remodeling markers to determine the risk of fracture.

Methods

Using "machine learning" to assess the prognosis of osteoporosis, 36 women and 21 men aged 30-50 years were examined. At the time of the examination, no one had complaints about their state of health. Biomaterial sampling was carried out once. 151 laboratory parameters of biochemistry, hemostasis, hormones, tumor markers, markers of osteoporosis, autoantibodies, immune status, antigens and antibodies of infectious diseases, DNA of microorganisms – pathogens of the urogenital tract, alleles of bone remodeling genes, allergens were determined for each of the examined.

Results

The study of the relationship of the body's immune response to the presence of infectious agents showed a positive correlation (+0.6) of the osteoprotegerin allele and IgA antibodies to Helicobacter pylori. Also, during the study, a relationship between the genes of bone remodeling and allergic status was found. In 19% of the examined, the IgE level was in the range of 105.7-117.8 IU/ml and a positive correlation of 0.5-0.7 allele of osteoprotegerin with allergens "cod", "wormwood", "dog", "cockroach", tick Dermatophagoides farinae was revealed.

Conclusions

As a result, we can conclude that there is a relationship between the alleles of the genes responsible for the increased risk of osteoporosis and data on a chronic disease of an infectious nature or aggravated allergic status.

doi: 10.1016/j.cca.2022.04.389

M306

Evaluation of 25-OH vitamin D on SNIBE MAGLUMI® 1000 immunoassay analyzer

S. Cuesta De Juan, J. Garcia Ayuela, R.A. Torrado Carrion, A. Mata Fernandez, M. Perez Alonso, M. Santos-Ruiz Rodriguez-Arias, N. Soriano Balcazar, C. Martinez Sanchez

Central Laboratory in Eurofins Megalab, Madrid, Spain

Background-aim

Vitamin D is a family of compounds that is essential for numerous functions of the body. The 25-hydroxyvitamin D is the major form found in the blood and because of its long half-life and higher concentration, 25-hydroxyvitamin D is commonly measured to assess and monitor vitamin D status in individuals.

There is growing awareness for the role of vitamin D, not only for its role in metabolic bone diseases, but also for the increasing recognition for its association with a variety of diseases such as cardiovascular diseases, autoimmune diseases, diabetes mellitus. So determinations of 25-OH vitamin D status of our body have significant meaning.

The aim of this work was to evaluate the concordance between two Chemiluminescence Immunoassay direct methods for the determination of 25-OH Vitamin D.

Methods

A total of 288 serum samples were included in this laboratory study.

The analytical performance of quantitative determination of 25-OH vitamin D in human serum was measured by SNIBE (Shenzhen New Industries Biomedical Engineering Ltd, China) MAGLUMI 1000 and Abbott (Abbott Park, Illinois, U.S.A.) ARCHITECT i2000SR. The results were analyzed with Passing-Bablok regression.

Precision for the MAGLUMI CLIA 25-OH vitamin D assay was obtained by testing 1 control pool and 3 human serum pools in three replicates with 30 samples per run at two independent runs per day for 5 testing days.

The statistical test used was the XLSTAT 2018 Software Statistical the Microsoft Excel®

Results

The correlation between these 2 methods showed a coefficient of correlation $r = 0.97$ ($p < 0.001$), the 95% confidence interval for r was 0.9574 to 0.9730, the intercept 3.8735 and the slope 0.8264 ($y = 3.8735 + 0.8264x$).

The result of the assay for the pool serum 1 was SD 1.216 mg/mL and CV .5.97%, for the pool serum 2 was a SD 1.330 mg/mL and CV 4.75%, pool serum 3 showed a SD 0.788 mg/mL and CV 0.84% and de pool control SD 1.755 mg/mL and CV 3.86%. All de pools serum showed a good repeatability between run and day. The total precision of the assay was 0.84%-5.97%.

Conclusions

According to the comparison study, these results show a high correlation between MAGLUMI assay and Abbott assay for the determination of 25-OH Vitamin D. MAGLUMI Chemiluminescence Immunoassay platform could be a good option for assessing 25-OH Vitamin D in laboratory use.

doi: 10.1016/j.cca.2022.04.390

M307

A comparison study between MAGLUMI and Abbott ARCHITECT intact parathyroid hormone (PTH) assay

S. Cuesta De Juan, J. Garcia Ayuela, A. Mata Fernadez, R.A. Torrado Carrion, C. Martinez Sanchez, R. Piqueras Picon, C. Dacosta Galan, M. Garcia-Alcala Hernandez

Central Laboratory in Eurofins Megalab, Madrid, Spain

Background-aim

The parathyroid gland secretes parathyroid hormone (PTH), a polypeptide, in response to low calcium levels detected in the blood. PTH facilitates the synthesis of active vitamin D, calcitriol (1, 25-dihydroxyvitamin D) in the kidneys. In conjunction with calcitriol, PTH regulates calcium and phosphate levels.

PTH effects are present in the bones, kidneys, and small intestines. As serum calcium levels drop, secretion of PTH by the parathyroid gland increases. Increased calcium levels in the serum serve as a negative-feedback loop signaling the parathyroid glands to stop the release of PTH.

PTH assay can be used for monitoring the surgery effect after resection of thyroid adenoma, evaluating the hypoparathyroidism, to help diagnose the osteoporotic and hyperparathyroidism due to excessive PTH secreted from adenomatous or hyperplastic parathyroid tissue.

The aim of this work was to evaluate the concordance between Snibe MAGLUMI and Abbott ARCHITECT for the determination of Intact PTH.

Methods

A total of 285 serum samples were included in this laboratory comparison study.

The precision was evaluated using 3 serum pools (low, middle, high level sample) and 1 control pool by assaying 20 replicates for each sample on MAGLUMI system.

The study compared results of 285 serum samples on MAGLUMI system with ARCHITECT system. Snibe MAGLUMI adopts sandwich chemiluminescence Immunoassay method for its Intact PTH assay, and Abbott ARCHITECT uses chemiluminescent microparticle immunoassay method for its Intact PTH assay.

The results of these 2 methods were analyzed by Passing-Bablok regression and Bland-Altman plot. The statistical test used was the XLSTAT 2018 Software Statistical the Microsoft Excel®

Results

The correlation between these 2 methods showed a coefficient of correlation $r = 0.96$ ($p < 0.001$), the 95% confidence interval for r was 0.9442 to 0.9647, the intercept -24.213 and the slope 1.101 ($y = -24.213 + 1.101x$).

The result of the assay for the pool serum 1 was SD 0.61 pg/mL and CV 4.18%, for the pool serum 2 was a SD 3.18 mg/mL and CV 5.36%, pool serum 3 showed a SD 2.67 pg/mL and CV 2.89% and de pool control SD 11.76 pg/mL and CV 2.80%. The total precision of the assay was 2.80%-5.36%.

Conclusions

Based on the test results of 285 serum samples, there is a good correlation for the method comparison, r equals to 0.96. Above all, this comparison study demonstrated the reliability of MAGLUMI Intact PTH assay and its good precision.

doi: 10.1016/j.cca.2022.04.391

M308

Seasonal variation of vitamin d levels in northwestern India - A retrospective study

S. Karli[a], S. Tomo[b], M. Banerjee[a]

[a] *All India Institute of Medical Sciences, Jodhpur, India*
[b] *Santosh Medical College & Hospital, India*

Background-aim

25-hydroxy vitamin D (25(OH) D) deficiency is prevalent worldwide including India. Earlier some cross-sectional studies have revealed Vitamin-D deficiency and its prevalence. The correlation of Vitamin D with trend analysis of the annual and seasonal variation of vitamin-D levels has not been reported earlier from north western India.

Methods

Analysis of vitamin D was performed in biochemistry laboratory between 2018 and 2020. Data was acquired from Electronic Medical records of the hospital. The months of the year have been separated into the following seasons for the purpose of analysing seasonal trends: Summer/monsoon (March–September), and winter/spring (October–March). Less than 20 ng/mL of serum vitamin D levels were defined as Vitamin D deficiency.

Results

A total of 11,428 assays of serum 25(OH)D were performed in the study. The median vitamin D was 17.2 ng/mL. We observed the prevalence of 60%, 24.1% & 15.9% of vitamin D deficiency, vitamin D insufficiency and sufficiency respectively in the total no of individuals tested. 56% male and 63% females were vitamin D deficient. Notably, the lowest median vitamin D value was found in the 21-30 age group (14.8 ng/mL). and the 31-40 age group (16.6 ng/mL). Significant difference of median vitamin D levels between the summer and winter seasons has been noticed. In both years (2018-20), the winter median vitamin D level was lower than summer season.

Conclusions

Vitamin D deficiency is common in all age groups and genders, according to our findings. Surprisingly, lowest levels were reported in young adults. These findings suggest that the criteria for determining the state of vitamin D insufficiency and deficiency in the Indian population should be reconsidered. Vitamin D levels were significantly lower in winter compared to summer.

doi: 10.1016/j.cca.2022.04.392

M309

A study of Anti-Mullerian hormone in post menopausal women with low bone mineral density

S. Das[a], V. Shailza[b]

[a] *Ram Manohar Lohia Hospital, India*
[b] *School of Medical Sciences and Research, India*

Background-aim

Osteoporosis is a bone disorder that increases a person's risk of fracture due to low bone mineral density (BMD). Anti-Müllerian hormone (AMH) is an ovarian biomarker that plays an important role in folliculogenesis. AMH is widely used clinically in reproductive medicin and is known to decrease with age. Vitamin D is a steroid hormone that is mainly synthesized in the skin after exposure to ultraviolet sunlight. It is debated whether vitamin D has the capacity to influence the AMH level. This study evaluates the association of Anti-Mullerian hormone and 25-hydroxyvitamin D serum levels with bone minerals density test results in post menopausal women.

Methods

This study was conducted in a tertiary care hospital and included one hundred post menopausal females, with age ranging from 45-75 years. These patients attended the Bone clinic and were classified in the Osteoporosis Group and Control Group on the basis of BMD Study. Anti-Mullerian hormone and Vitamin D were measured by chemiluminescence assay, and the bone mineral density was measured by dual energy x-ray absorptiometry for all the participants.

Results

Among the study participants, 56% reported normal results of bone mineral density measurement (Control Group) and the incidence of osteoporosis was 44 %. (Osteoporosis Group). AMH levels in the Osteoporosis Group was 0.19 ± 0.06 ng/mL and the Control group was 0.44 ± 0.05 ng/mL. Vitamin D levels in the Osteoporosis Group was 37.65 ± 3.26 ng/mL and the Control group was 9.84 ± 2.48 ng/mL. A significant positive correlation ($r = 0.86$, $p < 0.05$) was observed between lower serum levels of AMH and lower levels of Vitamin D in the participants with decreased BMD results (Osteoporosis patients).

Conclusions

Lower Vitamin D and AMH levels correlate significantly with decreased BMD in post-menopausal females. These results suggest that AMH is a potential biomarker of osteoporosis in post menopausal women.

doi: 10.1016/j.cca.2022.04.393

Clinica Chimica Acta 530 (2022) S49–S72

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Cancer, Tumor Marker, Circulating Tumor Nucleic Acid

T001

Serum SYPL1 is a promising diagnostic biomarker for colorectal cancer

L. Liu [b], Q. He [a], J. Shan [c], X. Sun [c], X. Song [a], Y. Guo [b]

[a] *Clinical Laboratory, Sichuan Cancer Center, School of Medicine, University of Electronic Science and Technology of China, China*
[b] *Medical Research Center, The Third People's Hospital of Chengdu, China*
[c] *The Digestive Department, The Third People's Hospital of Chengdu, China*

Background-Aim

Colorectal cancer (CRC) is one of the most common malignant tumor worldwide. At present, the overall sensitivity and specificity of blood biomarkers is hard to meet the diagnosis of CRC.

Methods

Two GEO datasets were employed for biomarker mining based on the positive ratio of genes in all the cancer patients. Among the filtered proteins, synaptophysin-like 1 (SYPL1) was designated as marker candidate, its mRNA or protein expression level were detected in colon cancer cell lines, CRC tissues and serum samples from healthy volunteers, adenoma and CRC patients, and the clinicopathological relationships were assessed simultaneously. The diagnostic performance of serum markers were evaluated by receiver operating characteristic (ROC) curves.

Results

Via the analysis of the genes with cancer positive rate ranking at the top 10% in both GEO datasets, SYPL1 was singled out from the 8 membrane proteins of secretory vesicle. The confirmation tests showed SYPL1 was upregulated in colon cancer cell lines and CRC tissues at both mRNA and protein levels, compared to the normal controls. Consistently, the serum SYPL1 (sSYPL1) was significantly higher in CRC patients ($n = 151$) than both healthy controls ($n = 89$) and adenoma patients ($n = 73$) ($P < 0.0001$), and associated with lymph node invasion ($P < 0.05$). While, the sSYPL1 was declined after radical operation ($P < 0.0001$). Further, ROC curve showed that the performance of sSYPL1 was prominent in distinguishing CRC patients from healthy controls (AUC: 0.9481, sensitivity: 86.09% and specificity: 91.01%) or adenoma (AUC: 0.8631, sensitivity: 98.68% and specificity: 78.08%). This was much better than the performance of carcinoembryonic antigen (CEA) or Carbohydrate antigen 19-9 (CA19-9) or their combination. Even for the patients in the gray zones of CEA under 2.2 ng/mL or 5 ng/mL, SYPL1 still kept the same high performance for the identification of CRC.

Conclusions

We propose that serum SYPL1 may be an outstanding marker to establish simple, fast, and robust approaches for CRC diagnosis, especially for those patients with low CEA.

doi: 10.1016/j.cca.2022.04.238

T002

Targeted modulation of CXCR4 signaling cascade to improve clinical outcome of TNBC patients

G. Sethi

Department of Pharmacology, National University of Singapore, Singapore

Background-aim

Breast cancer is a complex and heterogeneous disease with respect to histology, mutations, metastatic potential, disease progression, therapeutic response and clinical outcome. Triple-negative breast cancer (TNBC), defined as ER-negative, PR-negative and lacking overexpression of HER2, is an aggressive breast cancer sub-type with a high rate of proliferation and metastasis, as well as poor prognosis for advanced-stage disease. Despite recent advances in targeted therapies, patients with TNBC continue to have poor survival and early metastasis compared to other types of breast cancer, highlighting the urgency to identify novel therapeutic targets.

Methods

The potential anticancer effects of farnesoid X receptor (FXR) antagonist, guggulsterone on the viability, migration, invasion, apoptosis was analyzed by various pharmacological techniques such as MTT, live and dead and wound healing assays. The effect of this phytosteroid on CXCR4 expression, NF-kB signaling cascade and in vivo tumor growth was also investigated using pre-clinical models.

Results

Chemotherapy-resistant TNBC remains a major cause of mortality and currently lacks any proven targeted therapy and the current treatment options for chemotherapy-resistant TNBC are limited. We noted that guggulsterone can abrogate invasion, migration, as well as modulate CXCR4 expression through the abrogation of NF-kB signaling

cascade and can also interestingly enhance the anticancer effects of paclitaxel in breast cancer mouse model.

Conclusions

We prove the potential efficacy of guggulsterone as a novel CXCR4 antagonist with significant anticancer potential against breast cancer.

doi: 10.1016/j.cca.2022.04.239

T003

Circulating exosomal miR-520c-3p and miR-1274b identified from a multicentric case-control study as potential diagnostic markers for early-stage non-small cell lung cancer

Y. Zhong [b], X. Ding [b], W. Zhou [c], X. Wang [a], C. Zhang [d], J. Li [d], C. Wang [b], C. Zhang [b]

[a] *Department of Laboratory Medicine, the Affiliated Brain Hospital of Nanjing Medical School, Nanjing, China*
[b] *Department of Clinical Laboratory, Jinling Hospital, State Key Laboratory of Analytical Chemistry for Life Science, Nanjing University School of Life Sciences, Nanjing University, Nanjing, China*
[c] *Department of Clinical Laboratory, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing, China*
[d] *School of Life Sciences, Nanjing University, Nanjing, China*

Background-aim

Non-small cell lung cancer (NSCLC) is the most common and the leading cause of cancer-related deaths, and early diagnosis is crucial for improved care.Exosome-encapsulated microRNAs (exosomal miRNAs) are becoming increasing appreciated for their potential as excellent biomarkers for cancers; however, little is known about their diagnostic ability for early-stage NSCLC.

Methods

We performed the global plasma exosomal miRNA profile analysis by TaqMan Low Density Array (TLDA) followed by a quantitative reverse-transcription PCR assays (RT-qPCR) validation randomly arranged in several independent cohorts of 159 untreated NSCLC patients and 120 age/sex-matched healthy controls, and 31 benign nodule patients enrolled from three different clinical centres. In addition, paired serum samples before and after operation were collected from 38 of the patients.

Results

Circulating exosomal miRNA profile of NSCLC patients was markedly different from that of normal controls, a total of 78 miRNAs were upregulated in NSCLC. The levels of serum exoxomal miR-520c-3p and miR-1274b were verified to be significantly increased in patients with NSCLC ($p < 0.001$), even in stage I cases, compared with normal controls and benign nodule ($p < 0.01$), and then markedly declined after operation ($p < 0.001$). The AUC for stage Ⅰ and stage Ⅱ-Ⅳ of NSCLC was observed to be 0.845 (95% CI, 0.793-0.829) and 0.881 (95% CI, 0.831-0.932), respectively. Furthermore, the two-miRNA panel enabled the differentiation of NSCLC from benign nodule patients with 0.823 of AUC (95% CI, 0.730-0.915). Moreover, Cox regression analysis identified the two two miRNAs have strong relation to NSCLC (ORs,16.128; 95%CI, 6.702-38.813).

Conclusions

The Circulating exosomal miR-520c-3p and miR-1274b identified in our study are promising auxiliary diagnostic markers for the early detection of NSCLC and warrants future validation in prospective clinical trials.

doi: 10.1016/j.cca.2022.04.240

T004

Should we be looking at the usual values for tumor markers in kidney transplant recipients?

H. Benghezel [a], M. Sadelaoud [a], W. Toumi [a], H. Boukrous [b]

[a] *Sadelaoud Laboratory Batna Algeria*
[b] *University of batna 2 Batna, Algeria*

Background-aim

Given the high risk of cancer in kidney transplant patients, the use of serum tumor markers as part of screening, diagnosis or prognosis could be a possibility. However, the usual usual values of these markers may be erroneous due to the fluctuations in glomerular filtration observed in the transplant recipients.

Consideration of the glomerular filtration rate in establishing the usual values for serum tumor markers seems to be a necessity. However, the serum tumor markers affected by renal function remain to be identified.

Methods

To carry out this study, all of the adult kidney transplant patients from the Batna Algeria University Hospital will be included, whose data from medical records are sufficiently supplied and whose availability has enabled their carcinological monitoring and the assay of tumor markers and parameters. renal checkup. All patients who use tobacco or who have a tumor process, a NODAT or significant dyslipidemia will be excluded from the study.

The blood creatinine assay is performed on the Vitros 3600 Ortho Clinical diagnostics® automated system. For the estimation of the glomerular filtration rate we used the MDRD formula called “simplified”

The assays of the serum tumor markers ACE, AFP, CA 15-3, CA 19-9, CA125, total PSA, free, He4, Pro GRP, CA 724, Cyfra 21-1, NSE and ® HCG are carried out on COBAS E 411 Roche diagnostics®. We used IBM® SPSS V 20 software to perform statistical analysis of our data.

Results

132 patients (sex ratio M/F is 2) are divided into three groups: group I (stage G1 and G2), group II (stage G3a and G3b) and group III (stage G4 and G5) based on the 2017 KDIGO nomenclature. None no statistically significant difference was found between the three groups of patients with regard to the median values of AFP, CA199, CA724, NSE, Beta HCG PSAT, PSAf, CA 153 and CA 125 ($p > 0.05$). Nevertheless, we highlight a significant decrease in the median values of ACE, ProGRP and cyfra21-1 ($p < 0.05$) with the improvement of glomerular filtration capacities. 17% of our patients had high ACE values; especially for patients with a GFR is < 60 ml / min (Chi2 test $p = 0.045$). The association between elevated serum levels of Cyfra211 or Pro GRP and decreased GFR is significant for patients with GFR < 30 ml / min (Chi2 test $p < 0.05$). We observe an elevation of He4 is observable for patients belonging to group III ($p = 0.024$).

Conclusions

Our results are in accordance with the few rare publications existing and this although it is limited unlike our work in terms of number of samples. It would be important to establish usual values or at least take kidney function into account when interpreting the usual values of CEA, ProGRP, cyfra21-1 and He4.

doi: 10.1016/j.cca.2022.04.241

T005

Incidence of diagnosis of triple negative breast cancer in south Indian population

K. Vaidyanathan

Amrita Institute of Medical Science, Kochi, Kerala, India

Background-aim

Molecular phenotype of breast cancer is done on the basis of 5 markers, estrogen receptor (ER), progesterone receptor (PR), HER-2/neu, Cytokeratin (CK) 5/6 and epidermal growth factor receptor (EGFR). ER positive and/or PR positive and HER2 negative are classified as luminal A, ER positive and/or PR positive and HER2 positive are luminal B cancers, ER and PR negative and HER2 positive are considered as HER2 type. Cancers which are negative for ER, PR and HER2 are known as triple negative breast cancers (TNBC).

Methods

358 cases of sporadic breast cancer were enrolled into the study. ER, PR and HER-2/neu status were assessed by immunohistochemistry (IHC). On a subset of 55 patients, we used polymerase chain reaction (PCR) using primers designed for the purpose. 5 year survival analysis was also carried out on available patients.

Results

The incidence of TNBC was 23.2% by IHC (83/358) and 23.6% by PCR (13/55). Survival analysis showed that TNBC patients had poor survival compared to other patients.

Conclusions

We have identified 83 cases of TNBC out of a total of 358 cases, 23.2%. Though currently, immunohistochemistry is the diagnostic tool for these markers, PCR techniques can be implemented in future.

doi: 10.1016/j.cca.2022.04.242

T006

Increase in S100B and LDH as early outcome predictors for non-responsiveness to anti-PD1 monotherapy in advanced melanoma

L. Rozeman [b], R. Mortiz [b], S. Wilgenhof [b], H. Van Thienen [b], J. Haanen [b], M. Van Den Heuvel [a], C. Blank [b], H. Van Rossum [b]

[a] *Radboud Medical Center, Nijmegen, The Netherlands*
[b] *The Netherlands Cancer Institute, Amsterdam, The Netherlands*

Background-aim

Only a subset of advanced melanoma patients derive long-term benefit from anti-PD-1 therapy. Early identification of non-responsiveness would enable early switch to next line (combination) therapies. The main objective of the study was to assess if an early increase in S100B or LDH could be a predictive for non-responsiveness to anti-PD-1.

Methods

We retrospectively analysed advanced melanoma patients treated with anti-PD-1 mono-therapy. Serum S100B and lactate dehydrogenase (LDH) levels were measured at baseline and before every infusion. Non-response was defined as progression or death at 6 months. Marker cut-offs were defined based on specificity $> 95\%$ and feasibility to use in clinical practice. Sensitivity, specificity and predictive values were generated per follow-up time point. For validation an independent cohort was analysed.

Results

In total 313 patients were included (166 training, 147 validation). An increase of $> 50\%$ in LDH or a $> 100\%$ increase in S100 above the upper limit of normal compared to baseline was determined as criterion to positively test for NR. Obtained specificity ranged from 0.97-0.98 and the positive predictive value ranged from 0.92-0.96 for the studied follow-up intervals. Obtained sensitivity ranged from 0.25 (95% CI; 0.16-0.35) at 3 weeks of follow-up to 0.35 (95% CI; 0.24-0.47) at week 12. In the validation cohort specificity of the test at week 6,9 and 12 was $>95\%$ but sensitivity was lower (21-22%).

Conclusions

Early increase in S100 and/or LDH are strong parameters for non-responsiveness to PD-1 blockade in advanced melanoma. Prospective confirmation is needed before clinical implementation.

doi: 10.1016/j.cca.2022.04.243

T007

Serum CEA- and Cyfra 21.1-response based tests enable early detection of immunotherapy non-responsiveness in non-small cell lung cancer patients

M. Muller, R. Hoogendoorn, R. Moritz, V. Van Der Noort, M. Lanfermeijer, C. Korse, D. Van Den Broek, J. Ten Hoeve, P. Baas, M. Van Den Heuvel, H. Van Rossum

The Netherlands Cancer Institute, Amsterdam, The Netherlands

Background-aim

A high percentage of non-small cell lung cancer (NSCLC) patients treated with immunotherapy do not respond to treatment. This study investigated whether readily available blood-based tumor biomarkers allow early and accurate detection of NSCLC patients not responding to immunotherapy to enable early treatment switch.

Methods

Advanced NSCLC patients treated with nivolumab or pembrolizumab immunotherapy were followed-up using the serum tumor markers CEA

and Cyfra 21.1. Tumor marker responses expressed as relative change from pre-treatment, at various follow-up intervals were compared to the efficacy of immunotherapy assessed by RECIST1.1 at 6 months follow-up. A training set was used to design biomarker-response based tests and an independent validation cohort was used for validation. Furthermore, causes of false positive tests were investigated and overall survival (OS) and progression free survival (PFS) analysis were performed.

Results

A total of 376 patients (training:180, validation:196) were included in this study. Positive individual tumor marker responses were based on an increase of >50% combined with specified minimum concentrations. A stringent test with a specificity and sensitivity of 95.1% (86.3-99.0%) and 28.7 % (19.5 – 39.43%) respectively and a less stringent test with a specificity and sensitivity of 91.8% (81.9-97.3%) and 40.2% (29.9-51.3%) respectively at 6 weeks follow-up were obtained. Similar diagnostic performance was observed for all follow-up within the first 20 weeks of treatment. The median PFS and OS of patients were 153 days (139.0-167.0 days) and 58 days (45.8-70.2 days) for patients tested as non-responders and 450 days (346.9-553.1 days) and 237 days (184.8-289.2) for the residual group ($p < 0.001$) respectively.

Conclusions

Serum Cyfra 21.1 and CEA response based tests enable accurate and early detection of non-responding NSCLC patients that received nivolumab or pembrolizumab immunotherapy.

doi: 10.1016/j.cca.2022.04.244

T008

Therapeutic importance of astilbin for the treatment of renal injury and hyperuricemia: Biological importance of xanthine oxidase in the kidney disorders

D.K. Patel, K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

Flavonoidal compounds are important plant secondary metabolite mainly known for their biological potential in the plants material due to their anti-oxidant, anti-hyperlipidemic, anti-inflammatory and anti-apoptotic activities. Astilbin is a natural flavonoidal compounds found to be present in the medicinal herbs Astilbe chinensis Smilacx glabra, Engelhardis roxburghiana and Dimorphandra nollis.

Methods

Astilbin have been well known in the medicine for their effectiveness against diabetic nephropathy, contact hypersensitivity and immunosuppression potential. Here in the present investigation, biological potential of astilbin for the treatment of kidney disorders have been investigated through data analysis of numerous scientific research works. Medicinal importance of astilbin for the treatment of hyperuricemia and associated complication has been investigated through scientific data analysis of different scientific work. Biological importance of xanthine oxidase in the kidney disorders have been also investigated in the present research work through data analysis. However role of serum creatinine and blood urea nitrogen in the development of kidney complication have been also investigation through data analysis of different scientific work.

Results

Scientific data analysis of various research works revealed the biological importance of astilbin in the medicine for the treatment of kidney disorders as it decrease serum uric acid level in potassium oxonate-induced hyperuricemia in mice. In some other scientific research work astilbin significantly decreased the serum uric acid level and recovered serum creatinine and blood urea nitrogen. In another scientific study effects of astilbin on xanthine oxidase were studied and revealed less significant results. Other scientific research work revealed the anti-hyperuricemic effect of astilbin in potassium oxonate (PO)-induced acute hyperuricemia.

Conclusions

From the analysis of scientific data of different research work it was found that astilbin has significant hypouricemic effects and could be used for the treatment of different kidney disorders and associated complication.

doi: 10.1016/j.cca.2022.04.245

T009

Therapeutic potential of cirsimaritin in the medicine for their effectiveness as anti-breast cancer drug: Biological importance and pharmacological activities

D.K. Patel, K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

Cirsimaritin is flavonoidal compounds found to be present in the Cirsium japonicum and aerial parts of Centaurea kilaea. The natural flavonoidal compound cirsimaritin have been well known for their effectiveness in the medicine due to their antioxidant, antibacterial and cyclooxygenase-1 inhibitory activities. Cirsimaritin also showed beneficial effect in human gallbladder carcinoma GBC-SD cell line and kidney tubular pithelial LLC-PK1 cells.

Methods

Medicinal importances of cirsimaritin in the medicine for their effectiveness against various forms of human complication have been investigated in the present investigation through scientific data analysis of different research works of the scientific fields. Effectivness of cirsimaritin for the treatment of breast cancer have been investigated through scientific data base analysis. Medicinal importance of cirsimaritin has been correlated with their pharmacological activities to reveal their effectiveness against human breast cancer.

Results

Scientific data analysis of different research work revealed the biological importance of cirsimaritin in the medicine due to their effectiveness and pharmacological activities. Cirsimaritin have revealed the inhibitory potential on the viability of HUVECs and downregulation of

VEGF, p-Akt and p-ERK in MDA-MB-231 cells in another scientific study. In another scientific study, anti-proliferative activity of cirsimaritin have been investigated for their effectiveness against human breast carcinoma cancer cell lines and found to have potential therapeutic benefit against breast cancer.

Conclusions

Scientific data analysis of different research work revealed the biological potential of cirsimaritin as an alternative remedies for the treatment of breast cancer in the medicine and other allied health sectors.

doi: 10.1016/j.cca.2022.04.246

T010

Serum alpha-fetoprotein levels in intra-hepatic cholangiocarcinoma patients versus hepatocellular carcinoma patients

J. Jittapranerat [c], T. Poomontienchai [c], S. Namkanya [c], N. Thewaran [c], T. Somsoongnean [c], N. Homying [c], T. Trongwongsa [a], K. Kongsomboon [b], M. Raveesunthornkiet [a]

[a] *Department of Pathology, Faculty of Medicine, Srinakharinwirot University, Thailand*
[b] *Department of Preventive and Social Medicine, Faculty of Medicine, Srinakharinwirot University, Thailand*
[c] *Faculty of Medicine, Srinakharinwirot University, Thailand*

Background-aim

Nowadays, there are higher incidence rate and mortality rate of Intra-hepatic cholangiocarcinoma (I-CCA) in Thailand. Many patients had been misdiagnosed with hepatocellular carcinoma (HCC) because both I-CCA patients and HCC patients can have high serum alpha-fetoprotein (AFP) level. Nevertheless, the I-CCA was poorer prognosis than HCC and the treatment of them was so difference. The aims of this study were to compare serum AFP levels in I-CCA and HCC patients, determine an optimal cut-off level to differentiate I-CCA and HCC and identify factors that influence serum AFP in I-CCA patients.

Methods

In this cross-sectional study, available serum AFP levels prior to treatment of 125 patients who are pathologically diagnosed with I-CCA or HCC from 2009 to 2019 by pathologists at MSMC were collected and the ROC curve was done to find the cut-off point of serum AFP.

Results

The results from 45 patients diagnosed with HCC and 80 patients diagnosed with I-CCA showed a median of serum AFP in I-CCA patient was 2.71 IU/mL (Interquartile range was 1.9 – 7.2 IU/mL) and the median of serum AFP in HCC patient was 19.26 IU/mL (Interquartile range was 5.1 – 368.5 IU/mL). The cut-off level for differentiating between two types of cancer was ε 12.4 IU/mL (AUC 77.9%, 95% CI 68.8-87.0) with sensitivity 66.7%, specificity 83.8%, PPV 69.8% and NPV 81.7%. Additionally, Hepatitis B, tumor masses greater than 10 cm, or cirrhotic liver were prevalent in I-CCA patients with serum AFP ε 12.4 IU/mL

Conclusions

In conclusion, serum AFP levels in HCC patients were higher than I-CCA patients with an optimal serum AFP cut-off level of ε 12.4 IU/mL.

doi: 10.1016/j.cca.2022.04.247

T011

Correlation of tumor markers and enzymes in pancreatic carcinoma

L. Andrijevic [c], D. Dugandzija [b], B. Radovanovic [b], I. Andrijevic [c], T. Dugandzija [c], Z. Potic [a]

[a] *Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia*
[b] *Oncology Institute of Vojvodina, Sremska Kamenica, Serbia*
[c] *University of Novi Sad, Faculty of Medicine Novi Sad, Novi Sad, Serbia*

Background-Aim

Pancreatic carcinoma is a fourth leading cause of all cancer deaths with survival rate smaller than 5%. Pancreatic cancer is usually unresectable at the time of diagnosis. Endoscopic ultrasound is the gold standard in detecting and screening of the disease. Accent is still on finding biochemical tests that could enable earlier detection of cancer. Antigens that are used as tumor markers in diagnostics are Carbohydrate antigen 19-9 (CA 19-9) and Carcinoembryonic antigen, and important enzymes are Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), Cholinesterase (CHE) and Gamma-glutamyl transferase (©-GT).

Aim of this work was to determine correlation of tumor markers with enzymes in patients with pancreatic carcinoma, so that early detection and screening of the disease could be improved.

Methods

We used Elecsys tests, electrochemiluminescence immunoassay method (ECLIA) to measure tumor marker levels on Cobas e 411 and Cobas Integra tests on Cobas Integra 400+ Roche Diagnostics machine for levels of certain enzymes.

Results

The most important correlation is between ©-GT and CEA with correlation rate r = 0.787 and also between LDH and CEA with rate r = 0.633 in anatomy group Head and papilla Vateri. We didn't find significant correlation rates between other parameters.

Conclusions

Pancreatic carcinoma is usually located on head and duodenal papilla, than in body and tail of pancreas. Women are more affected than men, and its more frequent with population older then sixty years. The tumor marker that is more useful in diagnostics is CEA, when compared to CA 19-9.

doi: 10.1016/j.cca.2022.04.248

T012

Epithelial-type CTCS with a restricted mesenchymal expression are a major source of metastasis in NSCLC

Y. Zhang [b], Y. Men [a], Z. Hui [a], W. Cui [b]

[a] *Department of VIP Medical Services & Radiation Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China*

[b] *State Key Laboratory of Molecular Oncology, Department of Clinical Laboratory, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China*

Background-aim

To analyze the heterogeneous phenotypes in peripheral circulating tumor cells (CTCs) of non-small cell lung cancer (NSCLC) by the expression of epithelial and mesenchymal markers.

Methods

CTCs in 5 mL venous blood were enriched by using the Canpatrol CTC technique in 101 cases, included 82 NSCLC patients and 19 non-NSCLC patients. And then CTCs were subjected to RNA in situ hybridization with a combination of epithelial (EpCAM and CK8/18/19) and mesenchymal (vimentin and TWIST1) markers.

Results

CTCs were classified into three types: epithelial CTCs (E-CTC), hybrid epithelial/mesenchymal phenotypes (E/M-CTCs) and mesenchymal CTCs (M-CTCs). Hybrid E/M phenotypes CTCs were further divided into three subtypes, E > m CTCs, e = m CTCs and e < M CTCs.

Hybrid E/M phenotypes CTCs, especially e = m CTCs was the most common phenotype for each patient. The M-CTCs were significantly more in the advanced NSCLC patients than in the early patients. ROC curve demonstrated that when the cutoff value was 2 cells/5 mL blood, the sensitivity of M-CTCs in the diagnosis of advanced NSCLC was 58.62%, and the specificity was 77.42%.

The component of distant metastasis positive and negative NSCLC was further analyzed and found that the patients that detected pure E/M-CTCs had lower chance of distant metastasis, while those patients that detected both E-CTCs and E/M CTCs , or those detected pure M-CTCs had more chance of distant metastasis. CTCs dynamic monitoring in 12 advanced NSCLC patients received therapy suggested that E/M-CTCs was related to the primary tumor load, while M-CTCs suggested the possible progression of the disease.

Conclusions

We conclude that heterogeneous CTCs exists in peripheral blood of NSCLC patients, and E-CTCs with hybrid E/M phenotypes CTCs are important in metastasis for those therapy-naïve patients.

doi: 10.1016/j.cca.2022.04.249

T013

Correlation of CEA levels and metabolic tumor volume in patients with recurrent and/or metastatic colorectal cancer

P. Bochev [c], Y. Bocheva [b], G. Mateva [c], I. Valchanova [a]

[a] *Clinical laboratory, Acibadem City Clinic Mladost Hospital, Sofia, Bulgaria*
[b] *Medical University-Varna, Bulgaria*
[c] *Nuclear medicine department, Acibadem City Clinic Mladost Hospital, Sofia, Bulgaria*

Background-aim

Carcinoembrionic antigen (CEA) is a well established tumor marker in the management of patients with colorectal cancer (CRC) in staging, prognosis and surveilance. Its use in treatment response monitoring is less clear in terms of timing and correlation with the actual tumor volume. Fluorodeoxyglucose Positron emission tomography (FDG PET CT) is frequently used in treatment response assesment to represent metabolic tumor volume (MTV). Data on direct correlation between MTV and CEA levels is scarce and inconvincing.

Aim: To correlate the CEA levels with metabolic tumor volume in patients with recurrence/persistence/metastatic disease of CRC.

Methods

Inclusion criteria: CRC patients with a recurrence/persistence/metastatic disease, which have measurable MTV on FDG PET/CT and have been monitored with CEA serum levels; CEA levels tested within 4 weeks of PET/CT.

Exclusion criteria: CRC with measurable tumor volume, but normal CEA levels; patients with high CEA levels, but low FDG uptake; patients with peritoneal spread (inability to discriminate MTV from bowel activity); chemo- or radiotherapy, applied between PET scan and CEA measurement.

We retrospectively reviewed the database of Nuclear medicine department, Acibadem City Clinic Mladost Hospital, Sofia from Nov.2018 to Nov.2019. 17 patients met the inclusion criteria. All the scans of those patients, that were coupled with CEA measurement were considered evaluable points and were further analysed. A total of 37 evaluable points were identified. MTV and CEA level were tested for correlation both at group and individual (patients with three and more scans) level.

Results

At group level there is a moderate correlation of r = 0,49, which is doubtfully useful. On individual patient basis the correlation detected was strikingly high, r = 0,992 (0,976 to 0,999). Patients could potentially receive additional CEA testing and those with rising interim CEA levels under treatment or in surveillance may need earlier imaging re-staging than initially scheduled. (Potential early non-responder selection).

Conclusions

CEA levels have very strong correlation with MTV on an individual patient basis and may be useful as surrogate marker for tumor burden and response of treatment.

doi: 10.1016/j.cca.2022.04.250

T014

The significance of the study of detoxification efficiency of serum albumin for early diagnosis and monitoring of purulent-septic complications in cancer patients

T. Davydova, N. Borisenko, N. Pluzhnikova, A. Sytov, O. Somonova, A. Elizarova, U. Kornyushenko

N.N.Blokhin National Medical Research Center of Oncology, Moscow, Russia

Background-aim

The development of purulent-septic complications (PSC) in cancer patients after surgical operations aggravates the course of the postoperative period, worsens the prognosis and is a frequent cause of death. Therefore, the search for reliable and simple methods of timely diagnosis of septic conditions and effective control of treatment of the disease is

one of the most relevant areas of laboratory diagnosis of critical conditions in surgical practice.

Objective: to evaluate the possibility of using the detoxification activity parameter of serum albumin (DTE) for the diagnosis and monitoring of PSC.

Methods

In the intensive care unit, 15 patients with stage III and IV disease (73%) aged from 43 to 78 years (average age 65 years) with tumors of various locations (tumors of the head of the pancreas, gall bladder, stomach, esophagus, lung, kidney, bladder) were observed. All patients in the postoperative period developed PSC (peritonitis, pancreonecrosis, acute endobronchitis, acute cholangitis, pneumonia).

5 patients were successfully treated and discharged from the hospital and 10 patients died due to the development of septic shock, multiple organ failure.

Results

In the first day of PSC development, almost all patients had an increase in the quantity of white blood cells, bands, procalcitonin, presepsin, C-reactive protein, fibrinogen, D-dimer, fibrin monomer, and decreased levels of protein, albumin, and DTE.

Retrospective analysis and division of data into groups of cured and deceased patients revealed that in the first day of PSC development, only the decrease in the DTE parameter was statistically significant ($p < 0.05$), while the other studied indicators did not differ in the groups. The DTE indicator remained low during 7 days (15-20% of the norm). By 12-15 days there was an increase in DTE to 30-40% in most patients, but by 20 days the DTE level decreased sharply to $9.6 \pm 4.1\%$ ($p < 0.05$), which was an unfavorable prognostic factor.

Conclusions

The DTE parameter, which reflects the detoxification efficiency of serum albumin, is a laboratory indicator of the diagnosis and prognosis of PSC in cancer patients in the postoperative period.

doi: 10.1016/j.cca.2022.04.251

T015

Fibrin-monomer in the diagnosis of thrombotic complications in oncology

T. Davydova, A. Elizarova, O. Somonova

N.N.Blokhin National Medical Research Center of Oncology, Moscow, Russia

Background-aim

The high frequency of thrombotic complications in cancer patients, the leading role in the pathogenesis in which hemostatic systems are impaired, necessitates the identification of markers of intravascular coagulation.

Goal. To evaluate the clinical significance of determining fibrin - monomer, an early marker of intravascular coagulation in the treatment of cancer patients.

Methods

The study included 153 cancer patients with different locations of the tumor process. Patients are determined by the concentration of fibrin - monomers in blood plasma on an automatic hemostasis analyzer. The control group consists of 40 healthy people.

Results

The obtained data showed that the level of fibrin - monomer in the control group was 2.95 ± 0.1 g/ml, which is within the reference values. In patients with colon cancer (13.6 ± 1.2 g/ml), ovary (9.8 ± 0.5 g/ml) and metastatic liver cancer (8.5 ± 0.9 g/ml), which indicates a high risk of thrombotic complications. In patients with acute thrombosis, the concentration of fibrin - in the monomer was 5-6 times higher, compared with a patient without thrombotic complications. As a result of the studies, it was found that maintaining high fibrin-monomer values against the background of ongoing antithrombotic therapy is an unfavorable prognostic sign and can lead to a relapse of thrombosis.

Conclusions

The determination of fibrin - monomer is an informative method for the early diagnosis of intravascular coagulation and thrombotic complications in cancer patients.

doi: 10.1016/j.cca.2022.04.252

T016

Cholecalciferol induces apoptosis and regulates vitamin D metabolic pathways in the SiHa cervical cancer cell line

S. Bhoora [a], T.S. Pillay [d,e,f], R. Punchoo [b,c]

[a] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa*
[b] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa*
[c] *National Health Laboratory Service, Tshwane Academic Division, South Africa*
[d] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa*
[e] *National Health Laboratory Service, Tshwane Academic Division, South Africa*
[f] *Division of Chemical Pathology, University of Cape Town, South Africa*

Background-aim

Vitamin D displays anti-cancer actions in numerous studies. Cervical tissue expresses a localised autocrine vitamin D metabolising system (VDMS) implicated in cell growth regulation. This study investigated a vitamin D precursor, cholecalciferol, on cell growth, cell death and the VDMS in a cervical cancer cell line, SiHa.

Methods

SiHa cells were treated for 72 hours under standard conditions with a range of cholecalciferol doses (26 nM – 2600 nM). Cells were counted using the crystal violet assay, cell viability semi-quantified by trypan blue dye exclusion and cell proliferation quantified by flow cytometric expression of Ki67. Mitochondrial membrane depolarisation was assessed using the Muse™ MitoPotential assay. Early and late-stage apoptosis were assessed using the Muse™ Annexin V and Caspase 3/7 detection kits, respectively. Transmission electron microscopy (TEM) provided assessment of ultrastructural cell death. Protein expression of the VDMS was qualitatively determined by Western blots of vitamin D activating enzymes (CYP2R1 and CYP27B1), vitamin D receptor (VDR)

and vitamin D inactivating enzyme (CYP24A1). All experiments were performed in triplicate, on three biological repeats and statistical analyses performed by one-way ANOVA and Bonferroni post hoc test using Graphpad Prism (v7., USA) with $p < 0.05$ considered statistically significant.

Results

Cholecalciferol significantly inhibited cell count ($p < 0.05$), cell viability ($p < 0.001$) and cell proliferation ($p < 0.05$) at high-dose treatment (2600 nM) in comparison to negative controls. Significant early and late biochemical apoptosis ($p < 0.0001$) and apoptotic ultrastructural features were apparent at 2600 nM compared to negative controls. Western blots identified increases in CYP2R1, CYP27B1, VDR and CYP24A1 expression at 2600 nM compared to controls.

Conclusions

These data demonstrate that cholecalciferol decreases cell growth and induces apoptosis at high-doses. These actions are associated with increased expression of VDMS activating enzymes and VDR, suggesting autocrine mediation of apoptosis by VDMS at 2600 nM treatment.

doi: 10.1016/j.cca.2022.04.253

T017

Analytical and clinical assessment of prostate specific antigen by chemiluminescence HISCL-5000 immunoassay

J. Han [b], D.W. Jekarl [c], S. Lee [a], M. Kim [c], Y. Kim [c]

[a] *Department of Laboratory Medicine, The Catholic University of Korea, Incheon St. Mary's Hospital/Laboratory for Development and Evaluation Center, The Catholic University of Korea, Seoul, South Korea*
[b] *Department of Laboratory Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital, South Korea*
[c] *Department of Laboratory Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital/Laboratory for Development and Evaluation Center, The Catholic University of Korea, Seoul, South Korea*

Background-aim

Prostate cancer (PCa) is leading cause of incidence and death worldwide. Prostate specific antigen (PSA) is a surrogate biomarker for screening, diagnosis and prognosis of PCa.

Methods

Analytical and clinical evaluation of PSA were performed using HISCL-5000 immunoassay (Sysmex, Kobe, Japan) based on chemiluminescent enzyme immunoassay. For analytical evaluation, 510 samples for PSA were used, respectively, for precision, linearity, trueness, limit of detection (LOD), limit of blank (LOB), and method comparison. Clinical analyses were performed for BPH (benign prostate hyperplasia) and prostate cancer. The area under the receiver operating characteristic curve (AUROC) was used for evaluation of diagnostic potential.

Results

Total coefficient variation (CV) of low (3.808 ng/mL) and high (18.199 ng/mL) level PSA was 5.462 and 8.5%, respectively. The linearity was verified from 0.002 to 189.9 ng/mL for PSA. LOB and LOD was 0.007 and 0.02 ng/mL, respectively. Bias between expected true and measured value ranged from -8.377 to 11.552%. Regression equation by method comparison was $y = -1.0605 + 0.9223x$ ($R2 = 0.9909$). AUROC for diagnosis of PCa and BPH compared to non-malignant disease ranged from 0.538 to 0.746. When compared to healthy substitute, ROC ranged from 0.925 to 0.969.

Conclusions

PSA by HISCL-5000 immunoassay was reliable and could be applied in clinical setting, but clinical usefulness of PSA should be considered in relation to underlying conditions of patients.

doi: 10.1016/j.cca.2022.04.254

T018

Next-generation sequencing of molecular tagged circulating cell free DNA of hepatocellular carcinoma

E. Kim [a b], S. Lee [d], J. Park [c], J. Choi [d]

[a] *Brain Korea 21 PLUS Project for Medical Science, Yonsei University, South Korea*
[b] *Department of Laboratory Medicine, Yonsei University College of Medicine, South Korea*
[c] *Department of Internal Medicine, Yonsei University College of Medicine, South Korea*
[d] *Department of Laboratory Medicine, Yonsei University College of Medicine, South Korea*

Background-aim

According to a GLOBOCAN 2018 report, Liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death in the world. Liver cancer is known for higher incidence and mortality rates in men than that in women and; its incidence rate in Eastern Asia is higher than other region in the world. The most primary liver cancer is Hepatocellular Carcinoma (HCC) comprising about 80% of liver cancer. The main risk factors of HCC are chronic infection with Hepatitis viruses including Hepatitis B virus (HBV) and Hepatitis C virus (HCV), heavy alcohol intake, aflatoxin-contaminated foodstuffs, obesity, smoking and type 2 diabetes. Currently, Liver cancer is diagnosed by levels of protein markers including Alpha-Fetoprotein (AFP) and PIVKA-II are measured via blood testing; presence and size of liver nodules are determined by ultrasound scan and; tissue biopsy of liver nodule is proceed to determine a malignancy of the nodule. Although there are different methods to diagnose liver cancer, necessity of liquid biopsy and molecular biomarker is rising due to variable in blood testing and ultrasound scan and heterogeneity of tissue in tissue biopsy. Liquid biopsy is currently known for non-invasive method to determine biomarkers in molecular basis using circulating cell free nucleic acids (cfNAs), circulating tumor cell (CTC) and exosomes. In this study, circulating cell free DNA (cfDNA) of HCC is analyzed using Next-Generation Sequencing (NGS) to observe low-allele-frequency mutation in molecular biomarkers.

Methods

Total 95 Hepatocellular Carcinoma (HCC) patients and 39 non-malignant patients are enrolled. The blood of enrolled patients are collected in cfDNA collection tubes are centrifuged to collect plasma. The cfDNAs are extracted from the centrifuged plasma. The library of cfDNA with molecular barcode are constructed, captured and sequenced in an illumina platform.

Results

Clinical Characteristics of HCC patients. Total of 95 HCC patients, number of males are 81 (85.3%) and that of females are 15 (15.7%). The age range of the HCC patients is from 20s to 80s and age at 50s are the most numbered patients in the group (38.5%). The average age of the HCC patients is 59.6 with standard deviation (stdev) 10.79. The main causes of HCC in the patients are HBV infection (58.3%), heavy alcohol intake (9.38%), HCV infection (3.13%), and etc. The average AFP level of the HCC patients is 13602.7 ng/ml with stdev 32138.034. The average PIVKA-II level is 14081.98 mAU/ml with stdev 24542.766. Clinical Characteristics of non-malignant patients. Total of 39 non-malignant patients, types of non-malignants are Liver Cirrhosis (41.0%), Autoimmune Hepatitis (AIH, 20.5%), Hepatitis (10.3%), Angiomyolipoma (5.1%) and etc. The number of males are 12 (30.8%) and that of females are 27 (69.2%). The age range of the patients is equal to that of HCC patients with average age of 59.7 and stdev 13.71. The average AFP level is 6.46.ng/ml wit stdev 4.91 and the average PIVKA-II level is 22.5mAU/ml with stdev 7.94. Circulating Cell Free DNA reference material. Circulating Cell Free DNA reference materials are proceed to Next-Generation Sequencing to measure sensitivity of molecular barcoded library. The lowest Variant Allele Frequency (VAF) detected is 0.5%. Analysis of HCC and non-malignant cfDNA. The average concentration of HCC cfDNA and mutation detection rate in UICC stage III, IV-A, IV-B is higher than that in UICC stage I and II. The most common mutation is missense in both HCC and non-malignant groups. The most frequent mutation are detected in TP53 (30%) in the HCC group and APOB (18%) in the non-malignant group.

Conclusions

In diagnosis of HCC, blood testing and ultrasound scan are mandatory and tissue biopsy are processed if necessary. Some HCC patients, however, has lower level of the protein makers than cut-off. Furthermore invasiveness of tissue biopsy to the patients and heterogeneity of liver nodule are difficulties of diagnosis of HCC. The analysis of cfDNA of the patients and molecular biomarkers in cfDNA can be a useful tool to diagnosis HCC as a non-invasive method comparing with other diagnosis methods.

doi: 10.1016/j.cca.2022.04.255

T019

Peripheral leukocyte N6-methyladenosine as a potential non-invasive biomarker for lung cancer diagnosis

Y. Pei [b], X. Lou [b], K. Li [b], Y. Guo [c], D. Xu [b], Y. Guo [b], Z. Yang [b], D. Xu [d], W. Cui [b], D. Zhang [a]

[a] *Center for Molecular and Translational Medicine, Georgia State University, Research Science Center, China*
[b] *Department of Clinical Laboratory, National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China*
[c] *Department of laboratory medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, China*
[d] *Hematopathology Program, CBL Path, Inc, China*

Background-aim

N6-methyladenosine (m6A) triggers a new layer of epi-transcription. However, the potential non-invasive screening and diagnosis value of peripheral blood m6A for cancer haven't been reported yet. Herein, we are intend to investigate the potential diagnosis value of leukocyte m6A in lung cancer (LC).

Methods

Peripheral blood was collected from 133 LC patients and 74 age-matched healthy controls. Total RNA was isolated from leukocytes for m6A measurement and clinical information of participants were reviewed. The sensitivity, specificity and area under curve (AUC) of m6A for cancer diagnosis were evaluated by the receiver-operating characteristic (ROC) curve. Flow cytometry and Human Protein Atlas (HPA) database were used for characterization of m6A regulators in leukocyte subpopulations.

Results

Results: Leukocyte m6A level significantly increased in LC patients as compared with controls ($P < 0.001$). There is no significant association between m6A and age or gender. Elevated m6A level in LC was closely associated with late stage($P < 0.05$), low differentiation ($P < 0.05$) and could be largely reduced after surgery($P < 0.01$). ROC analysis revealed that leukocyte m6A could significantly discriminate patients with lung adenocarcinoma (LUAD) ($AUC = 0.736$, $P < 0.001$) and lung squamous cell carcinoma (LUSC) ($AUC = 0.963$, $P < 0.001$) from healthy individuals. m6A showed a superior sensitivity (0.746) for LUAD and a superior sensitivity (0.913) and specificity (1) for LUSC than commonly used serum tumor markers. Besides, we found m6A modification mainly occurs on T cells among all leukocyte subpopulations in LC. Further analysis in HPA database confirmed the active expressions of m6A regulators in T cells, as well as the high m6A demethylase level in CD4 + / CD8 + T cells, and high m6A methylase level in regulatory T cells (Tregs).

Conclusions

Peripheral leukocyte m6A represent a potential non-invasive biomarker for lung cancer diagnosis.

doi: 10.1016/j.cca.2022.04.256

T020

Targeting SALL4 by JQ1 in serous epithelial ovarian cancer

Y. Wu, Y. Pei, K. Li, X. Lou, W. Cui

Department of Laboratory Medicine, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China

Background-aim

SALL4 is a stem cell factor that is involved in maintaining self-renewal and pluripotency in embryonic stem cells. Numerous studies have shown that SALL4 is abnormally highly expressed in many malignant tumors. However, in epithelial ovarian cancer (OC), the role of SALL4 is rarely reported. Therefore, we aimed to explore the role of stem cell factor SALL4 in the development and treatment of epithelial OC.

Methods

Lentiviral expression vector was constructed to establish SALL4 stable knockdown and overexpressing OC cell lines; Western blot, real-

time qPCR was used to detectprotein and RNA levels, CCK-8 kit was used to detect the cell proliferation ability and the toxic effect of JQ1, flow cytometric analysis was used to assess cell cyle with propidium iodide DNA staining.

Results

Comparision of normal ovarian tissues revealed elevated SALL4 mRNA levels that were greater than two-fold in 94% epithelial serous OC tissues (65/69, $P < 0.001$). After knocking down SALL4 in A2780, cells showed decreased proliferation and colony formation ability. The cell cycle analysis showed that the proportion of cells in GO/G1 phase was significantly increased, and the proportion of cells in S phase and G2/M phase was significantly decreased ($P < 0.05$). SALL4 overexpression increased cell proliferative and colony formation ability. The cell cycle analysis showed that the proportion of cells in the GO/G1 phase was significantly decreased, and the proportion of cells in the S phase and G2/M phase was significantly increased ($P < 0.05$).

Further, we explored the role of JQ1 on SALL4. This is the first study to investigate the role of JQ1 on SALL4. We found that treatment of A2780 with 0.05 M JQ1 reduced 70% SALL4 RNA in only 6 h and SALL4 protein were almost gone after treatment with 2.5 M JQ1 for 24 h. Meanwhile, low concentration of JQ1 can significantly inhibit the colony formation ability (0.05 M) and proliferation (0.5 M) of A2780, and A2780 can be blocked in G0/G1 phase after treatment with 0.05 M JQ1 for 24 h.

In addition, we compared the drug sensitivity to JQ1 between endogenously high SALL4 expression cell A2780 and low/no SALL4 expression cell lines (SKOV3, CAOV3, OVCAR3) . We found A2780 showed much higher inhibition rate after 0.5 M, 2.5 M and 5 M JQ1 treatment for 96 h than SKOV3, CAOV3, and OVCAR3. Cell cycle results showed that no G0/G1 phase block effects were found after 0.5 M JQ1 treatment with SKOV3, CAOV3, and HO8910 for 24 h and 48 h.

Conclusions

SALL4 acts as an oncology gene role in OC. Treating SALL4 high expression cells by JQ1 showed similar effects as SALL4 knockdown, suggesting SALL4 may be one of the targets of JQ1. Also, endogenously high SALL4 cells are more sensitive to JQ1. Overall, our studies suggest that targeting SALL4 by JQ1 may be a novel approach for OC treatment.

doi: 10.1016/j.cca.2022.04.257

T021

Nagalase – New laboratory test for the discovery of malignancy and some serious viral diseases

M. Ilic, N. Stupar

Aqualab Plus Laboratories, Serbia

Background-aim

Nagalase, Alpha–N-acetylgalactosaminidase, NAGA is degrading enzyme of extracellular matrix, which is first all secreted by cancerous cells in the process of tumor growth. Nagalase breaks down certain glycoproteins and glycolipids. Increased NAGA activity has not been found in the blood of healthy persons, but it is known that it has a significant role and is very important for normal functioning of the organism. NAGA is also called biomarker of malignant diseases. It is also secreted from the cells infected by some viruses (influenza virus and Human immunodeficiency virus, HIV). Due to a short half life it is a suitable for the monitoring of different types of therapy. In some cases it is referred to autism. In comparison with healthy persons, the patients with autoimmune diseases, such as lupus, have increased NAGA activity. It has been also found that alcoholics have elevated activity. NAGA can be easily measured in the blood during very early phase of carcinoma.

Methods

Enzyme method from Serum

Results

The number of tested patients increases each year: by regular monitoring the increased values were obtained in around 45% patients (in 2017-from the total number of 52 tested patients 26 of them had increased value, which amounts to 50%; in 2018–from 78 of the total number of tested patients 38 patients had elevated value, which amounts to 49%; In 2019 (January-June–half a year) from the total number of 50 tested patients 18 of them had an increased value which amounts to 36%.

Conclusions

Nagalase should not be considered either as a primary screening test or diagnostic tool for any kind of cancer. This test enables the increased chance for the timely diagnosis and treatment of the disease. Our results point to the necessity of further investigation, determination and monitoring of NAGA.

doi: 10.1016/j.cca.2022.04.258

T022

The role of antioxidant therapy in the symptomatic treatment of pancreatic cancer patients

T. Davydova, A. Tumanyan

N.N.Blokhin National Medical Research Center of Oncology, Moscow, Russia

Background-aim

The presence of a tumor in the body suggests a high level of reactive oxygen species and the development of intracellular oxidative stress. In this regard, studies of metabolic intracellular processes occurring at the molecular level in the symptomatic treatment of cancer patients are relevant for the development of measures to improve the quality of life of patients and metabolic correction of detected disorders.

Methods

Before treatment and after decompression, detoxification therapy, the activity of superoxide dismutase (SOD), catalase, levels of vitamins E and A, ®-carotene, ascorbic acid (AA) and malondialdehyde (MDA) in the blood serum of 15 patients with pancreatic head tumors with metastatic liver damage and obstructive jaundice, of which 6 people did not receive antioxidants (control group), and 9 received an antioxidant complex (⟨-tocopherol 400 mg/day, ®-carotene 30 mg/day, AA 1 g/day) during 7 days in the time of the detoxification therapy after biliary tract drainage operations.

Results

Initially, high SOD activity (by 67%) and low catalase activity (by 25%), a deficiency of all the vitamins under study, and a high level of MDA (by 139%) compared with healthy people were found.

In the control group, after eliminating cholestasis, a decrease in MDA by 20%, an increase in catalase by 32%, and a slight increase in the concentration of the studied vitamins were observed. The use of antioxidants in the correction group led to more pronounced positive changes in antioxidant protection: a decrease in MDA by 73%, an increase in catalase by 43% and a decrease in SOD by 17%, an increase in AA, ⟨-tocopherol and ®-carotene by 20%, 23% and 10% respectively. While taking antioxidants, 33% of patients showed an improvement in subjective well-being: 16% of them quickly relieved itching caused by jaundice, 13% decreased the severity of anorexia and physiological weakness, 10% eliminated intestinal disorders, noted a reduction in the period of detoxification therapy from the moment of application biliodigestive anastomoses.

Conclusions

The obtained results indicate pronounced metabolic disorders of free-radical oxidation and antioxidant protection in patients with pancreatic head malignant tumors and allow recommending the inclusion of a complex of bioantioxidants in the treatment plan.

doi: 10.1016/j.cca.2022.04.259

T023

The pyruvate kinase metabolic activity in citrate plasma from peripheral and uterine blood samples of women with atypical hyperplasia or endometrial cancer

B. Zapala [b], M. Dziechciowski [d], M. Piwowar [a], O. Chmura [e], M. Duda-Wiewiorka [c], K. Skotniczny [c], K. Pitynski [c], B. Solnica [b]

[a] *Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Poland*
[b] *Department of Clinical Biochemistry, Jagiellonian University Medical College, Poland*
[c] *Department of Gynecology and Oncology, Jagiellonian University Medical College, Poland*
[d] *Department of Gynecology and Oncology, University Hospital Jagiellonian University, Poland*
[e] *Jagiellonian University Medical College, Poland*

Background-aim

Tumor M2 Pyruvate Kinase (Tumor M2-PK) was described by Eigenbrodt in 1985. The determination of Tumor M2-PK in EDTA plasma has been described as a tumor marker in a variety of different tumor types such as gastric, colorectal, pancreatic and lung cancer. In our study we would like to show the role of pyruvate kinase as a sensitive marker in precancerous and cancerous condition of endometrium.

Methods

Measurements were performed with a commercially available assay kit (BioVision, USA) in citrate plasma samples from peripheral and uterine blood of patients with athypia or endometrial cancer and healthy women.

Results

We have found statistically significant differences between pyruvate kinase metabolic activity between cancer and athypia samples, with the activity being lower in atypia than in both healthy and endometrial cancer groups. The highest metabolic activity was observed in endometrial cancer. We did not confirm any correlations between disease severity and pyruvate kinase metabolic activity.

Conclusions

The measurement of citrate plasma pyruvate kinase metabolic activity might provide an interesting, reliable screening tool for precancerous state as well as endometrial cancer. Further studies are needed to reveal the molecular mechanism behind the paradox between increased and decreased pyruvate kinase metabolic activity in different states of endometrial cancer especially in precancerous hyperplasia of endometrium.

doi: 10.1016/j.cca.2022.04.260

T024

The plasma levels and diagnostic utility of vascular endothelial growth factor (VEGF) and metalloproteinase 9 (MMP-9) before and after surgery of breast cancer patients

S. Lawicki [b], E. Gacuta [c], K. Glazewska [d], J. Motyka [d], P. Lawicki [e], E. Bedkowska [a]

[a] *Department of Haematological Diagnostics, Medical University of Bialystok, Poland*
[b] *Department of Population Medicine and Civilization Diseases Prevention, Medical University of Bialystok, Poland*
[c] *Department of Perinatology, Medical University of Bialystok, Poland*
[d] *Department of Population Medicine and Civilization Diseases Prevention, Medical University of Bialystok, Poland*
[e] *Medical University of Bialystok, Poland*

Background-aim

Breast cancer is one of the most common malignancies in the world. Considering the increasing number of new cases, early diagnosis is necessary. New tumor markers could be helpful in early cancer detection and in monitoring of total resection of tumor cancer.

The aim of this study was to investigate the plasma levels and diagnostic usefulness of VEGF and MMP-9, and CA 15-3 before and after surgery of breast cancer patients.

Methods

The plasma level of tested parameters were measured in 60 breast cancer patients (I stage in TNM) and in 60 benign breast tumor patients (all tested before and after surgery), and in 60 healthy subjects. VEGF and MMP-9 were determined using ELISA methods, CA 15–3 was measured by CMIA. Diagnostic utility has been determined based on parameters such as diagnostic sensitivity, specificity, positive and negative predictive values, and area under the ROC curve (AUC).

Results

Plasma levels of VEGF, MMP-9 and CA 15–3 were significantly higher in breast cancer patients when compared to the benign breast

tumor group and healthy subjects. The statistical decreasing of plasma levels of VEGF and MMP-9 was observed before and after surgery of breast cancer patients. The same observation was noticed between plasma levels of VEGF in benign breast tumor group before and after surgery, similarly as CA 15-3.

The diagnostic sensitivity of VEGF was higher than MMP-9 and CA 15-3 (58%, 47%, 45%, respectively). Diagnostic specificity was higher and equal for VEGF and MMP-9 (both 92%) than CA 15-3 (90%). The positive and negative predictive value of VEGF were higher than MMP-9 and CA 15-3 (88%, 85%, 83%; 54%, 42%, 40% respectively). Combined use of VEGF or MMP-9 with CA 15–3 resulted in evidently higher diagnostic sensitivity and negative predictive values (83%, 81%; 64%, 58%).

Our results showed that VEGF has highest diagnostic power in comparison to MMP-9 and CA 15-3 (AUC respectively: 0,7304; 0,7102; 0,7082). Combined use of VEGF or MMP-9 with CA 15–3 resulted in higher AUC values (0,8004; 0,7842) .

Conclusions

These results suggest a potential role of VEGF and MMP-9 as tumor marker for diagnostic and monitoring of surgery treatment of breast cancer patients, especially with tumor marker CA 15-3.

doi: 10.1016/j.cca.2022.04.261

T025

The influence of follicular and luteal phases of the menstrual cycle on the indicators of obesity, endocrine disruptors, reproductive and thyroid hormones in African black women with breast cancer

O. Ajayi [a], M. Charles-Davies [a], J. Anetor [a], A. Ademola [b]

[a] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[b] *Division of Surgical Oncology, Department of Surgery, University College Hospital, Ibadan, Nigeria*

Background-aim

There is increasing prevalence of breast cancer in the developing world. Although hormones, endocrine disruptors and obesity have been implicated in its aetiology, existing data are inconsistent while well controlled studies are sparse. This may be attributed to the influence of the follicular and luteal phases of the menstrual cycle on the indicators of these parameters, which is investigated in this study.

Methods

Participants (n = 107) aged 28-50 years comprising 54 newly diagnosed women with breast cancer (cases) age and menstrual phase matched with 53 apparently healthy women without breast cancer (controls). Anthropometric indices and blood pressure were obtained by standard methods. Serum hormones-oestradiol, progesterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), free thyroxine (FT4), free triiodothyronine (FT3) and thyroid stimulating hormone were determined by enzyme immunoassay. Endocrine disruptors-lead, cadmium and arsenic were determined by atomic absorption spectrophotometry.Data were analyzed using Student's t-test were considered significant at $p < 0.05$

Results

Progesterone, oestradiol and LHlevels were significantly higher at the luteal phase compared to the follicular phase in both cases and control. However, FSH and FT3 levels were lower at the luteal phase compared with the follicular phase in the controls only

Conclusions

The levels of all indicators of obesity and endocrine disruption and selected hormones (progesterone, LH and oestradiol) appear not affected by the variations in the menstrual cycle. However, the levels of FSH and FT3 are probably dependent on the phases of the menstrual cycle, which may be considered during blood collection in breast cancer studies

doi: 10.1016/j.cca.2022.04.262

T026

Quantitative immunosubtraction improves measurement of monoclonal immunoglobulin proteins in the beta region on serum protein electrophoresis

L. Schroeder, D. Keren

The University of Michigan, USA

Background-aim

Accurate measurement of monoclonal immunoglobulin protein (M-protein) is essential to stratify risks of progression and to follow response to therapies in patients with monoclonal gammopathies. M-protein quantification is routinely performed on serum protein electrophoresis (SPE) by demarcating the point at which the M-protein spike meets the underlying polyclonal immunoglobulins and dropping a perpendicular line to the baseline. When measuring in the gamma region, this information gives good reproducibility in proficiency testing samples. However, when the M-protein migrates in the beta region, poor reproducibility has been reported on proficiency testing due to the presence of two relatively large proteins native to that region: transferrin (beta1) and C3 (beta2). Current recommendations do not recommend measurement below 20 g/L in beta-migrating samples.

Methods

For the study, 20 sera were chosen based on the migration of a relatively M-proteins >10 G/L in the beta region. Immunofixation and Immunosubtraction (ISUB) was performed on all sera. Serial dilutions in a pooled human serum were made of all the diluted samples yielding 278 total samples for analysis. The samples and dilutions were coded and provided to the two interpreters at random. We performed SPE and SUB on each dilution with the Sebia Capillarys 2™. The ISUB image has the pre- and post-subtracted images. Except for the area with the M-protein, the curves generally overlap. By subtracting the post-curve from the pre-curve one finds a clear M-spike. To quantify, we used traditional SPE analysis to calculate protein concentration in a region including both M-protein and underlying non-immunoglobulins. We then imported ISUB images into Image J™ and performed region of interest analysis to calculate the involved immunoglobulin subclass contribution to the SPE region.

Results

By immunofixation, there were 4 IgGK, 1 IgGL, 6 IgAK, 4 IgAL, 3 IgMK, and 2 IgML M-proteins. qIS measurement on the dilutions were recorded from 0.5 G/L to 54.0 G/L. Using a quality target of 25% error, our analytical was measurable to 0.5 G/L with good correlation between readers. Passing-Bablok regression between qIS and the expected M-protein recovery produced a slope of 0.98 (95% CI 0.96 -1.03), r = 0.994.

Conclusions

Using the pre- and post-immunosubtraction bands on capillary electrophoresis provides an opportunity to improve measurement of beta-migrating M-proteins. We found that qIS achieved quantification of beta-migrating IgG, IgA and IgM M-proteins at concentrations an order of magnitude lower than traditional SPE methodology.

doi: 10.1016/j.cca.2022.04.263

T027

Significance of estimation LDH release from cell culture in-vitro conditions

V. Jurisic

University of Kragujevac, Faculty of Medical Sciences, Serbia

Background-aim

The lactate dehydrogenase (LDH) among biochemical parameters represents a very valuable enzyme, and its serum level is an important prognostic factor in patients with different cancer. Until now LDH is estimated in serum of different cancer patients and results indicated that elevated LDH is significantly increase in patients in advanced stage of disease. However, in this paper we focused on estimation speontaneous LDH release from in vitro culturd cells obtained from cancer patients.

Methods

The spontaneous LDH release activity was measured from culted cells in vitro, obtained after cell separation on density gradient (Lymphoprep, Norway) from peripheral blood or from immunmomagnetics cell separation using Milteny system or from cell cultures, Cells where cultured at identical concentration (4.0 ×106/mL) in RPMI medium without phenol red (Sigma; USA) supplemented with 10% FCS (Sigma, USA) and incubated in 96 microwell plates (Flow, USA) during 2 h at 37°C in humid atmosphere containing 5% CO2. After that, the plates were centrifuged for 5 min at 160g; 100 μL of supernatants from each well was transferred into 96 flat bottom microwell plates and 100 μL of lactic acid dehydrogenase substrate mixture was added. The substrate was composed of 5.4 × 10–2 M of L (+) lactate, 6.6 × 10–4 M of 2-p-iodophenyl-3p-nitrophenyl tetrazolium chloride, 2.8 × 10–4 M of phenazine metosulfate,and 1.3 ×10–3 Mof NAD (all from Sigma) in 0.2 M TRIS buffer at pH 8.2. The microtiter plate reader (Behringer EL-311, Germany) was used for the evaluation of the changes in absorbance at 492–630 nm. The results were expressed in absorbance and also as well as pecentage of total LDH values obtained after ultrasonification using identical cell concentration.

Results

Results indicated that spontaneous LDH release from cultured cells better correlated with clinical stage of cancer patients then serum LDH. In addition, experimental data shows that spontaneous LDH release in vitro conditions mostly depend on cell type (normal or tumor), cell concentration, or separation process. Using a stronger magnet for cell separation, more cells showed necrotic form of cell death and cell destruction with consequently significantly LDH release activity in cell culture medium (supernatants).

Conclusions

Together results indicated that LDH release in vitro conditions are very sensitive methods for estimation cell membrane damage from cultured cells and can be reccomended as not toxic, no expensive, very senzitive methods for many in-vitro experimental proceduure.

doi: 10.1016/j.cca.2022.04.264

T028

Practices of breast self-examination and associated factors among female residents in Tangail city, Bangladesh: Cross sectional study

M.M. Khatun, M.S. Habib, M.M. Islam, J.J. Shifa, R. Akter, M. Mozibullah, F. Yasmin, M. Sohel

Mawlana Bhashani Science and Technology University (MBSTU), Biochemistry and Molecular Biology Tangail 1902, Dhaka, Bangladesh

Background-aim

Breast self-examination (BSE) is an important treatment method of early detection of breast cancer. Secondary prevention through monthly breast self-examination is the best option to tackle the rising incidence of breast cancer in Bangladesh. This study was aimed at assessing practice and associated factors of breast self-examination (BSE) among female residents in Bangladesh.

Methods

A cross sectional study design was adopted of 500 but only 304 female respondents randomly selected in Tangail city. The study was extended for four months from (1st September - 25th November) 2019. The interview was face to face, utilizing the standardized questionnaire. The information include socio-demographic characteristics, knowledge, attitudes, practice of BSE, risk factors and barriers to practicing.

Results

Majority of the study participants, 229 (75.1%), were between 19 and 40 years old. Only 14 (26.9%) had a family history of breast cancer performed BSE regularly and 73.1% respondents had a family history of breast cancer didn't perform BSE on a monthly basis. Majority of the respondents, 266 (92.4%) had no knowledge about mammogram test and didn't perform BSE. Knowledge of breast cancer risk factors, early detection method (EDM), clinical breast examination (CBE) methods, family history of breast cancer and knowledge of breast cancer symptoms were significantly strongly associated with breast self -examination practice (BSE) ($p < 0.05$).

Conclusions

The study showed that the prevalence of breast self-examination was extremely low. Family history of breast cancer, knowledge about breast

self-examination (BSE) and self efficacy in practicing breast self-examination did have statistically significant association with breast self-examination practice. Therefore, it is important to develop health educational programs in the community based and nationwide to raise awareness about BSE and early detection of breast cancer so as to practice self-breast examination.

doi: 10.1016/j.cca.2022.04.265

T029

The effects of 17-allylamino-17-demethoxygeldanamycin (17- AAG) on matrix molecules and angiogenetic factors in gastric cancer cells

F. Kosova [b], T. Gürpınar [f], F. Özdal Kurt [c], N. Umur [a], S. Ulaş Cambaz [e], İ. Tuğlu [d]

[a] *Celal Bayar University School of Health Service, Turkey*
[b] *Department of Biochemistry of Celal Bayar University School of Health Service, Manisa, Turkey*
[c] *Department of Biology of Celal Bayar University Science and art Faculty, Manisa, Turkey*
[d] *Department of Histology and Embiryology of Celal Bayar University Medical Faculty, Manisa, Turkey*
[e] *Department of Midwife of Celal Bayar University Health Science Faculty, Manisa, Turkey*
[f] *Department of Pharmacology of Celal Bayar University Medical Faculty, Manisa, Turkey*

Background-aim

Migration, invasion, metastasis and angiogenesis associated with cancer depend on the surrounding microenvironment. Angiogenesis, the growth of new capillaries, is a regulator of cancer growth and a useful target for cancer therapy. We examined matrix protein interactions in a gastric cancer cell culture that was treated with different doses of 17-allylamino-17-demethoxygeldanamycin (17- AAG).

Methods

We also investigated the relations among the levels of vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), endostatin (ES) and trombospondin- 1 (TSP-1). Cytotoxity of 17-AAG was measured using the 3-(4,5-dmethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. We examined the behavior of cells on laminin and collagen I coated surfaces in response to the angiogenic effect of these matrix molecules. We examined the protein alterations of these matrix molecules immunohistochemically and measured the levels of VEGF, MMP-9, ES and TSP-1 using the ELISA test

Results

We showed that application of 17- AAG to the gastric cancer cell line on tissue culture plastic, laminin and collagen I significantly decreased the VEGF, TSP-1, ES and MMP-9 protein levels. VEGF, ES and MMP-9 levels of gastric cancer cells were no significant change but, TSP-1 also was increased signifi cantly when tissue was cultured on collagen I. Application of 17- AAG to cells on laminin coated surfaces significantly decreased all of the proteins except ES. ES levels was increased on the laminin covered surfaces. Discussion: We demonstrated the beneficial effect of 17- AAG on a gastric cancer cell line including inhibition of proliferation and induction of some proteins that might be related to decreased angiogenesis

Conclusions

17-AAG is a drug that has entered the works of phase–1 and phase–2; we thought that the usage of this drug can become a hope for gastric cancer treatment as a result of the phase–3 studies testing on more communities with patients.

doi: 10.1016/j.cca.2022.04.266

T030

HE4 and CA125 in ovarian malignancy

M. Perovic

GENERAL HOSPITAL VRBAS, Serbia

Background-aim

Ovarian cancer is common malignancy of the female reproductive system. Over 90% of all cases of all ovarian masses detected in premenopausal and > 60 % in postmenopausal women are benign. The early diagnostics of ovarian malignant tumor becomes a key factor in improving the survival rate of patients.

CA125 has been used as a tumor marker for diagnosis and monitoring of ovarian cancer for 30 years and is also used for efficacy evaluation and monitoring of recurrence. The aim of this study was to evaluate HE4 in comparing with CA125 and ROMA index(Risk of Ovarian Malignancy Algorithm) in ovarian cancer and benign gynecological diseases.

Methods

HE4 and CA125 serum levels were determined in 100 patients with benign gynecological diseases,27 patients with ovarian cancer and 96 healthy women. Serum CA125 and HE4 were detected using the full automatic chemiluminescence analyzer Abbott-Architect and the corresponding kit according to the manufacturer's protocol . Briefly , serum CA125 and HE4 were calculated for ROMA index value using Abbott ROMA index assessment software.

Results

HE4 had significantly higher concentrations in ovarian cancer than in benign gynecological disorders ($p < 0.05$).CA125 was significantly increased in benign tumor group relative to the healthy control group. ROMA index between groups of healthy patients and ovarian cancer was significantly higher in patients with ovarian cancer ($p < 0.05$).

Conclusions

In the diagnosis of ovarian cancer, HE4 had higher sensitivity than CA125 as a single tumor marker.CA125 and HE4 were significantly different from the ROMA index, and the ROMA index was significantly better than CA125 and HE4 in the diagnosis of ovarian cancer.

In conclusion , the application of the ROMA index and HE4 for the diagnosis of ovarian cancer was found to be effective and it has good clinical application value.

doi: 10.1016/j.cca.2022.04.267

T031

Human epididymis protein 4 as a biomarker for screening of ovarian cancer

H. Dubey, A. Ranjan, P. Tanwar

Laboratory Oncology Unit, Dr. B.R. Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, India

Background-aim

Ovarian cancer is a deadly disease often diagnosed in late stage. Currently used biomarker CAl25 has a number of fallacies. It is positive in benign conditions including pregnancy. Recently HE4 is an emerging biomarker to complement and even can replace CA 125. It has higher potency to differentiate benign tumours from malignant one.

Methods

This study is being conducted after getting Ethical clearance by IEC & is an ICMR, New Delhi funded project. We studied 123 cases of ovarian cancer confirmed by histopathological examination. Whole blood samples were collected at the time of diagnosis prior to therapy (chemotherapy or surgery). We tested them for serum level of CA 125 & HE4. Cut off values for HE4 and CA125 were <57.6 pmol/L and <39.6 U/mL respectively. Cut off values were calculated by ROC Curve analysis.

Results

Total 123 cases were evaluated for serum level of HE4 & CA125 prior to therapy.

Out of 123 cases 38 showed CA 125 values negative, whereas the same was 14 for HE4, indicating a better diagnostic performance by HE4. As studied by Drapkin et al 2005, serum level of HE4 will not be raised in 50% cases of clear-cell variant and almost all cases of mucinous tumors. It is positive in 93% cases of serous tumors and almost all cases of endometrioid tumors. It will not be raised in benign ovarian cysts.

Among premenopausal cases (42/123); 5 out of 10 CA125 negative cases were HE4 positive but only 2 out of 7 HE4 negative cases showed CA 125 positive results.

Among postmenopausal cases(81/123); 18 out of 25 CA 125 negative cases were HE4 positive but none of the HE4 negative cases showed CA 125 positive results.

Conclusions

Study shows HE4 is more accurate in diagnosing malignant ovarian tumours. Positive HE4 is indicative of malignancy, but negative result may need further evaluation.

doi: 10.1016/j.cca.2022.04.268

T032

QIP-MS: An alternative method to standard electrophoretic techniques for the identification of intact monoclonal immunoglobulins

K.B. Tan [c], J. Ashby [a], S. North [a], D. Barnidge [b], S. Brusseau [a], R. Patel [a], B. Du Chateau [b], G. Wallis [a], S. Harding [a], D. Sakrikar [b]

[a] *The Binding Site Group Ltd, Birmingham, United Kingdom*
[b] *The Binding Site Group, Rochester, MN, USA*
[c] *The Binding Site Group, Singapore*

Background-aim

For the last 3 decades identification of monoclonal intact immunoglobulins has had little innovation. Here we present performance data for Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS) for the detection of intact immunoglobulins.

Methods

Modified sheep polyclonal antibodies (anti-IgG, -IgA, -IgM, - and -) were covalently attached to blocked magnetic microparticles. The microparticles were incubated with serum, washed and treated with 20mM TCEP in 5% (v/v) acetic acid to reduce immunoglobulin heavy and light chains. Mass spectra were generated on a Microflex LT smart matrix assisted laser desorption ionisation time-of-flight mass spectrometry system. Mass spectra were acquired in positive ion mode between 10,000 and 30,000 m/z. Specificity was assessed using normal polyclonal serum and patient sera containing monoclonal immunoglobulins. Accelerated stability was assessed at 22°C over 12 weeks using normal human serum. Limits of blank (LoB) and detection (LoD) were determined by serial titrations of polyclonal human serum diluted in sheep serum and linearity was assessed by serial titrations of a known monoclonal immunoglobulin diluted in normal polyclonal serum. Sensitivity was compared to capillary zone electrophoresis (CZE) and immunofixation electrophoresis (IFE).

Results

Polyclonal molecular mass distributions for the light chains from IgG (median IgG □ /IgG □ ratio 2.3:1 (CV = 4.4%)), IgA (IgA □ /IgA □ = 1:1 (CV = 3.5%)), IgM (IgM □ /IgM □ = 1.5:1 (CV3.7%)), total □ , and total □ were observed. Normal human sera assessed at 22°C/12 weeks gave reproducible intensities for the polyclonal molecular mass distributions. The LoD for monoclonal proteins diluted into sheep serum were: 0.7mg/L for IgG, 1.4mg/L for IgA, IgM and total □ , and 0.17mg/L for total □ . Serial dilution of known monoclonal immunoglobulins into normal polyclonal serum gave acceptable linearity. In a blind study QIP-MS had a greater sensitivity for the detection of monoclonal immunoglobulins than CZE (100x) or IFE (10x).

Conclusions

QIP-MS is a highly reproducible, linear, and sensitive alternative to conventional electrophoresis. The ability to measure a unique molecular mass for any myeloma paraprotein offers an innovative addition for the identification and quantification of monoclonal immunoglobulins.

doi: 10.1016/j.cca.2022.04.269

T033

QIP-MS discriminates therapeutic monoclonal antibodies from endogenous m-proteins in patients with multiple myeloma

K.B. Tan [d], M. Lajko [c], D. Sakrikar [c], J. Ashby [b], S. North [b], G. Wallis [b], S. Harding [b], B. Du Chateau [c], D. Murray [a], D. Barnidge [c]

[a] *Mayo Clinic, Rochester, MN, USA*
[b] *The Binding Site Group Ltd, Birmingham, United Kingdom*
[c] *The Binding Site Group, Rochester, MN, USA*
[d] *The Binding Site Group, Singapore*

Background-aim

Therapeutic monoclonal antibodies (t-mAbs) have revolutionized the treatment of multiple myeloma (MM). Most t-mAbs have an IgG| isotype and present a challenge to laboratories using electrophoresis to monitor MM patients due to peak serum concentrations which can cause interference. Here we present quantitative immunoprecipitation mass spectrometry (QIP-MS) as a method utilizing molecular mass to distinguish endogenous monoclonal protein (M-protein) from t-mAbs daratumumab and elotuzumab.

Methods

Modified sheep polyclonal antibodies (anti-IgG) were covalently attached to blocked magnetic microparticles. An IgGk patient monoclonal protein was diluted to 2.0g/L in normal human sera and either daratumumab or elotuzumab was added at 0.5-5g/L. Separately, 2xIgG| and 2xIgG| MM patient sera were diluted to 1.0 g/L in pooled normal human serum containing either daratumumab or elotuzumab at 0.2g/L. Microparticles were added to samples, incubated for 15 min, washed and treated with 20mM TCEP in 5%(v/v) acetic acid to reduce immunoglobulin heavy and light chains. Mass spectra were generated on a microflex LT smart matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) system. Serum immunofixation electrophoresis (IFE) was performed in accordance with the manufacturer's instructions.

Results

QIP-MS was able to differentiate the endogenous monoclonal IgG| (2g/L) in the same mass spectrum from daratumumab and elotuzumab at 0.5-5g/L. By contrast, IFE only identified the t-mAb at >1g/L. In an expanded study, QIP-MS could distinguish the endogenous M-protein from t-mAbs at 0.2g/L in all samples. The +2 charge states were monitored for the monoclonal light chains from daratumumab and elotuzumab and were clearly distinct from the endogenous monoclonal light chains.

Conclusions

QIP-MS can distinguish t-mAbs from the endogenous M-protein, even in the presence of a high polyclonal background and at levels below the detection limit of IFE. Furthermore, this approach is agnostic to the therapeutic antibody and therefore can be used to monitor patients irrespective of their treatment modality, a distinct advantage over idiotypic gel shift assays.

doi: 10.1016/j.cca.2022.04.270

T034

Identification of serum microrna-25 as a novel biomarker for pancreatic cancer

Y. Yu, R. Lu, L. Guo

Fuscc

Background-aim

To identify serum microRNA-25 (miR-25) as a diagnostic biomarker for pancreatic cancer (PCa) and to evaluate its supplementary role with serum carbohydrate antigen 19-9 (CA19-9) in early identification of cancers.

Methods

80 patients with pancreatic cancer and 91 cancer-free controls were enrolled in this study. Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the expression level of miR-25. Levels of CA19-9, carcinoembryonic antigen (CEA) and carbohydrate antigen 125 (CA125) were measured by chemiluminescent immunoassay. The logistic model was established to evaluate the correlation of miR-25 with clinical characteristics. A risk model for PCa was conducted by R statistical software. Diagnostic utility for PCa and correlation with clinical characteristics were analyzed.

Results

The expression level of miR-25, in the PCa group was significantly higher ($p < 0.05$). Risk Model illustrated the relation between miR-25 and pancreatic cancer. With the combination of CA19-9, the performance of miR-25 in early stages (# + #) in the diagnosis of PCa was profoundly better than CA19-9 and miR-25 alone. This combination was more effective for discriminating PCa from cancer-free controls (AUC-ROC, 0.985; sensitivity, 97.50%; specificity, 90.11%) compared with CA19-9 alone or the combination of CA19-9 and CA125.

Conclusions

The expression level of miR-25 among pancreatic cancer patients was significantly higher than that in the control group. miR-25 existed as one of the most relevant factors of PCa. miR-25 can serve as a novel noninvasive approach for PCa diagnosis, and with the supplementary of CA19-9, the combination was more effective, especially in early tumor screening.

doi: 10.1016/j.cca.2022.04.271

T035

Development of an efficient assay system for monitoring chemical-induced carcinogenesis in human serum

K. Moon

BioMedicinal Chemistry Laboratory, Department of Clinical Pathology, Gwangju Health University, South Korea

Background-aim

N-nitroso-N-methylurea (NMU) and N-nitroso-N-ethylurea (NEU) are well known mutagens and carcinogens. Modulatory role of NMU and NEU, as well as the simpler alkylating agents, methyl bromide and ethyl bromide, on the activity of human serum nitric oxide synthase (NOS) was evaluated to investigate the possible involvement of NOS activity with their chemical carcinogenesis.

Methods

The generation of nitric oxide (NO) induced by chemical carcinogens was determined in human serum with or without treatment of NOS inhibitors, e.g., aminoguanidine (AG), a preferential inhibitor of inducible NOS and N^{-}-nitro-L-arginine methyl ester (L-NAME), an irreversible inhibitor of constitutive NOS, respectively.

Results

All alkylating carcinogens significantly inhibited the NOS activity in a dose-dependent manner until 240 min, at concentration of 1 mM.

These results suggest that carcinogenicity by alkylating chemicals may be associated with the inhibition of human serum NOS activity, and NO may play an important regulatory role in simple alkylator induced carcinogenicity in human.

Conclusions

The results may provide that this human serum-based assay system may serve as an efficient assay system for monitoring chemical-induced carcinogenesis in human serum.

doi: 10.1016/j.cca.2022.04.272

T036

Classification of breast cancer stadium using fuzzy neural network (FNN) model algorithm

R. Fajar, J. Prihantini, N.I. Kurniastuti

Department of Computational Biology, Yogyakarta State University, Indonesia

Background-aim

Breast cancer is a type of cancer found in breast tissue. Breast cancer is a disease that most attacks women. One way to detect the presence of breast cancer is by examination using mammography. After breast cancer is detected, classification is done to determine the stadium of the cancer. In this study, a Fuzzy Neural Network (FNN) model is used to classify breast cancer stadium. Fuzzy Neural Network (FNN) is a combination of fuzzy systems with Artificial Neural Networks (ANN). This study aims to explain how the breast cancer classification procedure using the FNN model.

Methods

The initial procedure for breast cancer classification using FNN is mammographic image extraction to obtain statistical parameters, namely energy, contrast, correlation, sum of squares, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference variance, difference entropy, maximum probability, homogeneity, and dissimilarity. Determination of input variables, namely statistical parameters from the results of mammographic image extraction and output variables, namely cancer classification. The division of data into two, namely 80% training data and 20% testing data.

Results

The best model of breast cancer classification using FNN is 11 input neurons, 1 hidden layer with 8 input neurons, and 3 output neurons. Based on the best model, breast cancer classification results obtained with the value of sensitivity, specificity, and sequential accuracy of 96%, 75%, 89% in training data and 77%, 83%, 79% in testing data.

Conclusions

The conclusion of this study can be said that the classification of stadium obtained good accurate results.

doi: 10.1016/j.cca.2022.04.273

T037

Inappropriate tumor marker requests in an university hospital

R. Nar, H. Aybek, E. Avci

Department of Medical Biochemistry, Faculty of Medicine, Pamukkale University, Denizli

Background-aim

Serum tumor markers (TM) are widely used for diagnosis, screening, prognosis, response to therapy and monitoring the recurrence of cancer. TMs may be ordered inappropriately by clinicians. In this study, we aimed to investigate the inappropriate TM requests in our hospital.

Methods

In this retrospective study patients were identified from the TM requests for December 2019, using the laboratory information system. A total of 2460 patients (1317 male, 1143 female) were included the study.

Results

The number of requests were 667 (%20.5 male, %79.5 female) for Cancer Antigen 125, 1042 (%100 male) for Prostate Specific Antigen, 751 (%18.4 male, % 81.6 female) for Cancer Antigen 15-3.

Our findings only according to the gender there is an inappropriateness of test requests. National and international guide¬lines state that CA125 should never be measured routinely in males and PSA should never be measured routinely in females. In our hospital for PSA there is a restriction that clinicans can not request the test from females.

Conclusions

Our data show that management and control of inappropriate requests with evidence-based laboratory medicine and using guidelines by laboratory professionals may be relevant to increase the clinical efficacy of TM testing. Inappropriate request of some TMs are clearly linked to the need for more education on their clinical applicability/limitations. Clinicans should be informed on the risk linked to the TM request for screening purposes or for avoiding more expensive diagnostic investigations.

doi: 10.1016/j.cca.2022.04.274

T038

Plasmatic metanephrines in diagnosis of pheochromocytoma and paraganglioma

S. Fares Taie, M.C. Pijuan, N.M. Goria, J.P. Espinosa

Fares Taie Instituto de Analisis, Argentina

Background-aim

Background: Pheochromocytomas and Paragangliomas are characterized by deregulated secretion of catecholamines into the bloodstream. They are neuroendocrine tumors that originate in chromaffin cells in the adrenal medulla (pheochromocytoma) or extra-adrenal (Paraganglioma). It is a rare entity, presenting a global incidence of 0.8-2 /

100,000 in adults but potentially fatal. Early diagnosis is important for treatment as it allows healing after surgical removal of the tumor.

The clinical practice guidelines present the highest degree of recommendation and evidence regarding the use of plasma or urine methanephrines for the diagnosis and monitoring of patients with clinical suspicion of pheochromocytoma or paraganglioma. On the other hand, plasma methanephrines and normetanephrines have the best combination of sensitivity (97%), specificity (96%), positive predictive value (98%) and negative predictive value (95%) compared to other pheochromocytoma biomarkers. Aim: Development and Validation of a Method by LC-MS / MS for the Determination of Free Plasma Metanephrines and Normetanephrines.

Methods

Materials and methods: For the development of the method, we used plasma-EDTA obtained by venous puncture of healthy people between 25 and 35 years old without medication and without signs of high pressure or palpitations. Merck's Cerilliant® "Catecholamine Mix 2 (Metanephrines) solution" standard was used on a Waters Aquity H-class chromatograph equipped with an Aquity C18 column and A Waters Xevo TQS-micro spectrometer.

Results

Results: The parameters that were checked during validation are: Sensitivity, Linearity, Limit of Detection and Quantification, Accuracy, Bias, Carry-over and Stability. The results obtained in this development are consistent with the international literature and indicate that LC-MS/MS is a robust and precise method for the determination of free plasma metanephrines and normetanephrines.

Conclusions

Conclusion: The use of LC-MS/MS has some advantages over other methods such as greater precision, lower bias, less susceptibility to interference and lower TAT (Turn Arround Time). This translates into more reliable results for the patient and the health-professional for making decisions. After this work, it will be possible to offer free plasma metanephrines and normetanephrines in the laboratory in addition of the already used urine fractionated methanephrines. In this way a significant benefit/comfort is offered to the patient who can avoid collecting urine for 24 hours in a bottle acidified with 10N HCl.

doi: 10.1016/j.cca.2022.04.275

T039

Pancreatic cancer insights: optimization of the diagnostic capacity of tumor biomarkers

J.A. Delgado RodrÍguez, M.A. Ballesteros Vizoso, M.M. Parera RosellÓ, J.M. BauÇa

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

Pancreatic cancer (PC) is one of the deadliest malignancies. Despite false positive and false negative values, serum biomarkers CA19.9 and CEA can help in the detection of this malignancy. The aim of this study was to determine the usefulness of CA19.9 in combination with CEA (CA19.9/CEA ratio) as a diagnostic tool for the differentiation of PC from benign pathology or other types of cancers in our hospital.

Methods

Retrospective observational study (2015-2019), including all laboratory requests with increased CA19.9 and CEA, but no previous diagnosis of neoplasia. ROC curve analyses were performed for the CA19.9/CEA ratio and CA19.9 alone for the detection of PC, and cut-off values for both strategies were selected separately and in combination for the maximization of the diagnostic performance. Diagnostic capacities were calculated and cut-off values with maximal discrimination power were used to calculate the odds ratio (OR) for PC.

Results

A total of 373 individuals were included (82% with malignant pathology, 29% colorectal, 17% pancreatic, and 15% lung cancer). The area under the ROC curve (AUC) for CA19.9/CEA was 0.872 (95% CI: 0.824-0.920), whereas AUC for CA19.9 was 0.847 (95% CI: 0.798-0.896). Cut-off values with the greatest diagnostic power were CA19.9/CEA > 40 and CA19.9 > 1130U/mL. The combination of CA19.9/CEA > 40 with CA19.9 > 550U/mL maximized the diagnostic accuracy for PC [sensitivity: 0.810 (95% CI: 0.713-0.907), specificity: 0.813 (95% CI: 0.764-0.854)]. This combination was found to have an OR of 17.7 (95% CI: 8.4-37.0) for PC.

Conclusions

Our results highlight the relevance of the measurement of serum CA19.9 and CEA in the detection of pancreatic cancer, as a complement to physical examination and imaging studies. Verification and optimization of cut-off values in larger prospective cohorts will increase the diagnostic capacity and clinical usefulness of these biomarkers.

doi: 10.1016/j.cca.2022.04.276

T040

Micrornas stimulate prostate cancer stem cell expansion by targeting genes of the apoptotic pathway

K.K. Shukla, G.R. Chowdhury, S. Misra, S. Sankanagoudar

All India Institute of Medical Sciences (AIIMS), Jodhpur, India

Background-aim

Recent studies on microRNA (miRNA) have revealed its prominent role in malignant transformation and invasiveness. However, the diagnostic value of miRNAs in prostate cancer (PCa) remains unclear. Therefore, we assessed the diagnostic importance of miR-10b in cancer stem cells (PCSC) through their target gene expression of the apoptotic pathway in PCA

Methods

We undertook micro RNA and target gene expression on a total of 120 individuals including 40 benign prostatic hyperplasias (BPH) as control, 40 localized, and 40 metastatic PCa histopathologically confirmed, untreated, and newly diagnosed. We employed quantitative real-time PCR (qPCR) for miRNAs and gene expression, The expression of stem cells, cell death markers like apoptosis, and total reactive oxygen

species (ROS) were studied using flow cytometry. Chemiluminescence for Interleukin-6 (IL-6) and tumor necrosis factor (TNF-⟨).

Results

The results showed that the mean expressions of MiR-10b and apoptotic pathway gene expression of Cyto C and Caspase were significantly higher ($p < 0.001$) localized PCa and metastatic PCa than in BPH. In contrast, the mean expressions of BCL2, BAX, Procaspase, and SMAC were significantly lower ($p < 0.001$) in localized PCa and metastatic PCa than in BPH. In addition, CD44 + /CD24 − and CD45 and CD326 showed higher expression in localized PCa and metastatic PCa than in BPH. The levels of PSA, testosterone, apoptosis, TNF-⟨, IL-6, and ROS also showed a significant increase ($p < 0.001$) in both metastatic PCa and localized PCa compared with BPH control. Moreover, all studied markers showed significant ($p < 0.001$) diagnostic potential in estimating cases of metastatic PCa and those of localized PCa except PSA.

Conclusions

The findings of this study suggest that miR-10b has a role in generating cancer stem cells to yield an invasive phenotype, and overexpression of these results in poor outcomes in metastatic PCa, and hence can be used as a diagnostic biomarker for metastatic PCa patients.

doi: 10.1016/j.cca.2022.04.277

T041

Multiple myeloma with unique presenting symptoms: A case report

V. Khurana, R. Verma, R. Saijpaul, S. Kaushik

Department of biochemistry, Maulana Azad Medical College, New Delhi, India

Background-aim

Multiple myeloma (MM) is a hematological malignancy primarily involving the bone marrow, which is replaced by malignant plasmacytes producing monoclonal proteins. Patients most commonly present with bone pain, fatigue and weight loss. In some cases metastatic deposits may occur outside the bone marrow (extra-medullary), but are extremely rare. Here we present one such rare case where the patient subsequently diagnosed as MM had only an axillary lump as the presenting symptom.

Methods

Case report: Our patient is a 55-year-old male who came to the general surgery outpatient department with a complaint of a non-subsiding axillary swelling since the past 6 months. The patient had no history of any other comorbidities and was otherwise in good health. On physical examination, the swelling was about 3x3 centimetres, non-tender and immobile. A biopsy was taken and baseline routine laboratory investigations were done. Biopsy report revealed infiltration by atypical plasma cells. On further work up, bone marrow biopsy depicted clonal plasma cells forming 16% of all nucleated cells including binucleate and immature forms. Serum protein electrophoresis (SPEP) and subsequent Immunotyping (IT) performed on Minicap flex-piercing (Sebia, France) showed evidence of IgG-lambda as well as free lambda light chains. The urine was examined for presence of monoclonal Bence Jones proteins by Heat test, further confirmed on Urine protein electrophoresis (UPEP) performed on Minicap flex-piercing (Sebia, France) which reported them as 54.2% of the total urinary proteins.

Results

Case report: Our patient is a 55-year-old male who came to the general surgery outpatient department with a complaint of a non-subsiding axillary swelling since the past 6 months. The patient had no history of any other comorbidities and was otherwise in good health. On physical examination, the swelling was about 3x3 centimetres, non-tender and immobile. A biopsy was taken and baseline routine laboratory investigations were done. Biopsy report revealed infiltration by atypical plasma cells. On further work up, bone marrow biopsy depicted clonal plasma cells forming 16% of all nucleated cells including binucleate and immature forms. Serum protein electrophoresis (SPEP) and subsequent Immunotyping (IT) performed on Minicap flex-piercing (Sebia, France) showed evidence of IgG-lambda as well as free lambda light chains. The urine was examined for presence of monoclonal Bence Jones proteins by Heat test, further confirmed on Urine protein electrophoresis (UPEP) performed on Minicap flex-piercing (Sebia, France) which reported them as 54.2% of the total urinary proteins.

Conclusions

An unusual case of MM has been described here, in which the histopathological study of a lymph nodule provided invaluable information for further workup and diagnosis. The clinicians and laboratorians thus need to be aware of the possibility of MM presenting in such a unique manner so that the diagnosis of the disease is not missed and treatment delays can be avoided.

doi: 10.1016/j.cca.2022.04.278

T042

Automated EXENT® mass spectrometry for the qualitative assessment of monoclonal immunoglobulins in urine

W.H. Tay [e], D. Barnidge [d], D. Sakrikar [d], S. Harding [c], M. Sherman [b], N. Cheedarla [b], A. Neish [a]

[a] *Emory University School of Medicine, Atlanta, GA, USA*
[b] *Emory University School of Medicine, Atlanta, GA, USA*
[c] *The Binding Site, Birmingham, UK*
[d] *The Binding Site, Rochester, USA*
[e] *The Binding Site, Singapore*

Background-aim

MALDI TOF mass spectrometry (MS) has become a suitable alternative to electrophoretic methods for the identification and quantification of monoclonal immunoglobulins (M-proteins), as reported in International Myeloma Working Group guidelines. Current studies have focused on using serum as the biological matrix for identifying M-proteins. Here we report the use of the EXENT MALDI-TOF-MS system for the identification of M-proteins directly from aliquoted urine, thus eliminating any pre-analytical manual sample handling.

Methods

Mass spectrometry analyses were carried out using the EXENT system (The Binding Site Group Ltd, UK). Samples were incubated with paramagnetic microbeads conjugated with polyclonal antibodies (anti-

IgG, -IgA, -IgM, -total- ▫ and -total-|_). After washing to remove unbound proteins, purified immunoglobulins were eluted and reduced to separate the heavy and light chains. Eluates were mixed with HCCA matrix, spotted onto MALDI target plates, and acquired using a Bruker MALDI-TOF-MS system. All samples were run using total | and total |_beads, with a select number run using anti-IgG, -IgA, and -IgM beads. Urine EXENT and IFE results from 102 clinical samples were compared. EXENT analysts were blinded to all IFE results.

Results

There was 95% agreement between EXENT and IFE for samples with a monoclonal light chain (97/102). The 5 discrepant samples contained a monoclonal light chain peak identified by EXENT that was not observed by IFE. Furthermore, 5 samples presented characteristic mass-shifted monoclonal light chains which may correspond with glycosylation modifications. Samples reflexed to anti-IgG, -IgA, and -IgM beads showed 100% agreement with IFE for isotyping the intact M-protein. Urine samples with high polyclonal background but no monoclonal immunoglobulin were also in 100% agreement between EXENT and IFE.

Conclusions

EXENT performs well for the detection of monoclonal immunoglobulin in urine, showing 95% concordance to existing IFE assays. The EXENT system identified potential post-translational modifications that are not recognizable by IFE. The analytical workflow does not require any pre-concentration step, significantly improving laboratory handling of these samples.

doi: 10.1016/j.cca.2022.04.279

T043

Exent mass spectrometry allows early identification of multiclonal MGUS compared to electrophoretic methods

W.H. Tay [b], H. Giles [f,g], N. Wright [a], M. Afzal [e], J. Birtwistle [a], O. Berlanga [a], S. North [a], M. Drayson [d], G. Pratt [c], G. Wallis [a], S. Harding [a]

[a] *The Binding Site, Birmingham, UK*
[b] *The Binding Site, Singapore*
[c] *University Hospitals Birmingham NHS Foundation Trust, UK*
[d] *University of Birmingham, Birmingham, UK*
[e] *University of Birmingham, UK*
[f] *University of Birmingham, UK*
[g] *University Hospitals Birmingham NHS Foundation Trust, UK*

Background-aim

Monoclonal gammopathy of undetermined significance (MGUS) has a risk of progression to multiple myeloma of 0.5-1.0% per year, making long-term monitoring necessary. We present results from a patient diagnosed with IgA| MGUS monitored >10 years who evolved into multiclonal MGUS by standard electrophoretic methods. Sera were retrospectively analysed with EXENT® to assess whether the added sensitivity of mass spectrometry (MS) could provide early identification of evolving clones.

Methods

Capillary zone electrophoresis (CZE) and immunofixation (IFE) were performed on Capillarys2™ and Hydrasys2 Scan™ platforms, respectively (Sebia, France). Serum free light chain (sFLC) measurements were performed with Freelite™ (The Binding Site, UK) on a Cobas c501 turbidimeter (Roche, Switzerland). Immunoglobulin purification was performed using the EXENT system, by incubating diluted serum with antisera specific for IgG/A/M, total ▫, total |_, free ▫ and free |_conjugated to paramagnetic beads. Purified immunoglobulins were reduced and eluted. Samples were mixed with matrix, spotted onto a MALDI target plate and acquired by MALDI-TOF MS.

Results

At presentation and first follow up, CZE and IFE identified an IgA ▫ paraprotein. At second follow up, the primary IgA ▫ and a new IgG ▫ paraprotein were visible by CZE and IFE, and a second IgA ▫ band showed only by IFE. No other qualitative changes were observed during follow-up, even though renal function deteriorated as indicated by increasing sFLC and serum creatinine levels.

However, at presentation, EXENT identified an IgA ▫ paraprotein with a mass-to-charge ratio (m/z) of 11657, along with additional IgG ▫ (m/z 11765) and two IgA ▫ (m/z 11931 and 12037) monoclonal peaks. All 4 paraproteins remained detectable by EXENT for the remainder of follow up. Furthermore, EXENT identified a persistent clonal ▫ sFLC with the same m/z as one of the IgA ▫ monoclonal proteins from baseline, when sFLC levels and ratio were normal.

Conclusions

EXENT identified the presence of evolving paraproteins and monoclonal sFLC earlier than standard methods. The ability to track monoclonal proteins using their unique m/z enables unequivocal identification and tracking of low-level monoclonal proteins and early differentiation of emerging clones.

doi: 10.1016/j.cca.2022.04.280

T044

Verification of the automated EXENT® mass spectrometry (MS) system for the identification and quantification of monoclonal immunoglobulins

W.H. Tay [c], N. Marrott [a], S. North [a], D. Mcentee [a], R. Stanton [a], D. Matters [a], D. Sakrikar [b], L. Ouverson [b], O. Berlanga [a], H. Montgomery [a], D. Barnidge [b], G. Wallis [a], M. Perkins [a], S. Harding [a], J. Ashby [a]

[a] *The Binding Site, Birmingham, UK*
[b] *The Binding Site, Rochester, USA*
[c] *The Binding Site, Singapore*

Background-aim

The utility of MALDI-TOF-MS for identifying monoclonal immunoglobulins has been published in IMWG guidelines. We report verification results of this technology using the EXENT system. Standardisation of monoclonal peak quantification was performed using immunoglobulin assay data calibrated to DA470k.

Methods

EXENT (The Binding Site, UK) was previously known as QIP-MS. Modified polyclonal antibodies (anti-IgG/A/M, -total-|, -total-|_) were immobilised on to paramagnetic beads. Patient samples were incubated

with the beads, washed, eluted, and spotted onto MALDI plates with HCCA matrix. Spectra were generated using the MALDI-TOF-MS system. Light chain spectra were obtained using EXENT software to yield immunoglobulin isotype, mass-to-charge ratio (m/z) and quantity (g/L). Quantitative IgG, IgA and IgM values were obtained using the Optilite® analyser.

Analytical performance was based upon CLSI guidelines: LLMI (EP17-A2), reference interval verification (EP28-A3c), precision (EP05-A2), linearity (EP06-A), interference (EP07-A2), and stability (EP25-A).

EXENT results were compared to 60 IFE-positive and 49 SPEP-positive samples.

Results

LLMI was 0.015g/L for all specificities. Published Hevylite reference intervals were verified. 20-day total precision for polyclonal samples (<7%) and for two monoclonal samples (~1g/L and 30g/L; <15% and <10%, respectively) was acceptable, with between laboratory precision <8% for all samples.

The assay was linear for IgG, IgA and IgM across a 0.015 to 100g/L range using a 10% non-linearity acceptance limit. No significant assay interference was observed. On-board stability of reagents was 5-24h. Open-vial stability was 2.6 months.

The assay was 100% concordant with IFE for the monoclonal type. Quantitative agreement with SPEP was acceptable (y = 0.91x-1.14), but there was substantial discordance <10g/L (y = 0.75x-0.87) compared to >10g/L (y = 0.95x-2.59), suggesting over-estimation by SPEP at lower levels.

Conclusions

EXENT showed acceptable performance for precision, interference, and agreement with existing assays. The role of the EXENT software in the identification and integration of peaks will ensure reliable and reproducible results across different laboratories, an important step in standardising this methodology.

doi: 10.1016/j.cca.2022.04.281

T045

Evaluation of chemokine CXCL1, CXCL12 and metalloproteinase MMP-9 as diagnostic markers of breast cancer

J. Motyka [c], M. Kulesza [c], A. Kicman [a], E.G. Będkowska [b], S. Ławicki [c], P. Ławicki [c]

[a] *Department of Aesthetic Medicine, Medical University of Bialystok, Poland*
[b] *Department of Haematological Diagnostics, Medical University of Bialystok, Poland*
[c] *Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Bialystok, Poland*

Background-aim

Currently, mammary cancer holds the greatest incidence rate of malignant lesions worldwide. Existing tumor biomarkers lack adequate sensitivity and specificity, therefore new parameters are being sought that can outperform existing ones. There are numerous scientific reports demonstrating the crucial involvement of certain factors, such as chemokines or matrix metalloproteinases (MMPs) in tumorigenesis. The aim of this study was to evaluate plasma level and diagnostic usefulness of CXCL1, CXCL12 and MMP-9 as tumor markers of breast cancer. Selected factors was compared against routinely used tumor marker CA 15-3.

Methods

For this study 2 groups were used: the study group composed of 30 patients with ductal breast carcinoma (BC) and the control group of 30 healthy women (HW). Plasma concentrations of chemokines and MMP-9 were determined by ELISA method, CA 15-3 antigen - by CMIA. With the use of PQStat software, comparative analysis was performed with the Mann-Whitney U test. Diagnostic sensitivity (SE), specificity (SV), positive and negative predictive value (PPV, NPV), and diagnostic power of the test were calculated based on the cut-off point determined by Youden index and Area Under the ROC (AUC).

Results

We observed statistically higher levels of MMP-9 (median 279.36 ng/ml; p = 0.001), similarly as CA 15-3 (21.1 IU/ml; p = 0.007) and significant lower levels of CXCL12 (2.63 ng/ml; p = 0.007) in the BC group vs the HW group (163 ng/ml; 17 IU/ml; 2.87 ng/ml, resp.). The highest diagnostic utility was determined for MMP-9 (AUC 0.7461; SE 86.67%; NPV 81.82%) and CXCL12 (0.7022; 70%; 70%, resp.), which exceeded those obtained for the CA 15-3 (0.7000; 46.67%; 61.90%, resp.). Among tested parameters the highest PPV was observed for CXCL12 (70%), which was still lower than CA 15-3 (77,78%). Analysis of selected parameters in combination with CA 15-3 was also performed, yielding an increase in diagnostic parameters over single factor analysis, reaching the highest value for joint analysis of three parameters CXCL12, MMP-9, and CA 15-3 (0.8278; 90.00%; 86.96%, resp.).

Conclusions

Taken together, our results suggest the possibility of applying both MMP-9 and CXCL12 plasma levels as breast cancer markers in the diagnostic process, especially at the combination with CA 15-3 marker.

doi: 10.1016/j.cca.2022.04.282

T046

Evaluation of the concentration and diagnostic utility of matrilysins (MMP-7 and MMP-26) in the diagnosis of endometrial cancer

M. Kulesza [d], J. Motyka [d], A. Kicman [b], E.G. Będkowska [c], S. Ławicki [d], T. Guszczyn [a], P. Ławicki [d]

[a] *Department of Pediatric Orthopaedics and Traumatology, Medical University of Bialystok, Poland*
[b] *Department of Aesthetic Medicine, Medical University of Bialystok, Poland*
[c] *Department of Haematological Diagnostics, Medical University of Bialystok, Poland*
[d] *Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Bialystok, Poland*

Background-aim

Endometrial cancer is the most common malignancy of the female genital tract. Detection of cancer at an early stage translates into an improved prognosis for patients. Metaloproteinases (MMPs) play an essential role in all stages of carcinogenesis. Therefore, the aim of this study was to investigate potential of plasma MMP-7 and MMP-26 as a new markers of endometrial cancer.

Methods

The study group consisted of 30 patients with endometrial cancer (EC), a comparison group of 30 patients with uterine myomas, and 30

healthy women group. The concentrations of MMP-7, MMP-26 were determined by ELISA method, the comparative marker CA125 by CMIA. Statistical analysis was carried out in PQStat software using the Kruskal-Wallis test with post-hoc Conover-Iman test. Diagnostic utility were calculated based on diagnostic sensitivity (SE), specificity (SV), positive and negative predictive value (PPV, NPV), and diagnostic power of the test - by Area Under the ROC (AUC).

Results

We noticed statistically higher concentrations of MMP-7 in the EC (4.214 ng/ml; $p < 0.001$) and myoma group (2.761 ng/ml; $p = 0.002$) when compared to healthy subject. Additionally, MMP-7 showed statistical differences between the EC and myomas ($p = 0.002$). Statistically higher levels of MMP-26 were only found for benign lesions (12.56 ng/mL; $p = 0.024$) when compared against heathy women group. Analysis of CA125 concentrations showed significantly higher values in the EC (19.6 IU/mL; $p = 0.006$) and myoma group (21.25 IU/mL; $p = 0.008$) when compared to the healthy subjects. The highest diagnostic utility measured by AUC, SE, PPV and NPV were demonstrated by MMP-7 (0.9456; 96.67%; 87.88%; 96.29%, resp.). These values exceeded those obtained for the comparative marker CA125 (0.7028; 56.67%; 70.83%; 63.89%). Furthermore, MMP-26 showed higher SE (93.33%) and NPV (81.82%) than CA125. The results of the combined analysis of tested parameters with CA125 increased the AUC and diagnostic parameters: MMP-7 + CA125 (AUC 0.9533; SE 93.33%; PPV 90.32%, NPV 93.10%), MMP-26 + CA125 (AUC 0.7111; SE 80.00%; NPV 72.73%).

Conclusions

The obtained results suggest the usefulness of MMP-7 and MMP-26 as new markers in diagnosis of endometrial cancer patients, especially in the combination with CA125.

doi: 10.1016/j.cca.2022.04.283

T047

Evaluation of cytokines M-CSF, VEGF and metalloproteinase MMP-9 as diagnostic markers of ovarian cancer

P. Ławicki [c], E.G. Będkowska [a], E. Gacuta [b], J. Motyka [c], M. Kulesza [c], A. Kicman [c], M. Chlabicz [c], P. Sowa [c]

[a] *Department of Hematological Diagnostics, Medical University of Białystok, Poland*
[b] *Department of Perinatology, Medical University of Białystok, Poland*
[c] *Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Białystok, Białystok, Poland*

Background-aim

M–CSF, VEGF and MMP-9 play a crucial role in the pathogenesis of cancer disease, for example in cell growth, proliferation and angiogenesis. MMP-9 plays an important role in degradation of the extracellular matrix which provides to the metastasis. We investigated the plasma levels of VEGF, M-CSF and MMP-9 in comparison to markers (CA125 and HE4) in ovarian cancer patients (serous sub-types – 60 women) and in relation to the healthy subjects (60).

Methods

Plasma levels of M-CSF, VEGF and MMP-9 were determined using ELISA method, comparative markers CA125 and HE4 - by CMIA. The statistical analysis was performed by Statistica 12.0 software with the use of Mann-Whitney U test. Diagnostic utility were calculated based on diagnostic sensitivity (SE), specificity (SV), positive and negative predictive value (PPV, NPV), and diagnostic power of the test - by Area Under the ROC (AUC). Cut-off points were determined by Youden Index.

Results

Plasma levels of M-CSF (630.04 pg/ml), VEGF (180.48 pg/ml) MMP-9 (284.20 pg/ml) and tumor markers (CA125 – 100.2 U/ml and HE4 – 60.45 pmol/L) were significantly higher in ovarian cancer patients as compared to the healthy subject (280.50 pg/ml; 147.04 pg/ml; 174.24 pg/ml; 22.15 U/ml; 40.97 pmol/L). The M-CSF, VEGF, MMP-9 and tumor markers SVs received high values (94%; 94%; 92%; 92%; 94%; resp.). The SE, PPV, NPV of VEGF (72%; 95%; 62%; resp.) were higher than for M-CSF (70%; 93%; 60%; resp.) or MMP-9 (50%; 90%; 47%; resp.), CA 125 (62%; 91%; 56%; resp.) and HE4 (53%; 90%; 50%; resp.). The highest AUC was observed for VEGF (0.9004) than for M-CSF (0.8991), MMP-9 (0.820), CA 125 (0.8888) or HE4 (0.8322). The combined use of tested parameters with tumor markers resulted in the increase of the SE, NPV and AUC, but highest values were obtained by analyzing combination of all tested parameters with both tumor markers (96%, 87%, 0.9564; resp.).

Conclusions

These results suggest a potential usefulness of M-CSF, VEGF and MMP-9 in diagnostics of ovarian cancer, especially in combine use with CA 125 and/or HE4.

doi: 10.1016/j.cca.2022.04.284

T048

CCL2 and CCL5 as new markers of early-stage endometrial cancer

E.G. Będkowska [a], E. Gacuta [b], S. Ławicki [c], J. Motyka [c], A. Kicman [c], M. Kulesza [c], K. Pańkowska [a], P. Ławicki [c], M. Chlabicz [c], M. Dąbrowska [a]

[a] *Department of Hematological Diagnostics, Medical University of Białystok, Poland*
[b] *Department of Perinatology, Medical University of Białystok, Poland*
[c] *Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Białystok, Poland*

Background-aim

Endometrial cancer (EC) is the most common gynecologic cancer in developed countries and the fourth most common cancer in women. Therefore, it is important to find new diagnostic markers that will allow the earliest possible detection of cancerous lesions, for example chemokines. Chemokines are a family of small-molecule proteins that have the ability to control the migration of various cell types. They may affect tumor growth through several important mechanisms: regulation of angiogenesis, modification of the antitumor response, and direct effects on tumor cell proliferation and apoptosis.

The aim of the study was to evaluate the concentration and diagnostic usefulness of selected chemokines (CCL2 and CCL5) in plasma of patients with EC in comparison to patients with benign lesion (myoma) and healthy women. In addition, the obtained results will be evaluated against the reference marker CA125 used in routine diagnostic process.

Methods

The study group consisted of 30 patients with endometrial cancer (15 patients in stage I and 15 in stage II disease), the comparison group - 30 patients with myomas and the control group - 30 healthy women. ELISA method was used to determine CCL2 and CCL5 concentrations, while CA125 antigen was determined by CMIA method. Diagnostic utility were calculated based on diagnostic sensitivity (SE), specificity (SV), positive and negative predictive value (PPV, NPV), and diagnostic power of the test - by Area Under the ROC (AUC).

Results

Plasma levels of CCL2, CCL5 and CA125 (292.7 pg/ml; 12.0 ng/ml; 17.7 IU/ml, resp.) were significantly higher in EC patients ($p < 0.0001$; $p < 0.0001$; $p < 0.0002$, resp.) and myoma groups ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$) in comparison to the healthy subjects. The CCL5 diagnostic specificity and positive predictive value were the highest (both 100%) among all tested parameters. The diagnostic sensitivity (80%) and negative predictive value (83%) were similar to those of CCL2 (87%; 85%, resp.) or CA125 (83%; 80%, resp.). The combined use of tested parameters with tumor marker resulted in the increase of the SE, NPV and AUC. We observed that CCL5 had also the largest area under the ROC curve (AUC) among all tested parameters (0.9067). We also showed that combining CCL5 with a reference tumor marker provided a further increase in the diagnostic power of the assay, achieving an AUC value of 0.9500.

Conclusions

Obtained results suggest a diagnostic usefulness of CCL5 in combined panel with CA125 in diagnostics of early stages of EC.

doi: 10.1016/j.cca.2022.04.285

T049

Role of GDF-15, ABCC5 and FOXM1 in the advancement of breast cancer stage and drug resistance

A. Modi [a], P. Purohit [a], D. Roy [a], J.R. Vishnoi [d], P. Pareek [c], P. Elhence [b], S. Sharma [a], P. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India*
[b] *Department of Pathology & Lab Medicine, All India Institute of Medical Sciences, Jodhpur, India*
[c] *Department of Radio-Therapy, All India Institute of Medical Sciences, Jodhpur, India*
[d] *Department of Surgical Oncology, All India Institute of Medical Sciences, Jodhpur, India*

Background-aim

Growth differentiation factor-15 (GDF-15) is an inflammatory cytokine, involvedcancer progression. ABCC5 is a surface protein involved in drug resistance. Intrinsic drug resistance is the primary reason for therapy failure. Forkhead box protein M1 (FOXM1) is a transcription factor and a master regulator in cancer metastasis. The current study analyzed the expression of GDF-15, FOXM1, andABCC5 in BC staging.

Methods

30 diagnosed BC patients, among which 13 were early-stage and 17 were advanced-stage, and 15 healthy controls were included in this study. Serum GDF-15 was analyzed through sandwich ELISA. Expressions of GDF-15, ABCC5, and FOXM1 in BC tissue were determined by real-time PCR. Interaction between these genes were analyzed in-silico.

Results

Expression of serum GDF-15 was observed to be significantly higher in the advanced stage compared to early BC patients and healthy control ($p < 0.001$). Further, the expressions of GDF-15, FOXM1 and ABCC5 were 1.6, 1.95, and 2.65 folds upregulated, respectively, in advanced stage compared to early-stage BC patients. PPI network analysis showed the interaction between these genes were significantly enriched.

Conclusions

GDF-15 is probably responsible for the advancement of BC stage and intrinsic drug resistance, mediated by FOXM1.

doi: 10.1016/j.cca.2022.04.286

T050

HCG increase in an individual with lung cancer

S. Sánchez Asís, L. Valiña Amado

Hospital Universitario Son Espases, Palma de Mallorca, Spain

Background-aim

HCG is a glycoprotein hormone synthesized by the syncytiotrophoblastic cells of the placenta. An increase in HCG concentration in the absecense of pregnancy should lead to suspicion of neoplasia. HCG is mainly used as a tumor marker (TM) in trophoblastic tumors and germline neoplasms of the testis and ovary.

Neoplastic production of HCG in non trophoblastic or germline tumors has occasionally been described, with very few cases related to lung carcinomas. High levels of this hormone in lung cancer have been mainly associated with adenocarcinoma and advanced stages.

Methods

Clinical case description.

Results

A 76-year-old man with a history of urothelial carcinoma attended the emergency room due to disorientation and frequent falls. After performing a body and cranial CT scan, a brain lesion was observed in the right occipital region together with a mass in the right lung, suggestive of neoplasia.

TM were measured yielding the following results: AFP, CA19.99, CA125 and PSA were within the normal ranges; CEA concentration was 11.36 ng/mL (< 5 ng/mL) (Alinity I, Abbott). NSE was also in normal ranges while HCG concentration was 38.1 mUI/mL (< 2,6 mUI/mL) (Cobas e411 Roche). CEA was slightly increased, however HCG was clearly indicative of neoplasia. Eventually, the patient was diagnosed with stage IV lung adenocarcinoma.

Conclusions

The role of HCG in lung cancer is unclear. Some studies suggest that it could act as a growth factor through the inhibition of apoptosis. This

could explain why HCG-producing tumors appear to be more agressive and have a worse prognosis. Elevated values of this marker are commonly seen in patients with metastatic tumor and have also been related to chemoterapy resistance.

In consideration with all of the above, this unusal secretion of HCG from lung cancer and other non germinal or trophoblastic tumors should be recognized to guide in the identification of cancer origin.

doi: 10.1016/j.cca.2022.04.287

T051

Neuron-specific enolase in pleural fluid aiding lymphoma diagnosis

L. Valiña Amado, S. Sánchez Asís, B. García García

Laboratory Medicine Department - Hospital Universitari Son Espases

Background-aim

Pleural effusions (PE) are common complications in lymphoma with an incidence of 20–30%. Neuron-specific enolase (NSE) is mainly expressed in neurons of both central and peripheral nervous systems, being one of the most specific tumor markers. NSE is abnormally expressed in neuroectodermic, gut carcinoid, neuroblastoma and small cell lung cancer. Increased NSE is found in 17–21% of non-HL and 6.5–23% HL. Tumor markers in pleural fluid have largely been studied. Molina et al proposed a strategy which uses a quotient TM in fluid/TM in serum > 1.2 as an indicator of local production and therefore cancer focus.

Methods

AFP, CA19.9, CA15.3, CEA and CA125 were analyzed in Alinity i platform (Abbott diagnostics)

NSE was performed in Cobas e411 (Roche diagnostics).

Results

CLINICAL CASE

Here is presented the case of an 86-year-old woman who attended Emergency Department (ED) because of progressive dyspnea and lower limbs oedema. NTproBNP was 363 pg/mL, so heart failure was unlikely. A month later the patient came back to ED, presenting a bilateral PE. Cytological examination of pelural fluid (PF) showed a malignancy free sample. 90 days later a recurrent bilateral PE was diagnosed. PF was again negative for cancer, as well as pleural biopsy. However, lymphoma was suspected as a retroperitoneal mass had been detected by PET/TC.

Tumor markers analysis was performed on the second PF sample, as well as in serum. The results were as follows: AFP, CA19.9, CA15.3 and CEA were normal both in serum and PF. Serum CA125: 96.7 U/mL (<35 U/mL) and PF CA125: 140U/mL, CA125 ratio: 1.3. Serum NSE: 15.7 (<17 ng/mL) and PF: 68.2 ng/mL; NSE ratio: 4.34.

Molina et at suggested a ratio cut off of 1.2 as indicator of local TM synthesis and therefore tumor presence in the pleura. Eventually, a lymph node was biopsied and the patient was diagnosed stage IIIB B cell non-HL.

Conclusions

The case presented is especially relevant because of the diagnostic challenge it supposed. NSE ratio was indicative of neoplasia, and this finding in the context of the patient is highly suggestive of lymphoma, helping to shorten the time to diagnosis. Outstanding in this case is the fact that pathology study was not sensitive enough, and it's necessary to emphasize that sampling bias in tissue can be aided by tumor marker study.

doi: 10.1016/j.cca.2022.04.288

T052

Tumor marker study in pleural fluid and tumor heterogeneity

L. Valiña Amado, B. García García, S. Sánchez Asís

Laboratory Medicine Department - Hospital Universitari Son Espases

Background-aim

Tumor markers (TM) in body fluids have been studied for years and several authors have proposed different cut-off. An apparently more accurate strategy is the one proposed by Molina et al. considering that the ratio TM in fluid with regard to TM in serum >1.2 indicates local production in the pleura, however if the ratio is <1.2 the presence of TM in the fluid would be explained by serum extravasation. Despite enough evidence to manage this biomarkers in body fluids, the practice is not widely extended in the clinical setting yet.

Methods

AFP, CA19.9, CA15.3, CEA ,CA125, PSA and SCC were analyzed in Alinity i platform (Abbott diagnostics)

HCG and NSE was performed in Cobas e411 (Roche diagnostics).

Results

Here we describe the case of a 69-year-old patient attending the Emergency Room due to pain in both hemythoraxes. Also remarkable was a wasting syndrome (5 kg weight loss in the past month). In Emergency blood analysis: VSG 50, PT 75%, DD 765 ng/mL, ferritin 368 ng/mL and LDH 385 U/L were outsdanding. Thorax radiology showed a pleural effusion. The patient was diagnosed with COVID19 bronchitis. TC scan evidenced pleural solid metastasis, multiple bone lesions and hepatic M1.

Serum TM: AFP, CA19.9, PSA, NSE, SCC and HCG were normal. CA125 2992,60 U/mL (<35), CA15.3 614,70 U/mL (<32), CEA 400.82 ng/mL (<5). Pleural fluid TM: CEA 284.32 ng/mL; CA15.3 2210.3 U/mL. TM ratio: CA15.3: 3.6 (>1.2) this result indicates local synthesis of CA15.3, therefore pleural metastasis; CEA: 0.7 (<1.2) indicates that the CEA found un the fluid was extravasated from serum.

Pathological examination was only positive for CK7 and mixt CK. All other markers were negative. It was concluded to be an undifferentiated carcinoma, cytologically reminding of an adenocarcinoma. Due to TTF1 and napsine negativity lung neoplasm could not be discarded.The patient was diagnosed with undifferentiated lung cancer stage IV.

Conclusions

This a good example of different molecular patterns reflecting tumor heterogeneity evidenced by protein expression by each lesion: Pleural metastases expressed high amounts of CA15.3, however not CEA. Hepatic metastases and probably main tumor in the lung expressed CEA and CA15.3. It is arguable whether CA15.3 was expressed at lower quantities from the main tumor or the dilution of the protein in the bloodstream results in lower concentrations in relation to the ones found in the pleura.

doi: 10.1016/j.cca.2022.04.289

Clinica Chimica Acta 530 (2022) S73–S97

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Cardiovascular Disease

T053

Evaluation of apolipoprotein profile in HIV/AIDS subjects in pre and post 12 months antiretroviral therapy using 1.5 NG/ML troponin diagnostic cut-off for myocardial infarction in Nauth Nnewi, South Eastern Nigeria

I.P. Ezeugwunne [b], J.E. Ahaneku [b], C.C. Onyenekwe [b], M. Ifeanyichukwu [c], S.C. Meludu [b], W. Nnaemeka [a]

[a] *Biochemistry Department, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria*
[b] *Chemical Pathology Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*
[c] *Immunology Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

Background-aim

It has been reported that acute myocardial infarction (AMI) might occur at 1.5 ng/ml troponin level. HIV infection has been documented to influence antiviral drugs, stimulates the production of proteins that enhance fatty acids synthesis. Information on cardiac status in HIV infected subjects in Nigeria is scanty.The aim is to evaluate the apolipoprotein profile of HIV subjects in pre- and post 12 months antiretroviral therapy (ART) using 1.5 ng/ml troponin diagnostic cut-off for myocardial infarction (MI) in Nnewi, South Eastern, Nigeria.

Methods

A total of 30 symptomatic HIV subjects without malaria co-infection with mean age of 40.70 ±10.56 years were randomly recruited, for this prospective case controlled study. Serum apolipoproteins (Apo A1, A2, B, C2,C3 and Apo E), troponin I and CD4 counts were measured using standard laboratory methods. Parameters were re-classified based on 1.5 ng/ml troponin diagnostic cut-off for MI. Analysis of variance and student paired t test were used for data analyses.

Results

Paired-wise comparison showed that there were significantly higher levels of CD4 counts, Apo A2, Apo C2, Apo E but lower levels ApoA1, ApoB and ApoC3 in symptomatic HIV subjects before antiretroviral therapy (ART) when compared with after therapy at $p < 0.05$ respectively. The troponin value was significantly higher amongst the group studied at $p < 0.05$ respectively.

Conclusions

The increased values of troponin observed among the groups were higher than the diagnostic cut off for AMI. This may imply that AMI may occur at any group of study. But the significant reduction in the serum levels of Apo A2, Apo B, Apo C3,Apo E and significant increase in serum levels of Apo A1, Apo C2 and blood CD4 counts as length of therapy lengthened, may indicate possible cardio-protective effects of the ART on the heart, which may connote recovery.

doi: 10.1016/j.cca.2022.04.520

T054

Therapeutic significance of hyperin on cardiovascular system for acute ischemic stroke beyond thrombolysis: Metabolomics and lipidomics approach

D.K. Patel, K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

Stroke constitutes lots of death and disability around the entire globe and for the treatment of ischemic stroke we have very limited medication including serine protease tissue plasminogen activator (tPA) which also have short therapeutic window. Flavonoids have an attractive candidate due to its health beneficial effect and safety issue. Hyperin, an important flavonoid have cardioprotective, antioxidant, anti-inflammatory, anticancer, antiviral, antibacterial, antiparasitic, hepatoprotective and antispasmodic activity.

Methods

Therapeutic strategies for the treatment of stroke have been developed through data mining by various in-vitro and in-silico analysis. Further importance of metabolomics, lipidomics, human chymase and tPA in the stroke were also summarized. The structure-based computational approach combining molecular docking was applied to develop new effective medicine against stroke. An in-silico study was performed to confirm effectiveness of hyperin against Human chymase and tPA. Further few virtual screening has been also done to select potential candidates for stroke.

Results

Hyperin produces significant hyperpolarization in rat basilar artery smooth muscle cells and reduced the brain infarct size and cerebral oedema in focal cerebral ischemia reperfusion injury. Further hyperin also modulate superoxide dismutase, glutathione peroxidase and LDH activities in the cerebrum of mice. Hyperin increase the spontaneous

beating rate of embryonic mouse myocardial cell sheets and increase prothrombin time. Importance metabolomics and lipidomics underlying stroke pathophysiology was also developed and designed. Potential ability of hyperin to bind chymase enzyme using docking study showed importance of hyperin against stroke and results revealed that the compound hyperin may interact with the active site of human chymase and tPA.

Conclusions

Emerging roles of metabolomics and lipidomics in the modern medical science for underlying ischemic stroke were developed with various in-silico molecular docking study. These studies are a promising starting point for potential development of future drugs for stroke.

doi: 10.1016/j.cca.2022.04.521

T055

Are we meeting the turnaround time for stroke code protocol?

M.E. Uez [b], M.A. Ballesteros [a], P. Argente Del Castillo [a], S. Tur [c], C. Jimenez [c], M.M. Parera [a], J.M. Bauça [a]

[a] *Department of Laboratory Medicine, Hospital Universitari Son Espases, Spain*
[b] *Hospital Interzonal General de Agudos O Alende, Mar del Plata, Argentina*
[c] *Neurology Department, Hospital Universitari Son Espases, Spain*

Background-aim

Stroke represents the 3rd cause of death in Spain, and is associated with high and severe morbidities. Ischemic stroke causes brain damage, which is potentially recoverable if reperfusion treatment is started early. Therefore, it needs to be considered as a neurologic emergency.

The Stroke Code was a coordinated protocol implemented in our hospital aiming to minimize the time between the onset of symptoms and the access to diagnostic and therapeutic options in a specialized healthcare center. Treatment should be started as soon as possible (< 6 h).

In our hospital, this protocol includes the request of specific biochemical and hematological analyses and a maximum intralaboratory turnaround time (iTAT) of 30min. Our aim was to assess whether we are meeting this agreed iTAT, as well as the preanalytical extralaboratory times.

Methods

This is an observational retrospective study. We included all Stroke Codes in our hospital between May-Sep 2018 (N = 126). Laboratory requests with additional tests were excluded.

Percentile 80 (p80) and p90 were calculated for total iTAT and for time slots (morning, afternoon, night shift), in different months, and also for the preanalytical extralaboratory time, defined as the period since online laboratory request until specimen reception in the laboratory.

All data was extracted from the laboratory information system (LIS) (GestLab, Indra) and all calculations were done on Microsoft Office Excel.

Results

A total of 96 requests were included. With regard to iTAT, p80 was 29min, and p90 was 38min. The month with a greater delay was August, probably due to higher staff turnover. Delays were greater in the morning shift (p90 = 45), compared to afternoon (p90 = 29) or night shifts (p90 = 37), maybe due to an increased workload.

The preanalytical extralaboratory time was 22min for 90% of requests, and 16min for 80%.

Conclusions

Up to 80% of total laboratory requests take 29min to be completed, meeting the established iTAT. Our laboratory should reach 90% compliance and reduce iTAT especially in the morning shift and during the summer season.

In order to help reduce such time, we included a green sign in the LIS for all patients under Stroke Code aiming to a better identification and follow-up of these requests.

doi: 10.1016/j.cca.2022.04.522

T056

Comparison, correlation and predictability of AOPP, IMA and cardiac markers CKMB activity and troponin T in patients with cardiac and non-cardiac chest pain

N. Pokhrel

Modern Diagnostic Center Nepal Pvt.Ltd., Nepal

Background-aim

Chest pain is the major symptom associated with patients suffering from cardiac ischemia. The cause of chest pain is diagnosed differentially using history of chest pain, ischemic changes in electrocardiogram and elevated cardiac biomarkers. But some patients with cardiac ischemia may not present with chest pain and/or typical ECG findings. Only the patients with infarctive changes in the heart have elevated levels of cardiac biomarkers depending on time of onset and time of collection of sample. AOPP (Advanced oxidation protein products) and IMA (ischemia modified albumin) are known to increase in inflammation and ischemia. In such patients AOPP and IMA can be used to predict the cardiac chest pain. Hence this study was conducted to compare, correlate and calculate the ischemia predictability of AOPP and IMA levels with cardiac bio markers in chest pain patients.

Methods

2 ml of plasma or serum was collected from patients with chest pain and AOPP, mAOPP, IMA and cardiac biomarkers CKMB and troponin were estimated. Patients were categorised into SA (Stable angina) or, UA (Unstable angina) (Group I), MI (Myocardial infarction) (Group II), SA or UA or MI (Group III) and Non cardiac chest pain (Group IV).AOPP was estimated in serum/plasma by modified method based on witko-sarsat assay of AOPP. mAOPP was also measured in in seum/plasma. IMA was estimated in serum by albumin cobalt binding nitrosonapthol method. CKMB activity was estimated only in serum by coupled immuno enzymatic method. Troponin T was estimated in plasma either EDTA or heparinised plasma by fluoroscent immunoassay method.

Results

AOPP, mAOPP, IMA were significantly different in patient suffering from MI and high in MI than IHD (SA, UA) and IHD values were higher than non cardiac chest pain (NCCP). AOPP, mAOPP, IMA correlate positively with CKMB, Trop T in MI patients. AOPP, mAOPP, IMA correlate with each other in MI, IHD as well as patients suffering from NCCP.

AOPP, mAOPP all above have good predictability for MI in all chest pain compared with CKMB and Trop T.

Conclusions

Our study is handicapped by the fact that patients with IHD and MI had not been followed for AOPP level dynamics. It is possible that the patients with STEMI and NSTEMI may have had higher AOPP concentration after certain time interval from the onset of MI. Further study should be done in large population and adjustment of HTN (Hypertension), DM (Diabetes Mellitus), RF (Renal failure) for analysis of AOPP, mAOPP, IMA. Negative control group should be included for determining the diagnostic cutoff value

doi: 10.1016/j.cca.2022.04.523

T057

The hypertension and the use of gene panels for multiplex DNA analysis of the next generation

A. Nagay, G. Khamidullaeva, L. Khafizova, R. Kurbanov

Department of Arterial Hypertension and Molecular Genetics Research Republican Specialized Scientific- practical medical Center of Cardiology Tashkent, Uzbekistan

Background-aim

Background and objectives: The work was initiated to assess risk of hypertension and its potential association with coronary diseases in patients with high pulse wave velocity.

Methods

Materials and methods. The study included 150 patients with hypertension in average age of 60.8 ± 7.05 years. Genotyping of the SNP was performed by polymerase chain reaction, multiplex Real-Time PCR. Essential hypertension panel – 19 genes. Vascular stiffness was determined on a Sphygmocor apparatus.

Results

Result: In our study, the highest genetic risk was found 55%. We found the pulse wave velocity tendency, to increase towards high genetic risk of susceptibility to the hypertension (12 ± 1.5 and 11 ± 2.8: 10.7 ± 5.3). In patients with high genetic risk, the systolic pressure was found 17 mm Hg higher than the one in the low risk group. Three genes of salt sensitivity were found to make a 70% contribution to the risk above (ADD1 1378 G/T, GNB825 C/T, CYP11B2 C344T). According to our findings, the deleterious alleles, such as CYP11B2, GNB and NOS3, made more frequent contribution to the hypertension risk and blood vessel deterioration. We suppose that the frequent occurrence of NOS3 mutations was associated with the endothelial dysfunction, a triggering mechanism for the vessel deterioration. In our study, mutations in NOS3:-786_T > C, GNB:825_C > T, AGTR2:1675G > A and AGT:704_T > C genes occurred most frequently in patients with high genetic risk.

Conclusions

Conclusions. The above mutations are supposed to cause alterations in phenotypic expression in the cells of blood vessels in every other hypertensive person. The alterations make up 55% of the whole population of hypertensive persons. In other cases, factors of ecological effect of the environment, probably, left behind the genetic inheritance regardless of age and sex. To our mind, there are significant ethnic differences in ADD1, GNB and CYP11B2 genes. Mutation points in the genes were associated with dangerous prediction of the hypertension. These conclusions confirmed the concept of variability of the mutations associated with the etiology of salt gradient disorder, geographical latitude and race phenotype. Thus, the panel we use can be a suitable genetic marker to identify subjects with high risk of hypertension living in the hot climate and having associations with ischemic stroke, diabetes and heart failure.

doi: 10.1016/j.cca.2022.04.524

T058

Homocystinemia and polymorphysm of the gene for methylentetrahydrofolate reductase (C677T) in patient with coronary artery disease

J. Brezovska-Kavrakova, S. Cekovska, K. Tosheska-Trajkovska, I. Kostovska, K. Krsteva-Jakimovska

Department of Medical and Experimental Biochemistry, Medical Faculty, Skopje, Macedonia

Background-aim

Background: Hyperhomocysteenemia, which commonly occurs either as a result of a methylene tetrahydrofolate reductase (MTHFR) gene mutation or vitamin B12 deficiency and folic acid deficiency, has been reported as a risk factor for coronary artery disease (CAD).

The purpose of this study was to determine the concentration of total homocysteine (tHcy) and the prevalence of C677T mutation of MTHFR in healthy subjects and patients with CAD. Also, has been analyzed whether elevated serum tHcy may, together with this mutation, affect this disease as a risk factor.

Methods

Methods: The investigation included 123 controls of healthy subjects, and 81 patients were confirmed by subsequent angiography as CAD. The two groups of healthy subjects and patients were divided by sex. Plasma tHcy concentration was determined by a cyclic enzyme method, and MTHFR gene polymorphism was analyzed by Chain Reaction Polymerase Chain Reaction (PCR) and fragment length fragment length polymorphism (RFLP) by Schneider.

Results

Results: The plasma tHcy concentration in healthy subjects and in patients in women was (8.3 ± 2.3 mol / L vs. 18.73 ± 5.71 mol / L), whereas in men (11.1 ± 5, 0 mol / L v.s 14.5 ± 6.16 mol / L) and statistically significantly higher tHcy concentrations in CAD patients than in healthy subjects ($p < 0.001$). The values of X2test ($x2 = 35.48$ and $p < 0.001$) for both groups showed a significant correlation between the tHcy concentration and CAD.

The highest frequency in healthy subjects and patients, for both sexes, was the most frequent mutation in the MTHFR C677T gene in the heterozygous CT genotype (46% vs. 50%), followed by the homozygous CC-type genotype (44% vs. 33%), and lowest in TT genotype with (10% vs. 17%). Analysis of differences showed that tHcy concentrations were statistically significant in relation with TT genotype in the CAD group ($p < 0.05$).

Conclusions

Conclusions: There is a significantly higher plasma level of tHcy in patients with CAD than in healthy subjects and a risk factor for CAD. Our findings have also shown that current mutations in the MTHFR C677T gene affect tHcy levels, but these mutations are not a risk factor for the development of CAD.

doi: 10.1016/j.cca.2022.04.525

T059

Performance evaluation of Atellica® IM CK-MB, myoglobin and BNP assays on the Atellica® solution

J. Cho, S.J. Han, M.H. Lee, Y. Uh

Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Republic of Korea

Background-aim

Creatine kinase MB (CK-MB), myoglobin, and brain-type natriuretic peptide (BNP) are recommended biomarkers for ruling out acute myocardial infarction and risk assessment of patients with ischemic symptoms. The objective of this study was to verify the analytical performance of the Atellica® IM CK-MB, myoglobin and BNP assays on the Atellica® solution, a recently launched immunoassay analyzer of Siemens Healthineers (Erlangen, Germany), compared to the Flex® reagent cartridge on the Dimension Vista® system, the previous model.

Methods

The within-device imprecision was determined by a 10-day study protocol (performed on 10 consecutive days, and 2 separated runs in duplicate for each day), using three levels of quality control materials for each assay. The linearity test was assessed using five levels of test materials provided by the manufacturer, and each test material was tested in triplicate. For method comparison, a total of 48 specimens were evaluated from the remaining patient samples after completing the routine tests submitted to our laboratory. The results obtained between the Dimension Vista® system (X axis, reference method) and the Atellica® solution (Y axis, comparative method) were compared.

Results

The within-day and within-device coefficient variations (CVs) were lower than the manufacturer's claims. The linearity was acceptable, showing $<10\%$ non-linearity for all levels. The slopes of Passing-Bablok regression equations for CK-MB, myoglobin, and BNP against the Dimension Vista were 1.195 (95% confidence interval (CI), 1.116-1.234), 1.003 (95% CI, 0.977-1.016), and 0.970 (95% CI, 0.933-1.026), respectively. For CK-MB, the 20% positive bias corresponds to an increase in the upper end of reference intervals (from <3.6 to <5.0 ng/mL).

Conclusions

The Atellica® IM CK-MB, myoglobin and BNP assays on the Atellica® solution showed acceptable precision and linearity, according to the manufacturer's claims, with analytical agreement compared to the Flex® reagent cartridge on the Dimension Vista® system.

doi: 10.1016/j.cca.2022.04.526

T060

Association of DNA methyl transferase 3B gene polymorphism with coronary artery disease

E. Eftekhar, F. Rafiee, S. Koochak Khani

Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Background-aim

Coronary artery disease (CAD) has increased dramatically in the last decade. Recently, it has been proposed that genetic variations have a critical role in the pathophysiology of CAD. DNA methyl transferase 3 B (DNMT3B) is one of the important genes involved in the pathogenesis of CVD. In the present research, the association between DNMT3B rs2424913 T/C gene polymorphism and CAD was investigated.

Methods

Based on the result of angiography, subjects ($n=130$) were divided in two group of non-CAD control ($n=70$, subjects with no coronary artery stenosis) and CAD patients ($n=60$, subjects with at least 50% stenosis in one of the major coronary arteries). . Fasting blood samples were obtained from subjects and DNMT3B rs2424913 T/C gene polymorphism was evaluated by RFLP- PCR method

Results

The frequency of TT, TC and CC genotypes were 18.5%, 65.5%, 20.1% in CAD patients and 13.8%, 55.8% and 30.4% in control group, respectively. Differences between groups were not statistically significant ($P>0.05$). The rs2424913 T/C polymorphism was not significantly associated with CAD.

Conclusions

The results of this study indicate that there is no significant association between rs2424913 polymorphism of DNMT3B gene and an increased risk of CAD, so it cannot be considered as a predictor of CAD. Further studies with more sample size are needed to more precisely and thoroughly determine the role of genetic variations of DNMT3B gene in the pathogenesis of CAD

doi: 10.1016/j.cca.2022.04.527

T061

Initial values of B – Type natriuretic peptide and high sensitive troponin – I in patients with acute myocardial infarction with and without ST – segment elevation

D. Ristic Georgijev[c], J. Zdravkovic[c], M. Rasic Popovic[b], K. Zdravkovic[a]

[a] *Health Center Vranje, Serbia, Daily Chemiotherapy Hospital, Serbia*
[b] *Health Center Vranje, Serbia, Department of Internal Medicine, Serbia*
[c] *Health Center Vranje, Serbia, Department of Laboratory Medicine, Serbia*

Background-aim

The biomarker of choice in use for diagnosis of acute myocardial infarction (AMI) is troponin (cardiac specific isoforms cTnI and cTnT), as a marker of myofibrillar damage and necrosis of cardiomyocytes.

The B-type natriuretic peptide (BNP), as a cardioendocrine marker, is secreted by cardiomyocytes in response to tension and stretching of the ventricular wall. Myocardial ischemia is a fully autonomous mechanism for BNP gene expression in the myocardium, independent of left ventricular dysfunction, leading to an early plasma BNP level increase in patients with AMI.

The aim of this study was to investigate whether there is a difference in the initial increase of BNP and high sensitive troponin-I (hsTnI) between patients with acute myocardial infarction with and without ST-segment elevation (STEMI and NSTEMI) in the early phase of AMI.

Methods

This retrospective study included 65 patients admitted to the coronary care unit, HC Vranje. A diagnosis of AMI was created based on current medical recommendations. The first group of 29 patients had STEMI (44.6%) and 36 patients were diagnosed with NSTEMI (55.4%). Blood sampling for BNP analysis was performed within 24 hours of admission and hsTnI was analyzed at the patient admission.

Results

The level of hsTnI was statistically significant higher in STEMI group (Me = 5510.0 (448.9-78642.0 pg / mL)) than in NSTEMI group (Me = 760.6 (25.0-32647.1 pg / mL)) ($p < 0.0005$). The increase in hsTnI levels is proportional to the size of myocardial necrosis, therefore were the initial values of hsTnI significantly higher in patients with STEMI, which is mainly caused by complete obstruction to coronary artery blood flow. There was no statistically significant difference in BNP levels between NSTEMI and STEMI groups (Me = 360.5 (12.8-4168.8pg/mL) vs. (Me = 334.6 (16.2-4089,5 pg/mL), retrospectively) ($p = 0,48$). Numerous studies have shown a higher increase in plasma BNP levels in early age NSTEMI (due to greater ischemia) than in STEMI.

Conclusions

The initial increased hsTnI level was significant higher in STEMI than in NSTEMI group. The BNP values showed no significant difference between the two observed groups.

Keywords: hsTnI: BNP: NSTEMI: STEMI

doi: 10.1016/j.cca.2022.04.528

T062

Influence of doxorubicin on heart failure parameters

J. Zdravkovic [a], D. Ristic Georgijev [a], K. Zdravkovic [b], M. Rasic Popovic [c]

[a] *Health Center Vranje, Serbia, Department of Laboratory Medicine, Serbia*
[b] *Health Center Vranje, Serbia, Daily Chemiotherapy Hospital, Serbia*
[c] *Health Center Vranje, Serbia, Department of Internal Medicine, Serbia*

Background-aim

Anthracyclines used in breast cancer therapy have an effect on the parameters of heart failure. This toxicity is most commonly manifested after application of higher, cumulative doses. Cardiotoxicity will be confirmed through monitoring of the ejection fraction and monitoring of heart failure biomarkers.

The aim was to determine the cardiotoxicity of doxorubicin in patients with breast cancer who have no cardiovascular disease before treatment.

Methods

The study included 30 patients with pathohistologically proven breast cancer, aged 30-60 years, who were treated with doxorubicin at the Helt Center Vranje. Heart failure parameter brain natriuretic peptide (BNP) were determined before the start of treatment with doxorubicin and then after cycle 1, 2, 4 and 6 months after the start of treatment. The left ventricular ejection fraction (LVEF) was measured before starting the therapy, after cycle 4 and 6 months after the start of treatment.

Results

Doxorubicin administration caused an increase of BNP in 40% of patients after the first cycle ($p < 0.0005$). After the second cycle, 50% of patients had an increase of BNP ($p < 0.0005$). After cycle four, 56% of patients had an elevated BNP ($p < 0.0005$). Six months after the start of doxorubicin treatment, in 86% of patients was registered the baseline level of BNP. The highest increase of BNP levels is recorded in group of patients older than 51 years and after administration of forth cycle ($p < 0.0005$). After completing 4 cycles, LVEF decreased in 63% of patients ($p < 0.0005$). Six months after the start of treatment, 89% of patients had baseline LVEF.

Conclusions

Cardiotoxicity expressed through increased levels of BNP and decreased values of LVEF was registered in study population during the therapy with doxorubicin. Recovery of cardiac function was recorded 6 months after the start of chemotherapy. This result indicates the importance of the cumulative toxic effect of doxorubicin, the correlation of BNP levels with the onset of subclinical cardiotoxicity and diastolic dysfunction, as well as reversibility of cardiac toxicity.

Keywords: doxorubicin; breast cancer; BNP; LVEF; reversible cardiotoxicity

doi: 10.1016/j.cca.2022.04.529

T063

Possible microRNA biomarkers for the diagnosis of hypertension in a South African community

D.M. Matshazi [c], R.T. Erasmus [a], A.P. Kengne [b], S. Hector [c], G.M. Davison [c], T. Matsha [c]

[a] *Division of Chemical Pathology, Faculty of Health Sciences, National Health Laboratory Service and University of Stellenbosch, Cape Town, South Africa*
[b] *Non-Communicable Diseases Research Unit, South African Medical Research Council, Cape Town, South Africa*
[c] *SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical Sciences, Cape Peninsula University of Technology, Cape Town, South Africa*

Background-aim

In this study, we set out to investigate possible differences in miRNA expression profiles of participants placed into three different study groups based on their blood pressure status.

Methods

Whole genome sequencing (WGS) was used to identify circulating miRNAs in the whole blood of 48 gender and age-matched normotensive (n = 12), screen-detected HPT (n = 16) and known HPT (n = 20) female

participants. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to validate the WGS findings.

Results

Using WGS and fold change calculations, miR-1299 and miR-30a-5p were the most significantly differentially expressed miRNAs. The former was significantly upregulated in screen-detected HPT compared to the normotensive group (fold change = 3.38) and this was confirmed by RT-qPCR (fold change = 3.25). On the other hand, WGS showed miR-30a-5p, to be more significantly expressed in known HPT compared to both the normotensive group (fold change = 2.24) and the screen-detected HPT group (fold change = 2.02). RT-qPCR also confirmed this (fold changes = 2.46 and 3.40 respectively). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses indicated involvement of platelet activation, calcium signalling and aldosterone synthesis pathways.

Conclusions

These results indicate that miR-1299 and miR-30a-5p may act as biomarkers of hypertension and may be targeted for diagnosis. Furthermore, their expression may be related to the need to regulate genes and biological pathways that are essential for blood pressure homeostasis as seen in the KEGG pathway analyses results.

doi: 10.1016/j.cca.2022.04.530

T064

Impact of cardiovascular exercise training on apolipoproteins and atherogenic indices of adult Nigerians

S. Usman [b], S. Meludu [c], C. Dioka [b], O. Onyema-Iloh [a], C. Obi-Ezeani [b], I. Onuora [b]

[a] *Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria*
[b] *Department of Chemical Pathology, Nnamdi Azikiwe University, Nnewi, Nigeria*
[c] *Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi, Nigeria*

Background-aim

Cardiovascular disease is a major public health problem & a leading cause of mortality in sub-Saharan Africa especially Nigeria, which has been largely attributed to the decline in physical exercise predisposing people to various forms of chronic ailments in general. The objective of this study was to determine the effect and compare results of cardiovascular exercise training on Apolipoprotein A-1 & B and atherogenic indices (Atherogenic Index of Plasma, Cardiac Risk Ratio & Atherogenic Coefficient) before exercise, four weeks, eight weeks and twelve weeks after exercise.

Methods

This research was a prospective study. Serum concentration of atherogenic indices, apolipoproteins A-I & B of both football playing group (44 male individuals who played football for 40 minutes daily three days per week) and jogging group (48 male individuals who engaged in jogging for 30 minutes daily for 5 days per week) were determined using spectrophotometric & Enzyme Linked Immunosorbent Assay (ELISA) techniques respectively. Ethical approval was obtained from the Nnamdi Azikiwe University Teaching Hospital Research Ethics Committee, Nnewi, Nigeria. All data were expressed as Mean ± Standard Deviation (SD) and analyzed with t-test.

Results

The mean serum level of apolipoprotein A1 was significantly & progressively higher while the apolipoprotein B & atherogenic index of plasma (AIP) level were significantly lower ($p = 0.001$) in both exercise groups all through the exercise duration. In the jogging group, the cardiac risk ratio (CRR) & atherogenic coefficient (AC) levels were significantly lower all through with the exception of the 8 weeks after exercise result while in the football group, the CRR & AC were also significantly lower 4 weeks, 8 weeks & 12 weeks after exercise as compared with the baseline results.

Conclusions

Cardiovascular exercise leads to significant changes in apolipoprotein level, though with better atherogenic indices observed in the jogging group, indicating its ability to better reduce cardiovascular disease risk.

doi: 10.1016/j.cca.2022.04.531

T065

Relationship between serum 25 hydroxy vitamin D and myocardial infarction

N. Baral [a], S. Tamrakar [a], O. Sherchand [a], S.A. Khan [a], P. Karki [b]

[a] *Department of Biochemistry, B. P. Koirala Institute of Health Sciences, Dharan, Nepal*
[b] *Department of Cardiology, B. P. Koirala Institute of Health Sciences, Dharan, Nepal*

Background-aim

World Health organization estimates that there are around 1 billion cases of vitamin D deficiency worldwide. In addition to its well defined role as a major regulator of bone and calcium metabolism, several studies have found associations of vitamin D deficiency with coronary artery disease (CAD) and heart failure, as well as positive correlation with hypertension, metabolic syndrome, atherosclerosis, peripheral arterial disease and cancer.

Scientific studies have shown that cardiovascular morbidity and mortality are 30-50% more in the regions of less sun exposure and mortality from CAD is highest in winter season. People with vitamin D deficiency have 60% greater risk of myocardial infarction than those with adequate levels. In spite of the rising proportions of CAD in Asians, only limited data are available on the relationship between Vitamin D, CAD and endothelial dysfunction. We conducted a study to determine the prevalence of vitamin D deficiency in patients with myocardial infarction and determine the correlation of serum 25-OH vitamin D with other factors (Cholesterol, triglycerides, HDL,LDL, VLDL, glucose, smoking, alcohol).

This study aimed to estimate the vitamin D levels, lipid profile and glucose in patients with Myocardial infarction (MI) and also see the prevalence of vitamin D deficiency in patients with MI.

Methods

It was the hospital based cross sectional study conducted in BPKIHS, Dharan from August 2018- July 2019. We enrolled 148 diagnosed cases

having biochemical markers of MI: Troponin I positive, CK-MB greater than 25U/l. Blood samples were collected in plain vials and test were conducted in Department of Biochemistry BPKIHS. Test for lipid profile was done by semi-automated biochemistry analyzer (Biolyzer100) and LDL-Cholesterol was calculated by Frieldwald Formula, serum glucose was estimated by Hexokinase method using Cobas 311 automated analyzer. Serum 25(OH) was estimated by chemiluminesence method using Maglumi 1000 (SNIBE Co LTD, China), CKMB and CK total was done by optimized kinetic method (IFCC) using Accent 200 analyzer. Qualitative measurement of Troponin I was done by using Paper Immuno-chromatography technique. Baseline characteristics were obtained using a semi-structured questionnaire. Anthropometric measurements were done using standard procedures.

Data was analyzed by SPSS version 11.5. Serum 25 (OH) was categorized as deficient (< 30 ng/ml) and sufficient (ε 30 ng/ml). The Baseline characteristics were analyzed using Chi-square test and pearson's test was used to find the correlation. Ethical clearance was obtained from Institute Review Committee.

Results

Out of 148 cases, 117 (79%) had vitamin D insufficiency. The median (25th, 75th centile of vitamin D and CKMB were 23.76 (19.5, 28.6), 62(37,120) respectively. The mean cholesterol was 145.29 ± 30.6 mg/dL, triglyceride was 204.80 ± 69.21 mg/dL HDL-cholesterol was 35.93 ± 7.52 mg/dL, and glucose was 157.34 ± 74.18 mg/dL. A negative correlation was found between Vitamin D with Body Mass Index, total cholesterol, triglyceride, HDL and glucose, however they were not statistically significant.

Conclusions

The study revealed the high prevalence of vitamin D insufficiency (79%) among the patient of MI. Larger cross sectional and cohort study are required to find causal relationship.

doi: 10.1016/j.cca.2022.04.532

T066

Characterization of genome wide aberrant DNA methylation patterns on LNC-RNA associated genes in hypertensive subjects of South Africa

D.D. Motshwari [d], R.T. Erasmus [a], A.P. Kengne [b,c], T.E. Matsha [d]

[a] *Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town, South Africa*
[b] *Non-Communicable Diseases Research Unit, South African Medical Research Council, Cape Town 7505, South Africa*
[c] *Department of Medicine, University of Cape Town, Cape Town 8000, South Africa*
[d] *SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa*

Background-aim

Epigenetic mechanisms are gaining interest for their contribution in regulation of physiology and disease by changing gene expression. The interplay between DNA methylation and LncRNA has been proposed to be an important player in epigenetic regulation, however its role in hypertension remains unclear. This study aimed to characterize the DNA methylation profile of LncRNA associated genes in hypertensive and normotensive South Africans.

Methods

Whole genome methylated DNA immunoprecipitation sequencing (MeDIP-Seq) in peripheral-blood-derived DNA from age, gender, body mass index (BMI) and ethnicity matched 13 normotensive, 19 known hypertensive (on medication), 16 screen-detected hypertensive individuals was performed. Blood pressure measurements were performed according to the 1999 World Health Organization (WHO) guidelines. Gene ontology analysis was performed to retrieve the functional profile of the gene sets.

Results

We identified 45 significant differentially methylated regions (DMR) in the promoters of LncRNA associated genes in hypertensive subjects compared to normotensive controls. For known hypertension versus normotensive, 20 significant DMR were detected, while for screen-detected hypertension versus normotensive, 21 significant DMR were detected. Four significant DMR were detected between known hypertension and screen-detected hypertension. The gene ontology analysis showed that gene sets were associated with some biological processes which may be involved in blood pressure regulation.

Conclusions

This study provides evidence for the first time from an African population suggesting the possible role of DNA methylation in regulation of genes involved in blood pressure regulation and likely hypertension occurrence. However, these findings still need to be validated in a larger study sample, before further exploration of their applicability for risk evaluation or therapeutic purposes.

doi: 10.1016/j.cca.2022.04.533

T067

Vincristine sulfate inhibits platelet activation via suppressing AKT and MAPK phosphorylation

W. Lu [a], L. Kang [d], J. Sheu [b], R. Chen [c]

[a] *Department of Medical Research, Taipei Medical University Hospital, Taipei, Taiwan*
[b] *Department of Pharmacology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[c] *Division of General Surgery, Department of Surgery, Taipei Medical University Hospital, Taipei, Taiwan*
[d] *Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan*

Background-aim

Thrombus formation, a phenomenon primarily related to increased platelet activation, plays a key role in cardiovascular and cerebrovascular diseases. Antithrombotic agents show considerable benefits for the treatment of thromboembolic diseases; however, these agents still have substantial limitations due to their serious side-effect. Vincristine sulfate, a natural alkaloid derived from the plant Catharanthus roseus (Vinca rorea L.), exhibits multiple biological activities such as anti-inflammatory and antitumor effects. However, whether the role of vincristine sulfate on platelet activation remains unclear. Thus, this study

aimed to investigate the detailed mechanism of vincristine sulfate in platelet activation and thrombus formation.

Methods

In this study, platelet aggregation, flow cytometry, and immunoblotting analysis were used to investigate the effects of vincristine sulfate on platelet activation ex vivo.

Results

This study investigated the effects of vincristine sulfate on platelet activation ex vivo. Vincristine sulfate (10-100 μM) exhibited more potent activity in inhibiting platelet aggregation stimulated by collagen (1 μ/ml) but not thrombin (0.02 U/ml), arachidonic acid (AA, 60 μM), and U46619 (thromboxane A2 analogue, 1 μM). In addition, vincristine sulfate inhibited protein kinase B (also known as Akt), protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs; p38 MAP kinases, extracellular signal-regulated kinase 2 and c-Jun N-terminal kinase 1), and markedly reduced the ATP-release reaction and intracellular calcium mobilization in collagen-activated platelets.

Conclusions

The findings of our study suggest that vincristine sulfate is a potential therapeutic agent for preventing or treating thromboembolic disorders.

doi: 10.1016/j.cca.2022.04.534

T068

Triglycerides concentrations profile after a collection of a new specimen in patients with high levels of triglycerides

G. Loureiro, S. Mena, J.d. Sa, N. Sumita

Grupo Fleury, Brazil

Background-aim

Several studies suggest that elevated triglycerides (TG) levels are a biomarker of cardiovascular (CV) risk. Moreover, high levels (above 1,000 mg/dL) of TG concentration are associated with acute complications (pancreatitis). TG determinations are also subject of great biological and pre-analytical variations. Our aim was to evaluate triglycerides variation following a collection of a new specimen in patients with high levels of TG concentration.

Methods

We retrospectively analyzed all lipid profile samples ordered in outpatient and inpatient settings during a 21-month period (January 2018 to September 2019) in a large laboratory center in Brazil. The samples with high levels of TG (>1,000 mg/dL) were selected and a subsequent tracking of the next collection was performed. The TG results of the new collection samples were then compared to the previous one.

Results

A total of 2,914,948 lipid profile samples were analyzed in the period and 1,612 (0.05%) samples presented high levels of TG concentration (mean TG 1,649 ± 1,001 mg/dL, median age 46.5 (0-93 years), 98.9% aged more than 19 years, 78.4% male). A collection of a new specimen was ordered for 873 patients and 145 (16.6%) samples presented a normal level of TG concentration (mean 125 ± 30 mg/dL) in a median time period of 69 (6-574) days; 154 (17.6%) patients had persistent high level of TG after a new collection (mean TG concentration 2,035 ± 1282) in a median time period of 101 (4-570) days; 574 (65.7%) patients had elevated TG concentrations between 175 and 999 mg/dL after a collection of a new sample.

Conclusions

Most patients (82,3%) presented a reduction in TG concentration below 1,000 mg/dL after a collection of a new specimen. Most of these patients, however, had a TG concentration above the upper limit interval on a new determination. Of note, 16,6% of patients presented normal TG levels after a new collection, suggesting pre-analytical issues or intense treatment. The remaining 17.6% cases that maintain high TG levels possibly are at risk for complications such as pancreatitis due to high levels of TG concentration.

doi: 10.1016/j.cca.2022.04.535

T069

Features of platelet hemostasis in patients with unstable angina and on pump coronary artery bypass grafting

E. Medvedeva, L. Gelis, M. Kaliadka, I. Lazareva, V. Shumovets, T. Ginko, T. Rusak

Republican Scientific and Practical Center "Cardiology", Belarus

Background-aim

To evaluate the features of the state of the platelet hemostasis system in patients (pts) with unstable angina (UA) and on-pump coronary artery bypass grafting (CABG).

Methods

The study involved 56 pts with UA with on-pump CABG. The mean age was 63.2 ± 6.8 years. The average number of affected arteries were 2.8 ± 0.6. CABG was performed on 6.6 ± 1.3 days after admission. The risk on the GRACE scale was 104.7 ± 5.4 points. All pts underwent a complete blood count to determine platelet count, platelet volume (MPV) on Uni-Cell DxH 800 (Beckman Coulter); assessment of coagulation hemostasis. All pts were performed to determine the level of cardiac enzymes, general blood test with determination of the number of platelets, assessment of platelet volume (MPV), assessment of coagulation hemostasis. Platelet function was evaluated on an analyzer Multiplate (ASPI-test, ADP-test).

Results

Upon admission to the hospital in pts with UA while taking antiplatelet agents, hyperaggregation was detected in 39 (68.4%) pts, normoaggregation in 11 pts (19.3%), hypoaggregation in 7 (12.3%) cases.. The initial platelet level was 204*109/l, MPV 8.6 fl, fibrinogen 5.6 g / l, D-dimers 0.56 = 0.14 ng / ml, AT III 98%. On 1-3 days after on-pump CABG we found suppression of platelet aggregation (AUC ADP-test 21U, AUC ASPI-test 16U), a decrease in platelet count to 146*109/l, a decrease in platelet volume (MPV 7.1 fl). On days 5-7, there was an increase in platelet and plasma hemostasis activation: an increase in platelet count to 450 * 109 / L, MPV 8.9f, an increase in induced platelet aggregation (AUC ADP test 45U, AUC ASPI test 39U), fibrinogen to 6, 2 g / l, the level of D-dimer to 0.76 ± 0.19 ng / ml and a simultaneous decrease in the activity of natural anticoagulants (AT-III, protein-C). On the 10th day after on-

pump CABG there was a significant increase in platelet count to 530 * 109 / L, MPV to 9.1f, fibrinogen 6.6 g / L, AUC ADP-test 48U, AUC ASPI-test 56U, and from of them in 18 (31.6%) pts of the AUC ADP-test 78 ± 8.1U, and in 14 (24.5%) of the pts of the AUC ASPI-test 69 ± 5.2U, which significantly exceeds normal values and may indicate the presence of decreased sensitivity to clopidogrel and ASA. The decrease in platelet aggregation to baseline occurs after 1 month of control.

Conclusions

Activation of platelet hemostasis in the postoperative period in on-pump CABG occurs on the 5-7th day of the postoperative period. There is also a significant increase in the degree of platelet aggregation compared to baseline values, which is associated with surgical trauma and activation of the external coagulation pathway. The decrease in platelet aggregation to baseline occurs after 1 month of control.

doi: 10.1016/j.cca.2022.04.536

T070

Features of hemostasis in patients with unstable angina and coronary artery bypass grafting

E. Medvedeva, L. Gelis, M. Kaliadka, I. Lazareva, V. Shumovets, T. Rusak

Republican Scientific and Practical Center "Cardiology", Belarus

Background-aim

To evaluate the features of the state of the platelet hemostasis system in patients (pts) with unstable angina (UA) and off-pump coronary artery bypass grafting (CABG).

Methods

The study involved 56 pts with unstable angina (UA) with on-pump CABG. The mean age was 59,9 ± 7,4 years. The average number of affected arteries were 2,6 ± 0,5. CABG was performed on 6.6 ± 1.3 days after admission. The risk on the GRACE scale was 106,7 ± 5,4 points. All pts were performed to determine the level of cardiac enzymes, general blood test with determination of the number of platelets, assessment of platelet volume (MPV) on UniCell DxH 800 (Beckman Coulter); assessment of coagulation hemostasis. Platelet function was evaluated on an analyzer Multiplate (ASPI-test, ADP-test).

Results

Upon admission to the hospital, the prevailing number of pts with activation of hemostasis. Hyperaggregation was detected in 29 (72.5%) pts, normoaggregation in 7 (17.5%) pts, hypoaggregation in 4 (10%) cases. The platelet level was initially 213*109 /l, MPV 7.6fl, fibrinogen 4.6 g / l, D-dimers 0.350 ng / ml, AUC ADP-test 51U, AUC ASPI-test 49 U. In pts with CABG off pump, the platelet count began to increase from 2 days after CABG to 280*109 / l, MPV to 8.2fl, fibrinogen to 5.3 g/l, D-dimers to 0.56 ± 0.19 ng / ml increased the degree of induced platelet aggregation (AUC ADP-test 60U, AUC ASPI-test 58U), despite ongoing antiplatelet and anticoagulant therapy. On the 7th day after CABG off pump, platelet count was 480*109 /L, MPV increased to 9.2fl, fibrinogen level to 6.3 g/L, D-dimers to 0.70 ± 0.27 ng / ml, increased the degree of induced platelet aggregation (AUC ADP test 69U, AUC ASPI test 66U). These changes were saved by the time of discharge from the hospital. The decrease in platelet aggregation to baseline occurs after 1 month of control.

Conclusions

An increase in platelet aggregation and activation of platelet hemostasis in the postoperative period occurs on the 2nd day with CABG off pump and reaches a maximum on the 7-10th day. The decrease in platelet aggregation to baseline occurs after 1 month of control.

doi: 10.1016/j.cca.2022.04.537

T071

Association of atherogenic indices with 25(OH) vitamin D status

S. Sharma, K. Taneja

Department of Biochemistry, Chacha Nehru Bal Chikitsalya Hospital, New Delhi, India

Background-aim

25 (OH) Vitamin D plays an important role in bone metabolism. A growing body of evidence shows low levels of Vitamin D to be related to increased risk of stroke, myocardial infarction and total cardiovascular events. Atherogenic indices have been used for assessment of risk of cardiovascular disease development. To date there are very few studies evaluating the relationship between atherogenic indices (Casteli Risk Index 1 and 2, atherogenic index of plasma (AIP) and atherogenic coefficient (AC)) with Vitamin D status. Hence the present study was aimed at determining the atherogenic indices (CRI 1and 2, AIP and AC) in Vitamin D deficient subjects and evaluation the relationship between them.

Methods

A cross sectional study was done which included a total of 235 patients attending the Executive Health Checkup of Medanta-The Medicity Hospital. 188 patients were found to be Vitamin D deficient whereas the remaining 47 had sufficient levels. For each subject CRI 1 and 2, AIP and AC was calculated from the routine lipid parameters.

Results

Atherogenic indices CRI 1 and 2, AIP and AC were significantly elevated in Vitamin D deficient subjects as compared to those who had sufficient levels (4.70 ± 1.4 vs 3.93 ± 1.42, $p < 0.001$; 2.90 ± 1.13 vs 2.27 ± 1.43, $p < 0.01$; 0.23 ± 0.2 vs 0.15 ± 0.2, $p < 0.05$; 3.70 ± 1.4 vs 2.93 ± 1.42, $p < 0.001$ respectively). A significant positive correlation of CRI 1 and 2, AIP and AC was observed with cholesterol (TC), triglyceride (TG) and LDL cholesterol ($p < 0.0001$) and significant negative correlation with HDL cholesterol ($p < 0.0001$). Furthermore the occurrence of dyslipidemias (decreased HDL, increased levels of TC, TG and LDL, AIP > 0.11, CRI 1 > 3.5, CRI 2 > 3.3 and AC > 3) was more in Vitamin D deficient subjects than those who had sufficient levels.

Conclusions

25 (OH) Vitamin D levels are closely associated with serum lipids and atherogenic indices CRI 1 and 2, AIP and AC. Vitamin D deficiency may be associated with an increased risk of dyslipidemia in these subjects.

doi: 10.1016/j.cca.2022.04.538

T072

High-sensitivity versus conventional cardiac troponin I in acute myocardial infarction diagnosis

N. Trakas [a], E. Karakou [a], V. Georgakopoulou [c], I. Vylliotou [a], P. Sklapani [b], D. Mermigkis [c], V. Panoutsou [a], X. Tsiafaki [c]

[a] *Biochemistry Department , Sismanoglio General Hospital, Athens, Greece*
[b] *Cytopathology Department, Hygeia-Mitera Hospital, Athens, Greece*
[c] *Department of Respiratory Medicine, Sismanoglio General Hospital, Athens, Greece*

Background-aim

Acute myocardial infarction (AMI) is the most common cause of death in the world. The role of the cardiac troponins in the diagnosis of AMI is very important. Recently, newer assays for cardiac troponin (cTn) have been developed which are able to detect changes in concentration of the biomarker at or below the 99th percentile for a normal population. The objective of this study was to compare the diagnostic performance of a new high-sensitivity cardiac troponin I (hs-TnI) assay to that of conventional cardiac troponin I (TnI) for the diagnosis of AMI.

Methods

94 patients (73.6 ± 16.9 years, 65% male) with AMI and 82 patients with other diseases (no AMI) were enrolled in the study. There were no significant differences in gender and age between the two groups. Serum hs-TnI (three-site sandwich immunoassay-direct chemiluminometric technology) and TnI concentration measurements were performed and compared (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics). hs-TnI and conventional TnI had an upper limit of reference range of 47 ng/L and 40 ng/L, respectively. The diagnostic performance of serum hs-TnI and conventional TnI levels in AMI were evaluated by the Receiver operating characteristic (ROC) curve analysis. Data analysis was performed using the SPSS v.19.0 software. ROC curves were plotted and statistical indexes, such as area under the ROC curve (AUC), sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV), were calculated. P-value < 0.05 was considered to be statistically significant.

Results

The diagnostic accuracy was evaluated using the AUC values of the ROC curve. The AUC, for the diagnosis of AMI, was 0.903 (95% CI: 0.779-0.990) for hs-TnI compared to 0.776 (95% CI: 0.602-0.950) for conventional TnI ($p < 0.05$). hs-TnI and conventional TnI had respectively a sensitivity of 92.3% versus 84.6%, a specificity of 88.2% versus 70.6%, a PPV of 85.7% versus 68.8% and a NPV of 93.8% versus 85.7%, for the diagnosis of AMI.

Conclusions

For the diagnosis of AMI, the diagnostic accuracy of hs-TnI was superior to conventional TnI. However, it is important to note, supported by our results, that an increased hs-TnI concentration alone is not sufficient to make the diagnosis of AMI.

doi: 10.1016/j.cca.2022.04.539

T073

Changes in insulin resistance and subclinical inflammation over a 7-year period in a South African population

S.F.G. Davids [c], T.E. Matsha [d], N. Peer [b], R.T. Erasmus [a], A.P. Kengne [b]

[a] *Department of Chemical Pathology, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town, South Africa*
[b] *Department of Medicine, University of Cape Town, Cape Town, South Africa and Non Communicable Diseases Research Unit, South African Medical Research Council, Cape Town, South Africa*
[c] *Department of Medicine, University of Cape Town, Cape Town, South Africa and SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical Sciences, South Africa*
[d] *SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical Sciences, Cape Peninsula University of Technology, Cape Town, South Africa*

Background-aim

Insulin resistance (IR) and sub-clinical inflammation are involved in pathological pathways leading to the development of biological cardiovascular risk factors and subsequent cardiovascular events. Therefore, monitoring these processes can provide advanced information on the trajectory of cardiovascular risk profile of a population, and inform prevention and control strategies. Herein, we investigated the change in high sensitive C-reactive protein (hs-CRP), Gamma-glutamyl transferase (©-GT) as measures of sub-clinical inflammation and IR in a population from Cape Town, South Africa between 2008/09 and 2014/16.

Methods

In a total of 2503, (n = 797, 2008/09) and (n = 1706, 2014/16) participants from mixed-ancestry communities in Cape Town, plasma glucose, insulin, hs-CRP and ©-GT were measured, and the homeostatic model assessment of insulin resistance (HOMA-IR) and Matsuda index used as an estimator of insulin resistance. Data analysis used linear and logistic regression models, with interaction tests applied to investigate the differential effects of gender on changes in markers levels across years.

Results

In 2008/09 and 2014/16, 620 (77.8%) and 1278 (74.9%) participants were women. The overall mean age of participants was 53.2 (2008/09) and 48.2 (2014/16), respectively. The median estimated IR markers level decreased between the two time-points from 1.60 (2008/2009) to 1.40 (2014/2016) for HOMA-IR ($p = 0.859$) and slightly increased from 7.87 to 8.43 for Matsuda index ($p = 0.685$). Consequently, the prevalence of IR decreased from 10% to 5.78% based on HOMA-IR, and from 9.95% to 7.48% based on Matsuda index (both $p < 0.041$). There was a significant differential effect of gender on those changes (interaction $p = 0.006$, 2008 and $p = 0.015$,2014). Sub-clinical inflammation prevalence increased slightly between 2008/09 and 2014/16, from 54.71% to 57.08% ($p = 0.264$) for CRP, and 29.56% to 33.41% ($p = 0.055$) for ©-GT defined sub-clinical inflammation.

Conclusions

The findings indicate that insulin resistance levels have significantly decreased while sub-clinical inflammation mostly remained stable in this mixed ancestry population. This could be accounted for at least in part by the much younger age of the study population during the second survey.

doi: 10.1016/j.cca.2022.04.540

T074

Omega-3 index: new approaches in laboratory diagnostic

V. Yurasov, I. Mamedov, I. Zolkina, N. Polovkov, Z. Starkova, A. Sadykov

Scientific laboratory "Chromolab", Russia

Background-aim

Omega-3 Index reflects the relative amount of polyunsaturated omega-3 fatty acids (PUFA) within red blood cell membranes (RBC) and evaluated as a ratio of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) sum to total amount of fatty acids, currently is being considered as a relevant physiological marker of the sudden cardiac death risk. As shown earlier, low Omega-3 Index is associated with increased risk of cardiovascular disease (CVD). The purposes of this study were to introduce Omega-3 index in routine and large-scaled clinical practice through replacing RBC with whole blood. Besides the aim of the research is to ensure that Omega-3 Index includes all key and significant Omega-3 PUFAs and the ranges adequately correspond to desirable target value for the Omega-3 Index. In addition, we have identified the effects of age and gender on the Omega-3 Index in population.

Methods

Whole blood samples were obtained from 20-70 years old males (n = 1395) and females (n = 2967). The content of PUFA in the blood was determined by using gas chromatography (GC) with flame ionization detector (Agilent GC-7890B). The method involved the preliminary derivatization by acetyl chloride followed by extraction with hexane. Omega-3 Index was expressed as the percentage to total amount of fatty acids.

Results

The pool of PUFAs in whole blood adequately reflects their content in RBC. The intermediate metabolite of EPA and DHA – docosapentaenoic (DPA) was found as a «lost» component. Analyzed Omega-3 Index frequencies distribution in the population were in opposite with traditional representations for so-named adequate Omega-3 Index level (> 8%). The share of people in the desirable «canonic» interval (> 8%) remains few at only 5,5%. Most people (58%) fall in the range of 4-8%.

Conclusions

Whole blood along with RBC membranes is a clinically valid and less complicated biomaterial for measuring the Omega-3 Index. DPA is essential for Omega-3 Index estimation too. The desirable interval border of the Omega-3 Index must be revised for shifting downward 8%. And finally, the Omega-3 Index is dependent only on age but not gender.

doi: 10.1016/j.cca.2022.04.541

T075

Telomere length and serum uric acid level in hypertensive patients according to the 10-year prospective randomized study

O. Podpalova [b], M. Kaliadka [b], A. Babenka [a], V. Tsimashkova [b], I. Generalov [c], A. Mrochek [b]

[a] *Belarusian State Medical University, Belarus*
[b] *Republican Scientific and Practical Centre "Cardiology", Belarus*
[c] *Vitebsk State Medical University, Belarus*

Background-aim

The aim of the study was to investigate the association between level of serum uric acid and telomere length in 10-year prospective randomized study of Belarusian urban population.

Methods

A 10-year prospective study started in 2007/2008 and included 3,500 persons selected by the random number method from polyclinics N° 3 and N° 6 in Vitebsk. In 2012/2013 according to the results of the second screening we examined 2888 people. In 2017/2018 a 10-years prospective analysis included 145 patients with new cases of hypertension that was revealed in the second screening. Examination of these subjects included standard questionnaires for detection of cardiovascular risk factors, measurements of blood pressure, blood chemistry (Architect c4000, Abbott), electrocardiography, echocardiography, ultrasound examination of brachiocephalic vessels, real time PCR for the detection telomere length of peripheral blood lymphocytes. Real time PCR conducted with CFX96touch (Bio-Rad, USA) and Maxima SYBR Green/ROX qPCR Master Mix 2X (Thermo, USA, Cat.K0222) according to manufacturers instructions and recommendations. For telomere length quantification used specific oligonucleotides and principle, provided by Cawthon R.M., 2002.

Results

2171 subjects with normal blood pressure and 1257 subjects with hypertension were examined in the first screening. In 2012/2013 we established 286 new cases of hypertension (14,4% of men and 12,2% of women). Multifactorial regression analysis showed significant positive relationship between new cases of hypertension and IV quartile of serum uric acid level (339-527 μmol/l) (df = 1; |2Wald = 5,1; p < 0,05) as well as other risk factors, adjusted for age and sex, in 5 years. According to regression analysis of 145 randomized subjects of 286 new cases of hypertension we revealed that the IV quartile of uric acid level (339-527 μmol/l), determined in the first screening, was also associated with the I quartile of telomere length adjusted for age and sex in 10 years (df = 1; |2Wald = 4,5; p < 0,05) .

Conclusions

Serum uric acid level ε 339 μmol/l was a risk factor for development of hypertension in randomized urban population in 5-year prospective analysis and detecting lower telomere length in patients with new cases of hypertension in 10-year prospective analysis.

doi: 10.1016/j.cca.2022.04.542

T076

The significance of elevated baseline high sensitive troponin I in middle-aged patients with atrial fibrillation

V. Koleva [a], A. Borizanova [b], E. Kinova [b], A. Goudev [b]

[a] *Clinical Laboratory Department, Acibadem Cityclinic Tokuda Hospital, Sofia, Bulgaria*
[b] *Department of Cardiology, UMHAT " Tsaritsa Yonna", Medical University, Sofia, Bulgaria*

Background-aim

Dynamic high-sensitivity troponin (hsTn) elevations in atrial fibrillation (AF) patients are associated with significant coronary artery disease (CAD) or with increased all-cause mortality in patients without CAD. However, the significance of increased baseline hsTn levels in these patients remain unclear.

Aim: To investigate baseline levels of hsTnI in middle-aged patients with new-onset and recurrent episode of AF.

Methods

Sixty consecutive middle-aged patients (52 ± 9 years) were divided into 3 groups: new onset of AF (n = 15), recurrent AF (n = 24) and healthy controls (n = 21). All subjects underwent 2D echocardiography with speckle tracking analysis 1 month after restoration of sinus rhythm and hsTnI levels were tested.

Results

There were significant differences between 3 groups (new onset AF, recurrent AF, healthy controls) in: LV global longitudinal strain (GLS) (-20,60 ± 2,93% vs. -21,33 ± 2,91% vs.-21,33 ± 2,91%, p < 0,011) ; LA maximal volume(59,52 ± 14,32ml vs. 68,00 ± 25,14ml vs. 38,13 ± 10,09ml, p < 0,001) and stiffness index (0,33 ± 0,15 vs. 0,42 ± 0,19 vs. 0,16 ± 0,03; p < 0,001) ; LA reservoir strain (31,75 ± 8,13% vs. 26,25 ± 11,88% vs. 44,7 ± 9,27%, p < 0,001) and contractile strain (-7,39 ± 2,91% vs. -7,55 ± 3,33% vs. -12,10 ± 3,10%, p < 0,001). Baseline hsTnI levels were significant elevated only in patients with recurrent AF in comparison to new onset AF and healthy controls (2,04 ± 2,13 pg/ml vs. 1,28 ± 1,06 pg/ml vs. 0,49 ± 0,38 pg/ml, p < 0,004) and there was a positive correlation with LA stiffness index (r = 0,475, p < 0,001) and an inverse correlation with LA reservoir strain (r = -0,401, p < 0,001) . There was no correlation between hsTnI and LV GLS. Multivariable regression analysis demonstrated that BMI (B = 0,101;p = 0,001), LA reservoir strain (B = -0,018;p = 0,013) and hsTnI (B = 0,112; p = 0,031) are predictors for recurrent AF with 57,7% (adjusted R^2) prediction of this model.

Conclusions

Reduced LA reservoir strain and increased baseline hsTnI in middle-aged obese patients with new- onset AF could be used as one of the predictors for recurrent episode. Ongoing atrial remodeling with myocyte loss through apoptosis and necrosis could be the underlying mechanism.

doi: 10.1016/j.cca.2022.04.543

T077

Laboratory-specific equation for calculated serum LDL concentration

R. Choi, M. Park, Y. Oh, S.H. Kim, S.G. Lee, E.H. Lee

Laboratory Medicine, Green Cross Laboratories, Yongin, Republic of Korea

Background-aim

Various equations for calculated low-density lipoprotein cholesterol (LDL) from a lipid profile test that includes measurements of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) have been proposed. The aim of this study was to develop a new equation for LDL calculation and validate it in comparison with other equations and LDL concentration directly measured in a Korean adult population.

Methods

We developed a new equation for calculated LDL estimation based on retrospectively reviewed clinical lipid profile test results (TC, HDL, TG, and LDL) performed between July 2017 and September 2018 in Korean adults. We validated the accuracy of the developed equation in comparison with 11 other equations and directly measured LDL using an independent cohort data from the same laboratory between September and November 2018 and other data from Korea National Health and Nutrition Examination Survey (KNHNES) 2017.

Results

We developed a new equation for calculating LDL using 5,204 lipid profile results. The developed equation was as follows: estimated LDL = TC - 0.8735 x HDL - 0.1347 x TG. We validated the developed equation using an independent cohort with 2,238 lipid profile results and 907 data points from KNHNES 2017. Among the 12 equations, in the validation cohort of 2,238 results, the developed equation showed the lowest mean systemic difference (-0.6 mg/dL, SD 6.2 mg/dL) and median absolute error (3.4%, 95% CI 3.2-3.5%). However, in the KNHNES 2017 cohort, the DeLong equation [estimated LDL = TC – (HDL + 0.16 x TG)] showed the lowest mean systemic difference (-0.4 mg/dL, SD 13.8 mg/dL). For samples with high TG concentration (> 400 mg/dL) in the KNHNES 2017 cohort, the Vujovic equation [estimated LDL = TC – HDL -(TG/6.58)] showed the lowest mean systemic difference (-2.2 mg/dL, SD 26.6 mg/dL) and median absolute error (14.0%, 95% CI 11.0-19.0%).

Conclusions

Considering that the accuracy of equations varied according to cohort and TG concentration, assay-specific equations for LDL calculation may be needed to estimate calculated LDL, and/or direct measurement of LDL is needed. Future studies are needed to identify their clinical significance in a Korean population.

doi: 10.1016/j.cca.2022.04.544

T078

Evaluation of the new Beckman Coulter access HS-TNI: 99th Percentile upper reference limits according to age and sex in the Korean population

S. Yoo [a], S. Kim [b], J. Kim [c]

[a] *Department of Laboratory Medicine, Inje University College of Medicine, Sanggye Paik Hospital, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, Inje University College of Medicine, Ilsan Paik Hospital, Goyang, Republic of Korea*
[c] *Health Promotion Center and Department of Family Medicine, Inje University College of Medicine, Sanggye Paik Hospital, Seoul, Republic of Korea*

Background-aim

Defining the 99th percentile upper reference limits (URL) of cardiac troponin I (cTnI) is critical because it is clinically important in determining myocardial injury, and is variable among reagents, age, sex, or races. The analytical performance of the new, high- sensitivity reagent Beckman Coulter Access hsTnI was evaluated, and its 99th percentile URL was determined in the Korean population.

Methods

Analytical performances of Access hsTnI assay were evaluated including imprecision and limit of measurements. To define the reference limits, 600 healthy subjects were included with similar proportions of sex and age groups.

Results

Beckman Coulter Access hsTnI assay presented a limit of blank, detection, and quantitation at 10% CV of 1.7 ng/L, 2.3 ng/L, and 5.6 ng/L, respectively, and 98.3% of the healthy population showed cTnI above the limit of detection. The 99th percentile URLs were 9.5 ng/L (90% CI 7.4–14.9), 7.8 ng/L (90% CI 5.5–19.2 ng/L), and 11.3 ng/L (90% CI 8.0 - 15.7 ng/L) in 600 healthy participants, 300 women and 300 men, respectively with imprecision less than 5% CV. The median values and 99th percentile URLs of hsTnI were higher in men and the age group ε50 years than in women and the age group <50 years, respectively.

Conclusions

Access hsTnI assay met the performance criteria of the IFCC for high-sensitivity cTnI assays. Their 99th percentile URLs in the Korean population were lower than the manufacturer's claims. cTnI values were significantly different among different sex and age groups.

doi: 10.1016/j.cca.2022.04.545

T079

High sensitive troponin assays in differentiating acute coronary syndrome from healthy individuals

Y. Kim, S. Kim, H.K. Lee, J. Lee, H.J. Kwon

Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea

Background-aim

Cardiac troponins have been used as a diagnositc marker for acute myocardial infarction (AMI). In this study, we examined high sensitive troponin T and I as a marker to differentiate the patients with angina from healthy individuals.

Methods

A total of 730 patients (19 AMI, 41 stable angina, 49 unstable angina, 621 healthy individuals) were included. All patients had glomerular filtration rate of ε 60 ml/min. Blood draw in the patients with angina or AMI was done before coronary angiography. Serum concentrations of high sensitive TnT (hs-TnT, Roche), high sensitive TnI (hs-TnI, Abbott), and high sensitive TnI (hs-TnI, Beckman) were exaimend.

Results

The mean concentrations of hs-TnT, hs-TnI (Abbott), and hs-TnI (Beckman) in healthy individuals were 3.8 pg/ml, 2.4 pg/ml, and 2.4 pg/ml. The mean concentrations of hs-TnT, hs-TnI (Abbott), and hs-TnI (Beckman) in the patients with stable angina were 9.0 pg/ml, 5.9 pg/ml, and 5.2 pg/ml. The mean concentrations of hs-TnT, hs-TnI (Abbott), and hs-TnI (Beckman) in the patients with unstable angina were 19.5 pg/ml, 72.3 pg/ml, and 15.1 pg/ml. The mean concentrations of hs-TnT, hs-TnI (Abbott), and hs-TnI (Beckman) in the patients with AMI were 781.5 pg/ml, 5826.4 pg/ml, and 2998.7 pg/ml.

Conclusions

High sensitive troponins could be a useful marker for the differentiation of angina from healthy individuals.

doi: 10.1016/j.cca.2022.04.546

T080

A dual marker approach for rapid rule out of myocardial infarction in acute chest pain patients

M.R. Kulshrestha [a], V. Tiwari [a], B.C. Tiwari [b], A. Raj [c]

[a] *Department of Biochemistry, DR Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India*
[b] *Department of Cardiology, DR Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India*
[c] *Department of Pathology, DR Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India*

Background-aim

With the development of hsTnI assays, most patients with an Acute Myocardial Infarction (AMI) can be reliably identified within 3 hour after admission. However, in patients with 3 hour values of hsTnI unchanged, but in whom pre-test likelihood of AMI is high, additional subsequent sampling (e.g., at 2 or 3 h) may still be advisable.

Recent studies suggest that Heart type Fatty Acid Binding Protein (H-FABP) has high sensitivity (95-100%) and better specificity (68-80%) than CK-MB and myoglobin. Its combination with the well-established markers like hsTnI may reliably rule out AMI at admission.

Methods

The study included 80 consecutive patients who had acute chest pain for less than 6 hours suggestive of coronary origin at the discretion of an emergency department physician assessment. The second sample was taken 3 hours later. The hsTnI levels were estimated on these samples by Electro-chemiluminescence Immunoassay (ECLIA) using commercial kits on Access 2 (Beckman Coulter, USA). H-FABP levels were analyzed by Enzyme Linked Immune-Assay (ELISA) using kits from Biovendor, Czech Republic. The final diagnosis was established by cardiologist's panel was taken as confirmatory.

Results

The dual marker approach using hsTnI values above Upper Reference Limit (URL) and HFABP ε 6.38 ng/ml at zero hour (at admission) was comparable (sensitivity 95.5%; NPV 97.4%) to double sampling approach (sensitivity 90.9%; NPV 96.6%) i.e. Two samples of hsTnI at zero hour and 3 hours (post admission) for rule out of AMI. But specificity of the former approach remains lower (63.8%) in comparison to the double sampling approach (98.3%). We further noticed raising the cut threshold of hsTnI to 29.45 ng/L (as indicated by ROC curve) increases the specificity (79.3%) with similar rule out ability (sensitivity 95.5%; NPV 97.9%).

Conclusions

A dual marker approach may significantly reduce the observation time and relieve the bed occupancy in emergency department. It may obviate the need of double sampling in the patients who had been ruled out at the time of admission itself.

doi: 10.1016/j.cca.2022.04.547

T081

Relationship between eryptosis and biological markers in cardiac surgical patients

A.V. Zubko, A.D. Vasilenko, A.A. Kupryashov, M.V. Lukich, O.L. Bockeria

A.N. Bakulev National Medical Research Center of Cardiovascular Surgery, Moscow, Russia

Background-aim

Purpose of study is to specify relationship between eryptosis and biological markers in cardiac surgical patients.

Methods

The authors have studied peripheral venous blood samples obtained from patients (N = 41) undergone cardiac surgery for prosthetics of the heart valves in cardiopulmonary bypass, at six control points: 1st point - before surgery, 2nd point - 30 minutes after clamping the aorta during the artificial blood circulation, 3d point - completion of the surgery, 4th point - 1 day after the surgery, 5 th point - 3 days after the surgery, 6 th point - 5 days after the surgery. To assess the degree of eryptosis, the Annexin V cytometric marker was used.

Results

The degree of eryptosis directly depends on the partial pressure of oxygen in the arterial blood (pO2): the higher the pO2, the lower the degree of eryptosis, moreover, with oxygen saturation of the arterial blood (sat O2) this relationship is less pronounced. The greatest effect on the severity of erythosis pO2 produces 30 minutes after clamping the aorta during the cardiopulmonary bypass.

The concentration of sodium (Na+) after 30 minutes from clamping the aorta during the cardiopulmonary bypass is inversely proportional to the degree of eryptosis before surgery.

The glucose level in the middle of the surgery is directly related to the degree of eryptosis on the third day after the surgery.

The concentration of albumin (Alb) and degree of eryptosis is two-fold: degree of eryptosis by the fifth day after the surgery is in direct dependence the on level of Alb at the end of the surgery, however, during this period the concentration of Alb is as lower as higher the degree of eryptosis. That is, the increased degree of eryptosis before and after surgery is the opposite of the concentration of Alb in the blood.

Similarly, the increased degree of eryptosis before and after surgery correlates with the increased level of creatinine and total protein on the fifth day after the surgery, and total bilirubin at the time of completion of the surgery. Moreover, a low concentration of total protein is associated with an increased degree of eryptosis.

Conclusions

Further study of the interconnections of the level of eryptosis and biological markers will determine its clinical significance.

doi: 10.1016/j.cca.2022.04.548

T082

Polymorphism (G83A) renin gene is associated with telomere length and arterial stiffness in patients with hypertension

O. Pavlova[b], S. Ogurtsova[a], A. Gorkavaya[a], I. Karabko[b], I. Russkikh[b], M. Kaliadka[b], A. Mrochek[b]

[a] *Institute of Bioorganic Chemistry of National Academy of Sciences, Minsk, Belarus*
[b] *Republican Scientific and Practical Centre «Cardiology», Minsk, Belarus*

Background-aim

The available evidences suggest that genetic polymorphism of the renin-angiotensin-aldosterone system (RAAS) associated with the changes of activity this system in blood and tissue. Telomere length (TL) shortening is a main mechanism underlying the complex process of cell senescence, indicating the higher risk of cardiovascular disease development. Arterial stiffness is an independent marker of the early vascular ageing.

Objective. To determine the association of polymorphism (G83A) renin gene with leukocyte TL and arterial stiffness in hypertensive patients.

Methods

The clinical, laboratory and genetic analysis examination, were performed in 91 people (44 hypertensive patients and 47 normotensive individuals). Mean age of hypertensives was 55.9 ± 9.4 and of normotensives was 49.6 ± 9.9 years. Relative TL (RTL) of peripheral blood leukocyte was measured using real-time polymerase chain reaction. Telomerase activity was determined by immunoassay. Arterial stiffness performed by volume sphygmography with assessing cardio-ankle-vascular index (CAVI) and ankle-brachial index.

Results

The distribution of G and A alleles of polymorphism (G83A) renin gene was similar in hypertensive patients – 73.9 and 26.1% in comparison with normotensive individuals – 75.5 and 24.5%. In group of hypertensive patients with GA and AA genotypes mean RTL telomere length was shorter than hypertensives with GG genotypes (G83A) gene REN (0.78 ± 0.17 versus 0.94 ± 0.08; P = 0.02). CAVI on right and left were significantly greater in hypertensive patients with mutant A allele (8.54 ± 0.93 and 8.34 ± 0.91) compared to patients with G allele (7.69 ± 1.21 and 7.58 ± 1.17; p = 0.02 and p = 0.03). There were no association polymorphism (G83A) renin gene with RTL and parameters of arterial stiffness in group of normotensives.

Conclusions

The presence of mutant A allele polymorphism (G83A) of gene REN compared with G allele was associated with leukocyte TL shortening as well as an increasing of arterial stiffness in hypertensive patients.

doi: 10.1016/j.cca.2022.04.549

T083

Role of inflammatory markers in the identification of prosthetic endocarditis

M. Kaliadka, A. Lukashevich, S. Spiridonau, R. Zhmailik

Republican Scientific and Practical Center "Cardiology", Belarus

Background-aim

Assess the significance of inflammatory markers in the diagnosis of prosthetic endocarditis.

Methods

The study included 60 patients with suspected endocarditis of the heart valve prosthesis. The method of "steam" is divided into 2 groups. Patients of both groups are comparable by sex, age (p = 0.210356), weight and height indicators (p = 0.129654). In the first group of 30 patients with prosthetic endocarditis who underwent standard laboratory diagnostics (determination of the number of leukocytes, stab neutrophils UniCel DxH800 Coulter (Beckman Coulter), ESR, procalcitonin (Architect i2000 (Abbot)) and presepsin (Pathfast, Mitsubishi). In the second group, 30 patients operated on for prosthetic endocarditis, who underwent the diagnostic procedure 18FDG PET-CT to standard laboratory diagnostic methods (before surgery, 1, 7, 14 days). Compared inflammatory markers in both groups and their impact on the diagnosis of prosthetic endocarditis.

Results

In patients with prosthetic endocarditis and without signs of inflammation (according to PET data), indicators of procalcitonin (U = 5.000, p = 0.366157), prespepsin (U = 11.0000, p = 0.522817), stab neutrophils (U = 11.500, p = 0.583883), white blood cells (U = 9.0000, p = 0.395092) there is no significant difference in the groups.

Conclusions

Changes in the blood were nonspecific and required additional examination (in the preoperative period, the implementation of FDG PET CT, blood culture for blood culture) for the final diagnosis and in the postoperative period, the results of a pathological study were taken into account.

doi: 10.1016/j.cca.2022.04.550

T084

Glomerular filtration rate but not uric acid and galectin-3 levels is associated with reverse left atrial remodelling following catheter ablation of atrial fibrillation

V. Koleva [a], V. Konstantivova [b], R. Radoslavova [b], D. Boychev [b], E. Puncheva [a], I. Zheleva [b], V. Gelev [b], V. Traykov [b]

[a] *Clinical Laboratory Department, Acibadem Cityclinic Tokuda Hospital, Sofia, Bulgaria*
[b] *Department of Cardiology, Acibadem Cityclinic Tokuda Hospital, Sofia, Bulgaria*

Background-aim

Catheter ablation is an established treatment modality for patients with paroxysmal or persistent atrial fibrillation (AF). Left atrial (LA) remodelling frequently occurs following the procedure. We aimed to study the significance of several routine clinical scores, laboratory tests and Galectin-3 levels for LA reverse remodelling following catheter ablation of AF.

Methods

We included 72 consecutive patients with AF [41 males, 58.97 ± 8.6 years, paroxysmal in 63 (87%)] indicated for catheter ablation at a single centre. Two-dimensional echocardiography with speckle tracking was used to measure LA size, LA volume index (LAVI), peak systolic LA strain and LA emptying fraction (LAEF) before and 30 days after the procedure. Clinical parameters -CHADS-VASc score, history of coronary artery disease, moderate/severe mitral regurgitation and history of thyroid disease were analysed. Galectin-3 was studied as a marker of LA fibrosis. Among routine laboratory tests uric acid levels and estimated glomerular filtration rate (eGFR) were analysed. LA reverse remodelling was defined as any of the following: improvement in LAEF or LAVI reduction.

Results

Overall, LA reverse remodelling at 30 days following a single catheter ablation procedure occurred in 54 patients (74.6%). There were no significant differences in the CHADS-VASc score and any other clinical variables among patients with and without LA reverse remodelling. Uric acid and Galectin-3 levels were not significantly different in the patients who demonstrated LA reverse remodelling compared to the rest of the cohort: 324.72 ± 88.81 vs. 348.38 ± 94.73 μmol/l, p = 0.37 and 15.24 ± 5.28 vs. 15.80 ± 4.65 ng/ml, p = 0.71, respectively. Patients demonstrating LA reverse remodelling had higher eGFR values: 83.0 ± 17.24 vs. 71.94 ± 15.4 ml/min/1.73m2, p = 0.026; (AUC = 0.694, p = 0.021).

Conclusions

LA reverse remodelling following catheter ablation of AF is more likely to occur in patients with higher glomerular filtration rate and is independent of uric acid levels and the presence of LA fibrosis as assessed by Galectin-3 levels.

doi: 10.1016/j.cca.2022.04.551

T085

Establishment of a sustainable measurement infrastructure for standardised measurement of cardiovascular disease biomarkers within the cardiomet consortium

C. Swart[g], M. Weller[b], V. Delatour[e], M. Quaglia[f], M. Öztug[i], A. Gallo[d], H. Schwalbe[c], C. Cobbaert[a], A. Reid[j], A. Kessler[h], G. Nordin[k]

[a] *Academisch Ziekenhuis Leiden, the Netherlands*
[b] *Bundesanstalt für Materialforschung und -prüfung, Germany*
[c] *Goethe Universität Frankfurt, Germany*
[d] *Inst Cardiometabolisme Nutrition ICAN, France*
[e] *Laboratoire national de métrologie et d'essais, France*
[f] *LGC, France*
[g] *Physikalisch-Technische Bundesanstalt, Germany*
[h] *Stiftung für Pathobiochemie und Molekulare Diagnostik, Germany*
[i] *Turkiye Bilimsel ve Teknolojik Arastirma Kurumu, Turkey*
[j] *UK-NEQAS Cardiac Markers, UK*
[k] *Uppsala Universitet, Sweden*

Background-aim

Cardiovascular diseases remain one of the main challenges for health care systems in the EU. Cardiac biomarkers help to confirm the diagnosis, provide prognostic information and, thus, enable successful treatment. Analysis of patient blood samples must be undertaken by accredited laboratories, each of whom uses a variety of measurement devices, which must, in turn, be calibrated to ensure accuracy, reliability and comparability between laboratories.

Methods

Liquid chromatography mass spectrometry (both elemental and molecular), immunoassays, biosensors

Results

Within the framework of the European Metrology Programme for Innovation and Research (EMPIR) leading European Metrology Institutes, universities and hospitals in the field of cardiovascular diseases have now combined their efforts in the CardioMet consortium.

So far progress has been achieved in the development of reference measurement procedures for the traceable quantification of:

- Apolipoproteins: Apolipoproteins and other advanced lipoprotein tests offer another route to evaluate CVD risk and to enable a more personalised treatment of patients. The creation of SI-traceability of apolipoprotein test results based on isotope dilution mass spectrometry (IDMS) is ongoing and will allow equivalence of test results in time and space and unconfounded evaluation of their clinical utility and impact on patient outcome.
- Cardiac troponin (cTn): acts as a biomarker for coronary heart diseases. Based on the efforts already undertaken by the International Federation of Clinical Chemistry and Laboratory Medicine a complementary and more sensitive method will be developed using IDMS for the quantification of cTn at very low concentrations in clinical samples. Additionally, we work on the development of a biosensor for the quasi-continuous monitoring of cTn.
- B-type natriuretic peptides (BNPs) including the development of IDMS approaches targeting the biomarkers NT-proBNP, 1-32 BNP and its metabolites as especially important biomarkers for the assessment of the status for heart failure.

Conclusions

This EMPIR project will help to standardise and improve commercially available quantification methods by establishing reference methods for biomarkers in serum/plasma such as apolipoproteins and cTn for cardiovascular disease and BNP for heart failure.

doi: 10.1016/j.cca.2022.04.552

T086

Risk stratification with novel biomarkers of adult patients with reduced ejection fraction undergoing open heart surgery

V. Shumavets, M. Kaljadka, I. Russkih, Y. Ostrovsky

Belarus Cardiology Centre, Minsk, Belarus

Background-aim

Novel biomarkers now exist to potentially improve preoperative stratification models. We aimed to assess the expression and significance of novel biomarkers perioperatively in open heart surgery in patients with low ejection fraction (EF).

Methods

358 patients with depressed left ventricle (LV) function (EF < 35%) and functional mitral regurgitation (MR) scheduled for elective surgery were included in the prospective study. Cardiomyopathy was ischemic (CAD) in 301 or idiopathic (IDCP) in 57 patients. Patients underwent either combined coronary artery bypass grafting (CABG) with mitral valve procedure (193 patients) or isolated mitral-valve repair or chordal-sparing replacement (94 patients) consequently. Plasma levels of cardiac biomarkers (ST2, NT-proBNP, hs-cTnI, Galectin 3, IL-6 and CRP) were measured preoperatively and at 1st, 7th and 30th postoperative days.

Results

LV EF were significantly worse in patients with IDCP compared with CAD (28 ± 4,4 % vs. 36 ± 3,9 %, p = 0,024). Higher baseline levels of sST2 were observed in patients with CAD (31,71 (21,5:50,26) vs. 24,09 (18,4:38,31), p = 0,034). Postoperatively bi-phases acute changing of sST2 level was detected. There were statistically significant increases for sST2 level at first postoperative day regardless from aetiology (28,6 (20,1:42,01) vs. 255 (155,5:382,4), p = 0,001). Then significant decreasing between first and 7th days were detected (p = 0,001) with median sST2 level – 64,47 (39,3:90,29). sST2 level on 30th day decreased (median 39,53 (27,7:60,91) but still was significantly elevated in comparison with preoperatively (p = 0,011). No significant correlation was detected between sST2 preoperatively and on postoperative days 1 and 7, with exception on postoperative day 30 (r = 0,658, p = 0,001).sST2 level higher 45 ng/ml was identified as independent predictors for cardiac-related complication after open heart surgery (OR – 5,345 (95% CI 3,6-9,78, p = 0,01).

Conclusions

Following isolated valve surgery or combined with CABG due to severe functional MR, ischemic and non-ischemic patients exhibited biphases acute changes in plasma sST2 levels with up to 10-fold increase immediately after operation. Preoperative level of sST2 compared with

NT-proBNP and hs-cTnI can be used to identify patients at increased risk of postoperative complications.

doi: 10.1016/j.cca.2022.04.553

T087

Research of immature platelets and aggregation in cardiological practice

E. Kryukov [b c], S. Kazakov [b,c], P. Shakhnovich [a], G. Tagirova [a], L. Shibina [d]

[a] *Burdenko Main Military Clinical Hospital of Ministry of Defense, Moscow, Russia*
[b] *Burdenko Main Military Clinical Hospital of Ministry of Defense, Moscow, Russia*
[c] *Russian medical Academy of Continuing Professional Education of Ministry of Health, Moscow, Russia*
[d] *Ltd «Sysmex Rus», Moscow*

Background-aim

To determine the diagnostic value of platelet parameters and their aggregation in patients with coronary heart disease with different therapeutic strategies, as well as in dynamics in the performance of percutaneous coronary intervention (PCI).

Methods

The studies were conducted in groups of healthy donors (n = 20), patients with ischemic heart disease (n = 52), including patients with the conservative strategy (n = 32) and patients with invasive strategy (n = 20), who were conducted PCI. The PCI group was divided into subgroups: before the intervention and 4, 24 and 72 hours after the intervention. The design of the study included the determination on the hematological analyzer Sysmex XN 1000 using the PLT-F channel of quantitative characteristics of platelets: the relative and absolute number of immature platelets IPF (%, 10^3 /μl), the percentage of large platelets (PLC-R).Platelet aggregation was investigated using an impedance method on the Chrono-Log (USA) model 590 with ADF and collagen inducers. The reliability of the differences was determined by the Wilcoxon criterion, a critical level of significance of $p < 0{,}05$. The data was presented in the form of Me (Q25; Q75).

Results

In a comparative analysis of the number of platelets, MPV, PLC-R, the relative and absolute amount of IPF in donors, patients with IHD and PCI statistically significant intergroup differences were not found. When comparing the absolute amount of IPF in the group of patients with the level of absolute IPF before the intervention 12.0 (8.5; 13.6) found a reliable increase in the absolute amount of IPF in 4 hours 15.0 (17.1; 13.4) and 24 hours 14.8 (15.9; 12.9) after surgery, as well as the relative amount of IPF in patients with PCI 4 hours after surgery 6.7 (5.8; 8.4) vs. 5,1(3,9;6,6). Reliable differences in platelet aggregation with collagen inducer were found in patients in 4 hours 3.5(2;13) om or 6.05 (3.3;33.4) om x min and 72 hours after intervention 12 (9;16) om or 34 (14.65;46.3) om x min.

Conclusions

Relative and absolute number of immature platelets in healthy individuals, as well as patients with coronary heart disease, regardless of the chosen therapeutic strategy, statistically differs statistically.4 hours after the transluminous balloonangioplasty, stenting of the coronary arteries, there is a statistically significant increase in the relative and absolute number of immature platelets, followed by their recovery within three days after surgical intervention. Collagen aggregation is the most sensitive indicator to assess the degree of recovery of platelet functional properties after intervention in patients with PCI.

doi: 10.1016/j.cca.2022.04.554

T088

The relationship among diabetes mellitus, oxidative stress biomarkers and prostate cancer

F. Adegeye [a], O. Akinosun [b], E. Bolajoko [b]

[a] *Chemical Pathology Department, University College Hospital, Ibadan, Nigeria*
[b] *Chemical Pathology Department, University of Ibadan, Nigeria*

Background-aim

Diabetes has been implicated in the generation of oxidative stress. The association between diabetes mellitus and prostate cancer are incompletely elucidated. Therefore, this study investigated the relationships between diabetes mellitus, oxidative stress biomarkers and prostate cancer in type 2 diabetes mellitus.

Methods

A total of 60 participants were recruited for the study, 40 diabetic participants (DM) and 20 non-diabetic participants (control). Ten millilitres of venous blood was collected from each participant into fluoride oxalate and plain bottles. Fasting plasma glucose (FPG), total antioxidant capacity (TAC) and total plasma peroxide (TPP) were determined using spectrophotometer and oxidative stress index (OSI) was calculated. Prostate specific antigen (PSA) was analysed using enzyme immunoassay. The data were analysed using Student't'-test and Pearson's correlation coefficients. Statistical significant was considered at $p < 0.05$.

Results

In this study, the mean values of age for the DM and control participants were 62.00 ± 9.39 and 56.80 ± 10.43 respectively. The mean values of FPG (8.14 ± 1.98 vs. 4.96 ± 0.60 mmol/L), TPP (40.33 ± 9.51 vs. 14.56 ± 10.83 μmol H2O2/L) and OSI (105.00 ± 56.87 vs. 30.59 ± 23.92 %) were significantly higher in the DM participants compared to the control participants. Similarly, the mean values for TAC (40.67 ± 10.26 vs. 49.51 ± 10.29 μmol/L) and PSA (1.64 ± 1.06 vs. 3.03 ± 1.52 ng/ml) were observed to be significantly lower in DM compared with the control participants. However, when the PSA level was compared among the different age categories, the values were found to be within the normal interval of 0-4.0 ng/ml except in age category 70-79 years, where a slight increase in PSA was found in all participants ($p > 0.05$). Non-significant associations were observed among the parameters in both DM and control participants.

Conclusions

This study reveals depleted total antioxidant capacity in type 2 diabetic participants. Therefore, these participants may be required to con-

sume more of leafy vegetables in other to boost their total antioxidants capacity.

doi: 10.1016/j.cca.2022.04.555

T089

Helicobacter Pylori and coronary disease

T.I. Nedeljkovic [a], I.I. Nedeljkovic I [d], Z.V. Vuckovic [a], D. Brakocevic [c], M.M. Petrovic [b]

[a] *Clinical Center Kragujevac, Kragujevac, Serbia*
[b] *Clinical Center of Serbia, Belgrade, Serbia*
[c] *Health Center Vracar, Belgrade, Serbia*
[d] *Specialistic Internistic Ordination Nedeljkovic, Novi Sad, Serbia*

Background-aim

Atherosclerosis is chronicle inlammatory answer on endotelial laesion. Clinical, serological and epidemiological data indicate on possible relation between HP seropositivity (HPS) and Coronary disease (CD). The Aim of this work is analysis of relation between HPS and CD.

Methods

In this study included 135 Patients (P) with determined IgG antibodies (IgGAb) in blood on HP determined by ELISA methodology.

For statistics we used hi 2 test and Student t-test.

Results

All P divided in 2 (two) groups: A group (85 P) with positive results (>50 U/mL) and B group (50 P) with negative results (<50 U/mL). HPS was finded by 63 % P. Between groups was not statisticaly significant diferentiations by age (years):group A = 54.39±14.67 (20-86) versus (vs.) group B = 50.48±11.21 (33-78) ; p=0.106 and by women : group A = 47 (55.3 %) vs. group B = 36 (72 %) ; p = 0.106.

In group A was 20 P with CD (23.53 %) and in group B was 2 P with CD (4 %) , p = 0.006 !

Conclusions

In conclusion, we indicate relation between HPS and CD in our study.

Keywords: Helicobacter pylori: Coronary disease: Comorbidity: IgGAb: ELISA

doi: 10.1016/j.cca.2022.04.556

T090

Correlation between NT-proBNP and haemoglobin values in steady state sickle cell disease patients in Nairobi, Kenya

J. Maganga [b], A. Amayo [b], F. Okinyi [b], K. Wafula [a]

[a] *Aga Khan University Hospital Nairobi, Kenya*
[b] *University of Nairobi, Kenya*

Background-aim

BACKGROUND: Sickle cell disease (SCD) is a global public health concern. As SCD patients live longer, chronic effects of sustained haemolytic anaemia lead to the development of end organ complications, which include the heart. Cardiovascular derangements are mostly diagnosed by use of ECHO, which is expensive and not found in many health facilities in developing countries. Biochemical markers of heart disease are however affordable and can be done in many hospitals. Utility of cardiac biomarkers in screening for cardiovascular disorders in SCD patients has not been reported in Kenya.

Objective: To determine serum NT- proBNP (NT-proBNP) for prediction of heart failure in asymptomatic steady state Sickle Cell Disease patients, and correlate NT-proBNP with haemoglobin levels.

Methods

Study design and setting: A cross-sectional descriptive study conducted in two health care facilities: the Kenyatta National Teaching and Referral Hospital, and an outpatient clinic in a high-density area in Nairobi (Baraka Medical Centre).

Methodology: Consenting Sickle Cell Disease patients in steady state seen at the clinics were consecutively enrolled into the study. Demographic and clinical information was obtained. NT-proBNP was measured using an automated Fluoroimmunoassay technique and Haemoglobin was measured using an automated blood cell counter.

Results

Results: A total of 90 SCD patients were recruited. Females were more (55.6%) than males (44.4%). The mean age was 12.1 (SD=6.8) years, and most (50%) were in the 5-10yrs age group. The NT-proBNP values ranged from 18.0pg/ml to 5852.5pg/ml, with a mean of 354.4pg/ml (SD=644.96), and median of 253.1 (IQR=223.62). One third of the study participants had NT-proBNP levels higher than 300ng/L, which is the cut-off used for risk of heart failure. The haemoglobin values ranged from 4.10 to 13.2g/dl, with a mean of 8.01g/dl (SD=1.37). There was a significant weak negative correlation between NT-proBNP and Hb.

Conclusions

Conclusions: Elevated NT-proBNP was found in a third of sickle cell disease patients in asymptomatic steady state condition indicating risk of heart failure. The negative correlation between NT-proBNP and Hb has been reported in several studies and indicates diastolic dysfunction due to anaemia. NT-proBNP may be a useful biomarker for diastolic dysfunction in steady state SCD patients.

doi: 10.1016/j.cca.2022.04.557

T091

Platelet-to-lymphocyte ratio as prognostic factor for patients with acute coronary syndrome

W. Tidjane

University of Medicine in Oran, North Africa University Hospital of Oran, Algeria

Background-aim

Evaluate the prognostic value and determine the positivity threshold of the PLR ratio in coronary patients compared to the risk of recurrence in the 3 months following the diagnosis.

Calculate the overall survival without recurrence at 3 months of coronary patients treated in the Cardiology service of the EHUO.

Calculate the mean survival and the median recurrence-free survival as according to the PLR ratio for coronary patients admitted to the Cardiology department of the EHUO.

Methods

This is a prospective prognostic survey including, in total, 60 coronary patients admitted to the Cardiology service and the Intensive Care unit of the EHUO, between December 1, 2016 and March 31, 2017. A Complet Blood Count was performed with calculation of the PLR Ratio within 24 hours of admission for each patient. Demographic and background data were collected using a standardized questionnaire. The search for a recurrence or death of cardiovascular origin was made by telephone interview, 6 months after admission. The survival study was carried out using the Kaplan Meier method. A multivariate analysis was done by regression of COX. The ROC Curve for the PLR Ratio was plotted by The Method of Delong et Al. Data entry and data analysis were performed on IBMS SPSS 20.0 and MedCalc15.1 software.

Results

The mean of age 60.09 ± 23.6 years, the mean PLR value 129.89 ± 16.9, SR = 3.6.

23% of patients recurred with a mortality of 5%. There is a statistically significant difference in the mean age between men and women ($p = 0.035$) and not significant between the low PLR and high PLR groups ($p = 0.192$). The survival curves are significantly different between the low PLR and high PLR categories ($p = 0.0001$). The Cox model reveals that the PLR (HR = 11.52) is a predictor of recurrence. The PLR threshold value = 161.11 (Se = 71.4%, Sp = de84.8%).

Conclusions

The Ratio PLR is an inexpensive biomarker of inflammation, readily available by performing a simple NFS. Allows stratification and optimal management of patients with risk of recurrence or death of cardiovascular origin.

doi: 10.1016/j.cca.2022.04.558

T092

Long term effect of different edible oils on serum lipid profile and atherogenic indices in Albino Wistar rats

A. Chaturvedi [a], A. Amuthan [b], A. Kiran [b]

[a] *Department of Biochemistry, Melaka Manipal Medical College (Manipal Campus), Manipal Academy of Higher Education (MAHE), Manipal 576104, Karnataka, India*
[b] *Department of Pharmacology, Melaka Manipal Medical College (Manipal Campus), Manipal Academy of Higher Education (MAHE), Manipal 576104, Karnataka, India*

Background-aim

Different types of dietary lipids affect serum lipid profile and lipid metabolism. Vegetable oils are known for their cholesterol-lowering effects when substituted for dietary animal fat; however, specific types of vegetable oils differ in their cholesterol-lowering capacity. In predicting CVD risk, especially when absolute values of lipid parameters are not markedly deranged, atherogenic indices contribute significantly. Since changes in the lipid profile have a role in cardiovascular events, in this study we decided to evaluate the long term effect of commonly used forms of dietary oils (rice bran, sesame, sunflower, coconut, mustard, and cow ghee) on serum lipid profile in Wistar rats.

Methods

Forty-two female rats were divided into seven groups of six animals each. Groups 1 to 7 received distilled water (DW), rice bran oil (RB), cow ghee (CG), sesame oil (SO), mustard oil (MB), sunflower oil (SUN) and coconut oil (CO) respectively for 120 days (5ml/kg/day, oral). At baseline, and the end of the experiment, under anesthesia, fasting blood was collected by retro-orbital puncture method for lipid profile estimation. Data were analyzed using paired t-test and Wilcoxon signed-rank tests based on normality distribution. P-value < 0.05 was considered significant.

Results

All the studied oils except cow ghee significantly ($p < 0.05$) decreased the total cholesterol (TC) levels post 120 days of oil administration compared to their baseline levels. SO, MO, SUN and CO significantly ($p < 0.05$) decreased the low-density lipoprotein levels compared to its baseline. RB, SO and CO increased the high-density lipoprotein levels, but this increase was statistically not significant. CG significantly ($p < 0.05$) increased the atherogenic index of plasma (AIP). RB, SO, MO, SUN and CO significantly ($p < 0.05$) decreased the cardiac risk ratio (CRR) and atherogenic coefficient (AC).

Conclusions

In this study, we have observed that except CG all oils reduce the TC levels. RB, SO, MO, SUN, and CO significantly decreased the atherogenic indices (CRR, AC), which indicates the beneficial effects of these dietary oils. Although few findings of this study are in line with previous reports, none of them studied the long term effect (120 days) of vegetable oils.

doi: 10.1016/j.cca.2022.04.559

T093

Prevalence of iron deficiency in patients with heart failure

N. Ould Bessi [b], W. Aksas [b], M.E. Mehni [c], I. Nouani [b], F. Benaiche [b], A. Chikouche [a]

[a] *Biochemistry Laboratory, Department of Medicine, Algiers-1 University, Pierre and Marie Curie Center, Algiers, Algeria*
[b] *Biochemistry Laboratory, Department of Pharmacy, Algiers-1 University, Pierre and Marie Curie Center, Algiers, Algeria*
[c] *Biochemistry Laboratory, Department of Pharmacy, Mouloud Mammeri university, Tizi-ouzou, Algeria*

Background-aim

Heart failure (HF) is a public health problem affecting 1-2% of the general population. Iron deficiency (ID), with or without anemia, is a frequent comorbidity of the HF: it increases morbidity in terms of re-hospitalization, reduction in exercise tolerance, impairment of life quality and mortality increasing. Since ID is the most common nutritional deficiency in the world and, in view of its deleterious effects on people with HF, it becomes necessary to detect it. Several studies are currently focused on the interest of systematic screening and treatment of ID in patients with HF as a new therapeutic target. We therefore wish, through

a descriptive study, to assess the prevalence of ID with or without anemia in a population of Algerian HF patients.

Methods

- This is a descriptive cross-sectional observational study.
- All patients with HF, aged over 18, admitted, from February 1, 2018 to March 1, 2019, in the A2 cardiology department of the Mustapha Pacha hospital, and consenting to participate in the study, were included.
- The blood sampling was done on:
- Heparinized tubes, on an empty stomach, for the biochemical analysis which was carried out at the Biochemistry laboratory of the Pierre and Marie Curie Center.
- EDTA tubes, for a complete blood count that was carried out at the hemobiology department of Mustapha Pacha hospital.
- The clinical data of patients were collected on information sheets.
- The determination of Iron, ferritin, the unsaturated iron binding capacity (UIBC) and CRP were carried out on Cobas® Intégra 400 PLUS by spectrophotometric methods.
- Our algorithm for the diagnosis of ID in HF was based on the HF definition given by the HF ESC guidelines, 2016 edition: Ferritinemia $<100\mu g$ /L = absolute iron deficiency (AID) ; Ferritinaemia between 100-299μg /L + transferrin saturation (TSAT) $<20\%$ = functional iron deficiency (FID).
- Diagnosis of anemia was performed from hemoglobin levels $<13g$ /dL in men and $<12g$ /dL in women.
- CRP levels have been used to eliminate a ferritin increase due to inflammation.

Results

The study enrolled 95 HF patients, including 65 men and 30 women aged from 21 to 93.

- 28.42% were not deficient in iron but 8.42% among them were anemic.
- 71.58% were deficient in iron, 45.26% of them had an associated anemia: 49.47% of the total population had a AID of which 31.57% were anemic; 22.10% of the total population presented with FID of which 13.68% were anemic.

Conclusions

ID is a common comorbidity of HF. Often overlooked, it is responsible for the deterioration of the life quality and the mortality increasing. Its management in Algeria is essential, considering its very high prevalence.

doi: 10.1016/j.cca.2022.04.560

T094

High-sensitivity cardiac troponin I (HSTNI) immunoassay: Will the new Siemens Atellica® IM analyzer perform better than Beckman Coulter DXI 800?

C.H. Yong, S.Y. Tan, A. Pajarillaga, N. Sahabdeen, C. Khoo, W.X. Wang, S. Saw, S. Sethi

National University Health System, Singapore

Background-aim

Our clinical biochemistry laboratory has been using Beckman Coulter DxI 800 for the analysis of hsTnI as a biomarker of cardiac injury. The need for reduced turnaround time and more reliable results could allow physicians to make faster and more confident decisions regarding patient management. The aim of this study is to evaluate and compare the hsTnI assay between the existing Beckman Coulter DXI 800 against an alternative Siemens Atellica® IM.

Methods

A comparison study was conducted by analysing 1068 serum specimens on DxI 800 and Atellica® IM, the same day the specimens were taken. Analytical performance of Atellica® IM was also evaluated based on imprecision, linearity and functional sensitivity. Analyse-IT software was utilized to generate linear regression fit and Bland-Altman difference plot.

Results

The hsTnI results from the Atellica® IM correlated well with the Beckman Coulter where linear regression fit produced a slope of 1.36, y-intercept of 79.8 ng/L and pearson's R value of 0.95. The coefficient of variation (CV) for Atellica® IM was 13.9%, 3.7%, 1.7%, 2.4% at hsTnI levels of 6, 30, 188 and 20790 ng/L respectively. Quality control material also demonstrated excellent precision of 2.5-3.2%. The analytical measurement range was demonstrated to be linear over the concentration range of 5.9-20748 ng/L, with an R value of 0.99 and recoveries from 95.7 to 104.4%. The manufacturer's claimed functional sensitivity of 2.51 ng/L at CV 10% was greater, in our experience at 7.8 ng/L.

Conclusions

Both analysers demonstrated comparable analytical performance. Total imprecision for the new assay across a wide range of hsTnI was $<15\%$. An advantage of the Attelica® IM is its turnaround time, where the hsTnI on Attelica® IM at 10mins is superior to the current assay time on DXI 800 at 18mins. Attelica® IM has been verified to be a suitable replacement for the current Beckman Coulter DxI 800.

doi: 10.1016/j.cca.2022.04.561

T095

Diabetes mellitus (DM) is a group of metabolic diseases. It is associated with well known complications linked to either microangiopathy, macroangiopathy or a combination of both. Some studies found that there were changes in respiratory system or PU

P. Ashok [a], N. Nerkar [b]

[a] *Bharati Vidyapeeth Medical College Pune, Dept of Physiology, India*
[b] *Dept of physiology Dr Vasantrao Pawar Medical College Adgaon, Nasik, 2, India*

Background-aim

Diabetes mellitus (DM) is a group of metabolic diseases. It is associated with well known complications linked to either microangiopathy, macroangiopathy or a combination of both. Some studies found that there were changes in respiratory system or pulmonary functions in dia-

betes so this study was planned to see the effect of type 2 DM on pulmonary functions.

Methods

The present study was undertaken in two groups. Hundred male individuals were included in the study with each group comprised of 50 individuals. For pulmonary function tests we used computerized Spirometer Statistical difference between the data obtained in various groups was evaluated by z test.

Results

Reduction in FVC-Forced vital capacity reduction is statistically significant. FEV1-reduction is statistically significant. FEV1/FVC (%) reduction is statistically significant.

Conclusions

So it is always better to detect the respiratory damage in diabetes patient at an early stage to prevent the further complications.

doi: 10.1016/j.cca.2022.04.562

T096

Analysis of inflammatory response of animal macroorganism during operations on the abdominal aorta using synthetic and experimental biological prosthesis

M. Kaliadka, R. Zhmailik, H. Popel, J. Ostrovsky

Republican Scientific and Practical Center "Cardiology", Minsk, Belarus

Background-aim

To analyze the systemic inflammatory response of an animal (white pigs) when the abdominal aorta is replaced with a synthetic and experimental biological prosthesis.

Methods

The inflammatory response of the animal's body was studied experimentally on 12 pigs weighing 50 ± 2.3 kg. The animals were divided into 2 groups of 6 animals each. Linear prosthetics of the infrarenal abdominal aorta was performed. The first group underwent replacement of the formed aortic defect with a synthetic prosthesis "Vascutek Terumo Gelsoft", in the second - with an experimental biological prosthesis. The inflammatory response was assessed by a general blood test (white blood cells, lymphocyte, monocyte, granulocyte, CRP, ESR). The effect on the hemostatic system was compared with the data obtained before the operation and with the results after the operation after 1,7,14,21 days. The experiment was carried out in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

Results

According to the data of the general blood test after the operation, there is no significant change in the level of leukocytes in the group with a biological prosthesis, compared with a synthetic prosthesis, which is characterized by a rapid phagocytic reaction to a synthetic prosthesis, however, these changes are not statistically significant and we can only talk about a trend ($T = 1.7358$, $p = 0.083$). An increase in the white blood cell count is observed up to 14 days in the group with a biological prosthesis, without a significant deviation from the trend line, which may correspond to the saturation of the biological vascular prosthesis with immune cells, as well as the development of an inflammatory response to surgical trauma. By day 21, the indicators return to the limits of the abstract interval (19.4-25.3) ($p = 0.05$). Changes in the indicators of leukocyte subpopulations are characteristic of this type of surgery in human practice and are characterized by an increase in granulocytes ($p = 0.15$), monocytes ($p = 0.34$) and a decrease in the level of lymphocytes ($p = 0.74$), which, first of all, says about the nonspecific immune response in animals of both groups by 21 days after the operation. Changes in the ESR level are marked by a stable increase in the indicator up to 7 days in the group with a biological prosthesis (Me 36.5 (31.0; 42.0)), with subsequent normalization of the indicator by 21 days (Me 12.5 (2.095; 19.05)) $p = 0.042$.

Conclusions

Given the absence of a pronounced inflammatory reaction, the use of biological prostheses is an actual alternative to synthetic vascular prostheses.

doi: 10.1016/j.cca.2022.04.563

T097

Novel predictors of cardiovascular risk in subjects with type-2 diabetes mellitus: Evidence from a cross-sectional study

M. Azharuddin [b], P. Kapur [a], M. Sharma [c]

[a] *Department of Medicine, Hamdard Institute of Medical Sciences and Research, Jamia Hamdard, New Delhi, India*
[b] *Department of Pharmaceutical Medicine, Division of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India*
[c] *Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India*

Background-aim

People with diabetes have an increased risk of cardiovascular morbidity and mortality. Cardiovascular disease is the leading cause of mortality in people with type 2 diabetes mellitus (T2DM), it has a detrimental impact on health outcomes as well. Therefore, the unmet need for early detection and diagnosis of cardiovascular risk in patients with T2DM is required to ensure adequate prevention of diabetic complications. The objective of this study was to find out the association between periostin level, anthropometric measurements, and inflammatory biomarkers in patients with type 2 diabetes mellitus.

Methods

A cross-sectional study, included a total of 130 T2DM patients. All anthropometric measures and biochemical parameters including periostin, high sensitivity C-reactive protein (hsCRP), and leukocyte count were estimated. Participant were divided into two groups by normal body mass index (BMI) and higher BMI group. Pearson's correlation analysis was performed to assess the significant association between periostin level and other biochemical parameters. P-values <0.05 were considered statistically significant.

Results

The mean age of included participants were 52.42 years. Serum periostin levels were higher among obese T2DM patients. Correlation anal-

ysis showed, positive correlation between serum periostin levels and waist circumference (WC), BMI, waist-hip ratio (WHR), fasting plasma glucose (FPG), postprandial glucose (PPG), glycated hemoglobin (HbA1c), triglyceride (TG), total cholesterol (TC), hsCRP, leukocyte count ($p < 0.05$) however, negative correlation was observed with high-density lipoprotein cholesterol (HDL-C) ($p < 0.05$). After adjustment, regression model showed hsCRP, BMI, TG were independent predictors of elevated serum periostin ($p < 0.05$).

Conclusions

The evidence of current study suggested that periostin levels were higher among patients with higher BMI group and it is significantly associated with lipid profile, anthropometric measures and inflammatory biomarkers. periostin may consider as a novel biomarker to measure the early cardiovascular risk. However, lacking of follow-up it further creates room for the future investigation. Therefore, further real-world studies warrant to robust the present findings.

doi: 10.1016/j.cca.2022.04.564

T098

Long term effect of different edible oils on serum lipid profile and atherogenic indices in Albino Wistar rats

A. Chaturvedi [a], A. Amuthan [b], A. Kiran [b]

[a] *Department of Biochemistry, Melaka Manipal Medical College (Manipal Campus), Manipal Academy of Higher Education (MAHE), Manipal 576104, Karnataka, India*
[b] *Department of Pharmacology, Melaka Manipal Medical College (Manipal Campus), Manipal Academy of Higher Education (MAHE), Manipal 576104, Karnataka, India*

Background-aim

Different types of dietary lipids affect serum lipid profile and lipid metabolism. Vegetable oils are known for their cholesterol-lowering effects when substituted for dietary animal fat; however, specific types of vegetable oils differ in their cholesterol-lowering capacity. In predicting CVD risk, especially when absolute values of lipid parameters are not markedly deranged, atherogenic indices contribute significantly. Since changes in the lipid profile have a role in cardiovascular events, in this study we decided to evaluate the long-term effects of commonly used forms of dietary oils (rice bran, sesame, sunflower, coconut, mustard, and cow ghee) on serum lipid profile in Wistar rats.

Methods

Forty-two female rats were divided into seven groups of six animals each. Groups 1 to 7 received distilled water (DW), rice bran oil (RB), cow ghee (CG), sesame oil (SO), mustard oil (MB), sunflower oil (SUN), and coconut oil (CO) respectively for 120 days (5ml/kg/day, oral). At baseline, and the end of the experiment, under anesthesia, fasting blood was collected by retro-orbital puncture method for lipid profile estimation. Data were analyzed using paired t-test and Wilcoxon signed-rank test based on normality distribution. P-value < 0.05 was considered significant.

Results

All the studied oils except cow ghee significantly ($p < 0.05$) decreased the total cholesterol (TC) levels post 120 days of oil administration compared to their baseline levels. SO, MO, SUN, and CO significantly ($p < 0.05$) decreased the low-density lipoprotein levels compared to its baseline. RB, SO and CO increased the high-density lipoprotein levels, but this increase was statistically not significant. CG significantly ($p < 0.05$) increased the atherogenic index of plasma (AIP). RB, SO, MO, SUN, and CO significantly ($p < 0.05$) decreased the cardiac risk ratio (CRR) and atherogenic coefficient (AC).

Conclusions

In this study, we have observed that except for CG all oils reduce the TC levels. RB, SO, MO, SUN, and CO significantly decreased the atherogenic indices (CRR, AC), which indicates the beneficial effects of these dietary oils. Although few findings of this study is in line with previous reports, none of them studied the long-term effect (120 days) of vegetable oils.

doi: 10.1016/j.cca.2022.04.565

T099

High-sensitive troponin I for the stratification of patients with a low risk for acute coronary syndrome in the emergency room

F. Berga Montaner, S. Sánchez Asis, D. Ramos Chavarino, A. Álvarez López, P. Gallego Angui, J.M. Bauçà Rosselló, I. Llompart Alabern, D. Morell-García

Department of Laboratory Medicine, Hospital Universitario Son Espases, Palma de Mallorca, Spain

Background-aim

The new guidelines from the European Society of Cardiology (ESC) 2020 recommend serial determinations of high-sensitivity troponin I (hsTnI) for the rapid diagnosis of acute coronary syndrome (ACS) in individuals with initial values above the quantification limit. The main limitation of such algorithm is the low sensitivity for the classification of low risk patients, defined as quantifiable hsTnI values below sex-dependent percentiles 99 (p99).

Our aim was to evaluate the ESC algorithm adherence in our Emergency Room and the role of initial hsTnI levels for the stratification of patients with a low risk for ACS.

Methods

Retrospective study performed between Dec-2019 and Feb-2020 in a tertiary care hospital. Adult patients with symptoms compatible with an ACS, and a hsTnI value below p99 were consecutively included (p99: 16 ng/L for women; 34 ng/L for men; Architect ci16200, Abbott Diagnostics).

Epidemiological and clinical values were recorded, along with performance of 3-hour hsTnI measurement and reconsultations within a 6-month period.

A bivariant analysis was performed (Student's t-test), and sex-dependent hsTnI values for the presence of ACS in low risk patients were calculated.

Results

Two hundred patients were included: 100 women with a mean age of 57.8 ± 20.2 years, and 100 men with a mean age of 57.8 ± 18.6 years.

ACS was diagnosed in 2 men (median initial hsTnI: 10.2 ng/L). Urgent reconsultation rate due to chest pain before 6 months was 18% for men vs 4% for women, without sex differences.

Taking the sample as a whole, initial hsTnI values were higher in individuals who were reconsulted due to chest pain than those who were not (9.75 ng/L vs 6.46 ng/L, $p = 0.037$). In our study, values above 9.75 ng/L have a higher 6-month reconsultation rate. ESC algorithm adherence (0-3 hours) was 21% for men and 14% for women patients ($p = 0.14$).

Conclusions

We observed an algorithm adherence below 25% for the urgent diagnosis of ACS in low risk individuals, without sex differences. Initial hsTnI values could be an useful tool for establish an association with ACS risk in these population, and to improve the adherence to international recommendations.

doi: 10.1016/j.cca.2022.04.566

T100

Expression profiling of epigenetics and chromatin remodeling factors in human heart failure: The road towards precision medicine

S. Mahmoud [a], G. Labib Abu Elenain [c], N. Al Yacoub [b], M. Al Zaabi [a]

[a] *Fatima College of Health Sciences, United Arab Emirates*
[b] *King Faisal Specialist Hospital & Research Center, Saudi Arabia*
[c] *Parasitology Department, Theodor Bilharz Research Institute, Giza, Egypt*

Background-aim

Cardiovascular diseases remain a leading cause of mortality worldwide including the United Arab Emirates. It is associated with multiple genetic and environmental factors that can only explain a small part of its variability. Epigenetic has emerged as one of the most promising areas that will address some of the gaps in our current knowledge in the development of many cardiovascular diseases. Epigenetic mechanisms include DNA methylation, chromatin alterations, histone modification, and microRNA. This proposal aims to establish a new paradigm in regulating heart failure.

Methods

We will examine epigenetic changes in several histone modifications and chromatin remodeling factors in human failing hearts tissue/cell models using specific expression arrays for epigenetic factors utilizing microarrays, ChIPseq and ATAC-seq technologies. Afterwards, we will attempt to elucidate the potential mechanism(s) by which these factors govern the process of gene expression and consequently the induction of cardiac hypertrophy cascade that leads to heart failure development.

Results

Results from this study revealed a new concept in epigenetics regulation in the heart and provided new insights into understanding the development and progress of heart diseases. Moreover, our data demonstrated that epigenetic mediated gene expression changes are nodal points for the control of cardiac hypertrophy and represents a novel regulatory mechanism in the heart. While transcriptional events in heart muscle have been chosen to illustrate the importance of this new mechanism, similar events might exist with other epigenetic factors expressed in other tissues.

Conclusions

Our observations revealed novel epigenetic targets that are involved in the regulation of heart failure, thus contributed to increasing our understanding of the interplay between genetic and epigenetic factors. This will consequently lead to developing new approaches that can lead to innovative therapeutic tools which will reflect positively on enhancing human health.

doi: 10.1016/j.cca.2022.04.567

T101

HE4 and heart failure. A novel biomarker?

C. Castillo Perez, M. Cebrian Ballesteros, B. Torrubia Dodero, L. Rodriguez Alonso

Laboratory Medicine Department, Hospital Fundacion Jimenez Diaz, Spain

Background-aim

Cardiac failure is a major health problem worldwide that concerns the health systems of developing countries. Cardiac failure is a clinical diagnosis based in specifics signs and symptoms but several laboratory markers had been proposed for its diagnosis. NT-proBNP is the only biomarker used for the diagnostic and prognostic of cardiac failure and it had been included in specific guidelines. There are a few studies that have seen association between HE4 (human epididymis protein 4) and acute heart failure severity in patients without tumoral pathology and normal renal function.

Methods

50 patients presenting with myocardial infarction were selected. Determination of NT-proBNP was made in the first 24 hours seeking for a laboratory diagnostic of hearth failure. The determination of NT-pro BNP and HE4 was made using an immunologic assay. To analyze the data we have used SPSS 19. Patients were divided in two groups using the NT-proBNP diagnostic value as recommended by the European Society of Cardiology with 88% positive predicting value:

1. Heart failure group (HFG). 27 patients (18 men and 9 women).
2. non Heart failure group (nHFG). 23 patients (12 men and 11 women)

We calculated the medians and the IQR of both groups and the area under
the curve (AUC), sensitivity and specificity were estimated

Results

The mean age of HFG group was 65.23 and for the nHFG group was 71.56. No sex or age differences were observed. The HE4 median and IQR of HFG group and nHFG group was 166.78 (98.96) pmol/L and 54.10 (7.84) pmol/L respectively. We found statistically significant differences between both group ($p < 0.001$).

The AUC was 0.86 with 76% sensitivity and 95% specificity with a cutoff point of 92.3 pmol/L.

Conclusions

The present study suggests a positive association between increased HE4 levels in acute cardiac failure. Further studies are needed to investigate the value of HE4 as a biomarker in acute hearth failure.

doi: 10.1016/j.cca.2022.04.568

T102

Has the SARS-COV-2 pandemic affected cardiovascular risk parameters in the general population?

M. Chlabicz [c], J. Jamiołkowski [c], A. Szum-Jakubowska [c], P. Sowa [c], M. Dubatówka [c], M. Łapińska [c], M. Kondraciuk [c], J. Motyka [c], M. Chlabicz [c], A. Szpakowicz [a], S. Ławicki [c], A. Moniuszko-Malinowska [b], K. Kamiński [c]

[a] *Department of Cardiology, Medical University of Białystok, Białystok, Poland*
[b] *Department of Infectious Diseases and Neuroinfection, Medical University of Białystok, Białystok, Poland*
[c] *Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Białystok, Białystok, Poland*

Background-aim

The world is currently facing a pandemic caused by the acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological factor of the novel coronavirus disease of 2019 (COVID-19). The mechanisms of how SARS-CoV-2 may cause myocardial complications are not clearly understood. The aim of the study was to assess the impact of a COVOD-19 pandemic on cardiovascular (CV) risk parameters in general population.

Methods

The Białystok Plus Study was conducted in 2017-2021 on a sample of Bialystok (Poland) residents aged 20-79. Many parameters related to CV risk were assessed in the COVID-19 population (136 people) and compared with the pre-COVID-19 population (518 people) using the Mann-Whitney test. March 18th 2020 was considered the beginning of the COVID-19 pandemic in Poland. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated from the following formula: score = fasting insulin (U /mL) x fasting glucose (mmol/L)/22.5.

Results

The conducted analysis did not show any statistically significant differences between the analyzed groups with regard to the parameters related to cardiovascular risk: N-terminal pro-brain natriuretic peptide (NT-proBNP) $p = 0.442$; total cholesterol $p = 0.165$, low-density lipoprotein cholesterol (LDL-C) $p = 0.116$; high-density lipoprotein cholesterol (HDL-C) $p = 0.094$; triglycerides $p = 0.119$; high- sensitivity C-reactive protein (hs-CRP) $p = 0.775$; fasting glucose $p = 0.166$; the 120 min glucose in oral glucose tolerance test (OGTT) $p = 0.515$; hemoglobin A1c (HbA1c) $p = 0.692$, HOMA-IR $p = 0.362$; creatinine clearance using Cockcroft-Gault Equation (CrCl) $p = 0.481$.

Conclusions

The SARS-CoV-2 pandemic did not affect the parameters associated with CV risk in the general population.

doi: 10.1016/j.cca.2022.04.569

T103

Evaluation of uric acid's role in cardiovascular disease stratification among type 2 diabetics

B. Myke-Mbata [a], S. Meludu [b], J. Okwara [b], C. Olisah [c]

[a] *Department of Chemical Pathology, Benue State University, Makurdi, Nigeria*
[b] *Department of Chemical Pathology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*
[c] *Department of Medical Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria*

Background-aim

BACKGROUND: In the light of challenges of management of diabetic cardiovascular complications which is complicated by its huge economic demand, increased mortality and morbidity in addition to inadequate manpower to combat this menace especially in developing countries, more attention should be given to the use of biomarkers in order to ease diagnosis and aid early management. Biomarkers may be needed to aid the screening of cardiovascular disease in diabetes mellitus to enable early detection and foster early intervention in the management of chronic complications in diabetes mellitus. Hyperuricemia has been correlated with diabetic cardiovascular complications and has been implicated in the development and manifestation of cardiovascular diseases. Hence this study intends to evaluate its role in cardiovascular diseases stratification among diabetes mellitus.AIM: Evaluation of the value of uric acid in cardiovascular diseases stratification among type 2 diabetics.

Methods

METHODS: This is a cross sectional study made up of 100 Type 2 DM and age matched control participants. The diabetics were classified into known and no cardiovascular complications. The cardiovascular complication observed were diabetic retinopathy, peripheral neuropathy, cardiovascular accident and ischaemic heart diseases. Blood samples were collected for HBA1c and serum uric acid

Results

RESULT: Uric acid increase in diabetics (0.24 ± 0.12) was not significantly different from control (0.18 ± 0.04) while uric acid increased significantly in diabetics with cardiovascular complications (0.36 ± 0.11) than those without (0.30 ± 0.11). Additionally, it was found to increase in those with peripheral neuropathy (0.37 ± 0.09) than those without (0.31 ± 0.13). Those with poor glycaemic control also had significantly elevated uric acid(0.37 ± 0.08) than those with good glycaemic control (0.30 ± 0.13)However, the receiver operating curve shows no significant diagnostic sensitivity to diabetic cardiovascular complication (AUC = 0.612; P = 0.060) while binary logistic regression showed that

uric acid has no significant predictive value for cardiovascular diseases (B = -4.006; Exp(B) = 0.018; P = 0.036).

Conclusions

CONCLUSION: Uric acid may not be diagnostically relevant in screening for cardiovascular diseases in the bid to ease early diagnosis among diabetics.

doi: 10.1016/j.cca.2022.04.570

Clinica Chimica Acta 530 (2022) S98–S112

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Critical Care, Blood Gases, POCT

T104

An accuracy of blood glucose obtained from glucose meter [LDQUO] Standard Gluconavii® GDH Blood Glucose Meter [RDQUO] by testing with arterial and neonatal blood samples

K. Thongsukkeang [b], W. Treebuphachatsakul [c], N. Apiratmateekul [a]

[a] *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*
[b] *Laboratory Medicine, Phu Khiao Chalermprakirt Hospital, Chaiyaphum, Thailand*
[c] *Reference Material and Medical Laboratory Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*

Background-aim

Blood glucose testing by glucose meters is useful for monitoring of glycemic control in diabetes mellitus (DM) patients to prevent the complications. The novel, STANDARD GlucoNavii® GDH Blood Glucose Meter, is based on amperometry-GDH with using co-enzyme, FAD and developing in technology for blood glucose testing in arterial and neonatal samples. This study was to evaluate an accuracy of blood glucose obtains from STANDARD GlucoNavii® GDH Blood Glucose Meter when tested with arterial and neonatal samples by following ISO 15197: 2013 guidelines.

Methods

Arterial and neonatal blood samples were collected at Phukieo Chalermprakiat Hospital, Chaiyaphum Thailand. Protocol was approved by the Ethics Committee of Human Research, Naresuan University (IRB#No.0993/61). All glucose data from three different lot numbers of STANDARD GlucoNavii® GDH Blood Glucose Meter was compared to those from reference glucose, YSI 2700 analyzer.

Results

More than 95 % of biases of paired data sets with glucose <100 mg/dL (5.55 mmol/L) and glucose ε 100mg/dL (5.55mmol/L) were within $\pm$ 15 mg/dL or $\pm 15\%$ for both neonatal and arterial samples. All paired glucose data sets obtained from neonatal and arterial samples were within zones A of the Consensus Error Grid.

Conclusions

This study concluded that "STANDARD GlucoNavii® GDH Blood Glucose Meter" was accepted by ISO 15197: 2013 criteria on accuracy in blood glucose testing with using neonatal and arterial blood samples.

doi: 10.1016/j.cca.2022.04.572

T105

POCT in critical care: An accuracy check!

S. Sengupta [a], A. Handoo [b], S. Mehta [d], M. Kaushik [c]

[a] *Consultant (Clinical Biochemistry) & Quality Manager (Laboratory Services), BLK Superspeciality Hospital, Delhi, India*
[b] *Department of Laboratory Services, BLK Super Speciality Hospital, Delhi, India*
[c] *Operations & Planning, BLK Superspeciality Hospital, Delhi, India*
[d] *Head- Medical Services, BLK Superspeciality Hospital, Delhi, India*

Background-aim

Quality in POCT was never previously questioned. However, regulatory bodies like the CAP, CQLA & JCI now recommend that POCT be brought under the direct supervision of the Central Laboratory and subjected to strict quality practices. Our journey in POCT in BLK Super Speciality Hospital, Delhi, India, also started when the hospital applied for accreditation by the JCI (Joint Commission International), of which it is a requirement as per the Standard AOP 5.1.1.

Methods

In addition to other POC devices, a total of seventy three glucometers were in use across all floors of the hospital, including wards, intensive care units out-patient departments, emergency and ambulances. CLSI guidelines were followed to design policies for Internal Quality Control and Proficiency Testing for glucometers. The available data were reviewed after a year. Mean, SD, z-scores and percent variation were calculated. Bland Altman graphs were plotted and percent variations reviewed as per ISO standards. Clark Grid Error Analysis was also performed to evaluate the impact of inaccuracies in glucometers on clinical outcome.

Results

Six glucometers in October'18 and two in January'19 were identified as unacceptable for use as per ISO criteria. 3.2% of glucometers were in Zone B of Clark Grid error Analysis, meaning that the difference in their values would alter clinical action with or without any effect on patient outcome.

Conclusions

Innovations in glucometer technology have enabled obtain better accuracy in recent times. However, handling by different testing personnel, lack of adherence to manufacturer's instructions, presence in varied locations across the hospital and absence of defined quality assurance practices influence the performance of glucometers and may produce

deviant results. It is essential to subject the glucometers to accuracy and precision checks at regular intervals. This would ensure inter-instrument comparison and comparison of the glucose values measured in glucometers to those determined by the reference method in the Central Laboratory. Use of Clark Grid Error Analysis is necessary for evaluation of the effect of deviant results on clinical outcome.

doi: 10.1016/j.cca.2022.04.573

T106

Significant process improvement after implementation of point-of-care creatinine testing at outpatient radiology services

M.Y. Lee, S.F. Lim, L. Lam

Department of Laboratory Medicine, Ng Teng Fong General Hospital, Singapore

Background-aim

In our hospital, patients requiring CT or MRI scans must have their renal function reviewed at least 1 and 6 months prior respectively to minimize the risk of contrast-induced nephropathy. Unfortunately, the average process time for patients without recent renal function tests is 126 minutes. Our objective was to reduce the average process time to less than 60 minutes over 5 months.

Methods

Data from November 2018 to February 2019 was retrieved from our EMR to establish our process baseline. Current processes and steps from the time patients were registered until completion of radiological investigation were mapped. A fishbone diagram was constructed to analyse root causes and possible solutions.

Results

Stakeholders agreed to first explore point-of-care (POCT) creatinine testing at outpatient radiology services as this was the easiest option to implement with the highest potential impact. The new POCT creatinine workflow was started from March 2019. Post-implementation data (March – June 2019) showed significant reduction in average process time to 10 minutes with the new workflow decreased to 12 steps compared with 22 previously. In approximately 68% of the cases in the old workflow, we found that our clinicians ordered a full renal profile instead of just creatinine. Therefore, despite the introduction of POCT creatinine, we are expecting overall positive savings per patient.

Conclusions

We have demonstrated a significant process improvement after implementation of POCT creatinine testing at Outpatient Radiology Services, resulting in a cost-effective workflow, improved turnaround time and better patient care. We also observed increased satisfaction among our clinician users and patients.

doi: 10.1016/j.cca.2022.04.574

T107

Comparison of sodium and potassium concentrations measured on blood gas analyser and biochemistry laboratory autoanalyser

M. Andjelkovic [b], M. Petrovic [a], I. Nikolic [b], M. Zaric [b], M. Stanojevic Pirkovic [b]

[a] *Clinical Center Kragujevac, Department of Laboratory Diagnostics, Serbia*
[b] *University of Kragujevac, Faculty of Medical Sciences, Serbia*

Background-aim

Blood gas analysis are frequently used in clinical practice, especially in critically ill patients. When it comes to electrolite and glucose analysis, blood gas analyzers' advantage over biochemical laboratory autoanalyzers is faster availability of results. We purposed to evaluate the agreement in results of sodium and potassium concentrations obtained by blood gas analyzers (BGA) and biochemistry laboratory autoanalyzer (BLA).

Methods

Total of 60 patients' blood specimens were analysed. Sodium and potassium concentrations were measured from venous whole blood samples using BGA analyser (GEM Premier 3000) and from serum specimens that were processed by biochemistry laboratory autoanalyser ARCHITECT c2100 (Abbott Laboratories, Abbott Park, IL). Data were analysed using MedCalc Statistical Software version 19.0.7.

Results

Sodium concentations measured on GEM Premier 3000 Blood gas analyzer were between 125 and 153mmol/L (median 138, 95% CI for median 136-139mmol/L), while on Architect c2100 they ranged in 128 and 158mmol/L (median 138, 95% CI for median 138-139mmol/L). Potassium concentations measured on GEM Premier 3000 Blood gas analyzer were between 2.8 and 5.4mmol/L (median 4.2, 95% CI for median 3.9-4.3mmol/L), while on Architect c2100 they ranged in 3.2 and 6.3mmol/L (median 4.2, 95% CI for median 4.0-4.3mmol/L). The results were highly correlated ($r = 0.597$ for sodium and $r = 0.806$ for potassium) according to Spearman's correlation testing. Bland-Altman analysis for sodium and potassium concentration showed that in both cases more than 95% of points are between ± 1.96 SD of the mean (limits of agreement for sodium were between -6.1 to 8.9 and the mean difference was 1.4, and for potassium limits of agreement were between -0.61 to 0.70 and the mean difference was 0.04. Passing-Bablok analysis for sodium showed intercept (95% CI) of 14.75 (1.00-46.5) with slope (95% CI) of 0.900 (0.667-1.000), while for potassium intercept (95% CI) was 0.288 (0.100-0.643) with slope (95% CI) of 0.941 (0.857-1.000).

Conclusions

Results of this research showed acceptable agreement between sodium concentration measurement on GEM Premier3000 blood gas analyser and ARCHITECT c2100 platform as well as potassium levels measured on examined analysers.

doi: 10.1016/j.cca.2022.04.575

T108

Interferences evaluation on nova biomedical (StatStrip) hospital-based glucometer

A. Alanazi [a], R. Alabdaly [a], H. Dibekkaya [b], O. Ali [c], C. Bueno [a], N. Alhussain [a], F.I. Santos [a], N. Nicolas [a], A. Alharbi [a], M. Pharaon [a], W. Tamimi [a]

[a] *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical city, King Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*
[b] *Nova Biomedical, Waltham, MA, USA*
[c] *Redwan Medical Company, Riyadh, Saudi Arabia*

Background-aim

Monitoring of blood glucose levels is important for managing hospitalized patients. There is substantial evidence demonstrating that the accuracy of most of the commonly used meters is adversely affected and interfered by hematocrit (Hct), ascorbic acid, N-AcetylCysteine (NAC), maltose and galactose which may result in incorrect glucose readings leading to errors in patient care. Nova Biomedical (StatStrip) has developed a specialized POC Glucose Sensor specifically for hospitalized patients. In this study, we evaluated the effects of multiple interfering substances in the StatStrip glucometer and compared with another manufacturer.

Methods

The StatStrip POC device was compared to another POC hospital setting device (Device A) and both were compared to the chemistry analyzers Abbott Allinity. Protocols from CLSI (NCCLS) guidelines and ISO 15197 (2003) standards were followed. Multiple levels of product-specific manufacturer controls were run on all meters during each day of testing. A total of 30 patients samples were used in the comparison evaluation. For the Hct, ascorbic acid, NAC, maltose & galactose testing, whole blood samples were tested first as a neat, and then spiked separately with varying levels of interference substances, and then tested across three glucose levels ranging from low to very high.

Results

For Hct, ascorbic acid, NAC, maltose and galactose, the average percent-error (deviation from reference) was found to be 3.4%, 5.8, 6.8%, 8.5% and 8.0% for StatStrip respectively, and 6.4%, 35.8%, 23.3%, 14.5%, and 67.8% for device A respectively. The correlation of StatStrip and device A reference method has shown an R2 of 0.986 and 0.928 respectively with overall bias values of 1% and 21.8% respectively. Under ISO 15197 standards, the device A has shown positive bias of 50% and 93% below and above 4.2 mmol/L respectively, however, no bias trend was observed for StatStrip at this the cutoff value. Both of the two levels of QC met the stated/published manufacturer within-run precision specifications of the mean and standard deviation for QC1 (3.4 ± 0.12) and QC2 (16.5 ± 0.37) with CV of 3.5% and 2.2% respectively.

Conclusions

The StatStrip meter had an excellent correlation with the glucose reference method. It has shown lower accuracy errors with minimum interference effects from hematocrit, ascorbic acid, NAC, maltose and galactose levels.

doi: 10.1016/j.cca.2022.04.576

T109

Evaluation of the GEM® Premier™ 5000 with intelligent quality management 2 (IQM®2) at Beijing Tsinghua Changgung Hospital, Tsinghua University (Beijing, China)

Y. Xu [a], X. Tang [b]

[a] *Beijing Tsinghua Changgung Hospital, China*
[b] *Werfen China, China*

Background-aim

Beijing Tsinghua Changgung Hospital is a 1,000-bed, non-profit teaching institution focused on reducing operating costs while improving healthcare service and efficiencies. In an aim to continue to deliver quality results efficiently in blood gas testing, the Intensive Care Unit (ICU) team performed a clinical evaluation of the new GEM Premier 5000 with iQM2 (Instrumentation Laboratory). The cartridge-based system provides rapid analysis of blood gases, electrolytes, metabolites, CO-Oximetry and total Bilirubin. Additionally, the system features iQM2 for automatic detection, correction and documentation of errors in real-time.

Methods

A method comparison study was performed across all analytes: pH, pCO2, pO2, Na+, K+, Cl-, iCa, Hct, Glu, Lac, tHb, O2Hb, COHb, MetHb, HHb, and sO2 on the GEM Premier 5000 and the system in clinical operation. De-identified ICU patient were performed by clinicians daily, for six weeks, on each system according to Clinical Laboratory Standards Institute EP09-A3, totalling 111 samples.

Quality management performance and usability were analysed based on the system's ability to automate labor- and skill-intensive tasks in blood gas testing such as consumable replacement, error detection and troubleshooting. Additionally, clinicians performing the testing rated the system on overall usability.

Results

Method comparison between systems demonstrated excellent correlation across all analytes with an $r > 0.95$ and slopes between 0.93 to 1.05. iQM2 automatically detected, corrected and documented interfering substances on 6 samples for CO-Oximetry, including identifying Methylene Blue, without operator intervention. Replacement of the single consumable completed warm-up and validation in as little as 54 minutes. Time-to-results in 45 seconds also offered an opportunity for increased throughput. Finally, the clinicians provided favourable feedback, affirming suitability for routine clinical use.

Conclusions

Strong clinical performance, iQM2, and system ease-of-use, make the GEM Premier 5000 a suitable platform for our busy ICU. The GEM Premier 5000 specifically offers marked performance in terms of quality and simplicity—iQM2 ensures lab-quality results on a single and standardized platform and maintenance-free technology enables more efficient blood gas testing.

doi: 10.1016/j.cca.2022.04.577

T110

Comparison of blood-glucose measurements using blood gas analyser and biochemistry laboratory autoanalyser

M. Petrovic [a], M. Andjelkovic [b], M. Stanojevic Pirkovic [b], I. Nikolic [b], P. Canovic [b], I. Zelen [b]

[a] *Clinical Center Kragujevac, Department of Laboratory Diagnostics, Serbia*
[b] *University of Kragujevac, Faculty of Medical Sciences, Serbia*

Background-aim

Glucose concentrations are measured both by blood gas analysers (BGA) and biochemistry laboratory autoanalyser (BLA), but there is significant time gap between the availability of both these results, with the BGA obtaining faster results than the BLA. We investigated the agreement between the results of glycemia measured on BGA and BLA.

Methods

Total of 63 patients' blood specimens were analysed. Glucose concentrations were measured from venous whole blood samples using BGA analyser (GEM Premier 3000) and from serum specimens that were processed by biochemistry laboratory autoanalyser ARCHITECT c2100 (Abbott Laboratories, Abbott Park, IL).

All statistical analyses were performed using MedCalc Statistical Software version 19.0.7. The correlation study was performed using Spearman's correlation test. Comparison of assays was performed using Passing-Bablok regression analysis. The difference of measurements was estimated by calculating the bias using Bland-Altmann plots.

Results

Glucose concentrations measured on GEM Premier 3000 Blood gas analyser were between 2.7 and 17.2 mmol/L (median 5.8, 95% CI for median 5.5-6.2), while on Architect c2100 they ranged from 2.7 to 15.3 mmol/L (median 5.2, 95% CI for median 4,7251 to 5,4000). The results were highly correlated (r = 0.815) according to Spearman's correlation testing. Bland-Altman analysis (where the differences of the two paired measurements are plotted against the mean of both measurements) for glucose concentration showed that more than 95% of points are between ±1.96 SD of the mean (limits of agreement were between -0.6 to -0.36 and the mean difference was -0.5). Passing-Bablok analysis on the whole group showed intercept (95% CI) of -0.046 (-0.050-0.3784) with slope (95% CI) of 0.923 (0.851-1.000).

Conclusions

Results of this research showed acceptable agreement between glucose concentration measurement on GEM Premier3000 blood gas analyser and ARCHITECT c2100 platform. However these results should be evaluated in larger studies in order to be sure whether clinicians could initiate an appropriate therapy based on blood gas analysers' results without awaiting a control measurement.

doi: 10.1016/j.cca.2022.04.578

T111

Analytical performance characteristics of the i-SmartCare 10 analyzer for point-of-care testing

S. Kim, J. Oh, H. Park

Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Background-aim

Analysis of blood gas and electrolytes is crucial for management in critically ill patients. The i-SmartCare 10 (i-SENS, Seoul, Korea) is a novel blood gas analyzer providing rapid analysis of whole blood samples with less amount of specimen, in point-of-care as well as in a central laboratory setting. Here, we evaluated the analytical performance of i-SmartCare 10 according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Methods

Ten evaluation items are as follows: pH, partial carbon dioxide pressure (pCO2), partial oxygen pressure (pO2), sodium (Na+), potassium (K+), chloride (Cl-), ionized calcium (iCa++), glucose, lactate and hematocrit (Hct). The precision was evaluated according to CLSI EP5-A3. Carry-over was estimated with replicate measurements of the high-low sequences. Linearity was assessed by measuring the quality control material at five levels of concentration four times each by following CLSI EP6-A. The comparative evaluation between i-SmartCare 10 and RapidLab 1265 (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) was performed with residual blood samples (n = 182~209) after regular medical examination according to CLSI EP9-A3.

Results

For imprecision in nine test items, a within-run and a total CV (coefficient of variation), ranged from 0.00 to 2.20% and 0.00 to 4.31%, respectively. Lactate showed a within-run CV of 0.45-6.65% and a total CV of 2.02-9.41%. The i-SmartCare 10 showed no carry-over in all test items. Linearity was validated in analytical measurement ranges for each parameter. In method comparison with the RapidLab 1265, correlation coefficient (r) ranged from 0.90 to 0.99 except pO2 (r = 0.87). In seven items, the 95% confidence interval for the slope included 1. Two items, pCo2 and Hct, showed a difference between the two equipment at both concentrations of medical decision points.

Conclusions

The i-SmartCare 10 showed excellent precision, linearity, and carry-over, and acceptable agreement with the conventional blood gas analyzer except for some items. These results show that i-SmartCare 10 can provide reliable measurement results and can help to apply it to the clinical settings.

doi: 10.1016/j.cca.2022.04.579

T112

Accuracy assessment of three different glucometers used at the point of care testing (POCT) by using potential interferences

A. Alqahtani [a], O. Alghamdi [a], F. Alseraye [d], O. Saweed [d], F. Albloui [a], M. Alqahtani [e], M. Abodahish [d], z. Alshehry [c], W. Tamimi [b]

[a] *Biochemistry Unit, Pathology and Laboratory Medicine, Security Forces Hospital, Riyadh, Saudi Arabia*
[b] *Biochemistry unit, Pathology and Laboratory Medicine, King Abdulaziz Medical City (KAMC), Riyadh, Saudi Arabia*
[c] *Biochemistry unit, Pathology and Laboratory Medicine, King Fahad Medical City (KFMC), Riyadh, Saudi Arabia*
[d] *Pathology and Laboratory Medicine, Security Forces Hospital, Riyadh, Saudi Arabia*
[e] *Radiological Sciences Department, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia*

Background-aim

A main advantage of using point-of-care (POC) blood glucose devices is that the results are immediately available to the patient so that urgent medical decisions about further diagnostic and therapeutic procedures can be made.

Methods

Three different interferences could be present in the patient's sample: ascorbic acid, hematocrit (HTC) and galactose were evaluated at a variety of glucose levels. Three different POC glucometers—StatStrip from Nova Biomedical, Accu-Chek Inform II from Roche and FreeStyle from Abbott—were compared using reference method cobas 8000 (hexokinase method). Approximately 19 venous blood samples were collected into heparin tubes from a single healthy male donor. The percentage differences were calculated at different glucose levels with the presence of these interferences in different levels and concentrations.

Results

The presence of interferences were evaluated in three POC devices by comparing the reference method at different glucose levels. HTC interference showed significant variation on two glucometer devices at different glucose levels. The percentage difference for the Accu-Chek Inform II was 25–43%, while the Freestyle was 21–37%. The StatStrip did not show any significant difference. Galactose interference significantly affected the Accu-Chek Inform II device, which gave a 34–446% variation at all glucose levels. Furthermore, the Freestyle showed about a 20% variation at low glucose levels, while the StatStrip shows good agreement with the reference method. Ascorbic acid interference affected all POC glucometers used in this study.

Conclusions

The correlation of the POC glucometer with the reference method shows significant bias as a result of the presence of the interferences, which affect the sensitivity of the glucometer. According to the Clinical and Laboratory Standards Institution (CLSI) and the International Organization for Standardization (ISO) recommendation for the comparison between POC glucometer and reference laboratory method, the variation for glucose results less than 4.5 mmol/L should be within 5% and ±20% for more than 4.5 mmol/L. Therefore, in this study, the effect of interreferences appeared clearly in the Accu-Chek Inform II and Freestyle devices. All interferences that could affect the glucose measurement should be taken into consideration before using a POC glucometer in a hospital setting.

doi: 10.1016/j.cca.2022.04.580

T113

Are repeat measurements required for critically high POCT glucose results?

J. Shaw

Division of Biochemistry, The Ottawa Hospital and Eastern Ontario Regional Laboratories Association, Department of Pathology and Laboratory Medicine, The University of Ottawa, Canada

Background-aim

It is common practice in the clinical laboratory to repeat critical results prior to verification. This is to prevent clinical decision making on a potentially inaccurate result. In our organization, a policy exists such that all critical point-of-care (POC) glucose results must be repeated prior to clinical action, in particular critically high results where treatment of insulin inappropriately could be very dangerous. The repeat can either be by the glucose meter or by sending a specimen to the central laboratory. Audits have revealed that compliance with this policy by clinical operators is low. In light of this, we sought to determine whether repeat of critical POC glucose results is necessary.

Methods

All POC glucose data from January-June 2018 were audited across the sixteen hospitals part of the Eastern Ontario Regional Laboratories Association. These range from small rural hospitals to large tertiary academic centres. This comprised approximately 340,000 POC glucose results. The data were extracted from Roche Cobas IT1000 POCT middleware at each hospital. Repeat of critically high glucose results either by POCT or in-lab was audited.

Results

There were 1022 critically high glucose results during the audit period across the sixteen hospitals. A critically high measurements is defined as >28 mmol/L in adults. Repeat measurements occurred for 358 of the critical results (35%). Where repeat measurements were performed, 24% of the repeat results were found to differ significantly from the initial result (ε 20%). A prospective audit of all critically high POCT glucose results from September to November 2019 identified twenty-one discordant repeat results. Follow-up with the Nurses performing these tests identified the top reason for discordance as the patient's hands not being cleaned sufficiently prior to the initial measurement.

Conclusions

The findings here suggest that repeat of critical POCT glucose results prior to taking clinical action is an important step in preventing the use of potentially inaccurate measurements. Continuous audit, education and follow-up with clinical operators performing POCT is key to maintaining compliance with policies and procedures.

doi: 10.1016/j.cca.2022.04.581

T114

Europium based, quantitative, point of care immunoassay, Fluoro-CheckTM for measurement of procalcitonin has a high correlation with Elecsys® BRAHMS PCT

S. Chae, J. Chung, H.J. Huh

Department of Laboratory Medicine, Dongguk University Ilsan Hospital, South Korea

Background-aim

Procalcitonin (PCT) is a biomarker of bacterial infections and increasingly used for early risk stratification with suspected sepsis and pneumonia. Point-of-care (POC) test is performed for early decision-making, POC for PCT is useful to diagnose bacterial infection. However, most POC devices for PCT measurement are semi-quantitative methods, they have limitations to monitor serial PCT level to evaluate antibiotics response. The Fluoro-CheckTM PCT (Nano-Ditech Corp, NJ) is a Europium based immunoassay for the quantitative determination of PCT. In accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines, within-laboratory precision of Fluoro-Check PCT is less than 15% coefficient of variation at a low, mid-, and high level. We compared Fluoro-Check PCT with Elecsys BRAHMS PCT (Roche Diagnostics, Mannheim, Germany) using clinical samples.

Methods

The study was approved by the Institutional Review Board and a total of 125 specimens, which PCT results by Elecsys BRAHMS PCT ranged from 0.03 to 16.44 ng/mL, were evaluated. 10 uL of serum sample transferred into the sample well. After adding sample, 2 drops of developer solution applied onto the developer well immediately and the PCT molecules in the sample bond to both biotinylated and Europium particle coupled with anti-PCT antibody at the end of the membrane. Test kits were inserted into Fluoro-Checker TRF reader, PCT results were derived by analysis of fluorescence intensity, proportional to the concentration of PCT. Data from the Fluoro-Check PCT were compared with the results using cobas E601 analyzer (Roche Diagnostics) according to CLSI EP 09-A3.

Results

The range of Fluoro-Check PCT values was 0.08 – 18.08 ng/mL and distribution was as follows; 21 samples with < 0.5 ng/mL, 33 samples with 0.5-2.0 ng/mL, 56 samples with 2.1 -10.0 ng/mL, and 15 samples with >10.0 ng/mL. Passing-Bablock regression analysis of Fluoro-Check PCT and Elecsys BRAHMS PCT showed a high correlation. Correlation coefficient was 0.921 (95% confidence interval [CI], 0.889 ~ 0.944), slope was 1.236 (95% CI, 1.181 ~ 1.299), and intercept was -0.046 (95% CI, -0.1339 ~ -0.008).

Conclusions

Fluoro-Check, the europium-based fluorescence immunochromatographic assay, has the advantages of POC. Fluoro-Check PCT showed a high correlation with Elecsys BRAHMS PCT at the measurable range of 0.08-18.08 ng/mL. Quantitative analysis of PCT using Fluoro-Check appears useful for clinical applications to diagnose and monitor patients with bacterial infection.

doi: 10.1016/j.cca.2022.04.582

T115

Hematocrit is a predictive marker for inaccurate interpretation of arterial blood gas analysis caused by inadequate sample mixing

H. Hong [a], E.J. Lee [b], J. Hong [c], H. Kim [c], W. Lee [c], S. Chun [c], W. Min [c]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, Hallym University College of Medicine, Dongtan Sacred Heart Hospital, Hwaseong-Si, Republic of Korea*
[c] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

Because arterial blood gas analysis (ABGA) is performed using whole blood, mixing of specimens prior to analysis is required to reverse any settling of erythrocytes that may have occurred. However, no recent studies focused on the effects of inadequate mixing on ABGA. Also, there is no known indicator for verifying proper sample mixing when interpreting ABGA. We intended to investigate the effect of inadequate mixing and identify an indicator for confirming that sample had been mixed properly in ABGA testing.

Methods

One hundred samples were kept vertically for 1 h to create a condition in which they were not mixed. Eleven ABGA parameters were measured using a RAPIDPoint 500 in unmixed status. Within 5 min of these measurements, the samples were mixed according to IFCC guidelines and then the same parameters were again measured in mixed status. The measured values of the two groups were compared using paired t-test. To find the indicator for sample mixing, the correlation analysis was performed between the difference of ABGA parameters and that of hematocrit, which reflects the homogeneity of sample. We confirmed concordance of the ABGA interpretations between the two different mixing status.

Results

Between the two mixing status, the pCO_2, pO_2, HCO_3^-, potassium, calcium, glucose, lactate, and hematocrit showed significant differences (all $P < 0.001$ except calcium: $P = 0.04$). Among them, the differences of calcium, glucose, HCO_3^-, and potassium had significant correlations with that of hematocrit (all $P < 0.001$). Between the two different mixing status, 35.2% (37/105) samples showed discrepancies in the interpretation of ABGA results.

Conclusions

We confirmed that inappropriate mixing can have significant effects on the ABGA measurement and their interpretations. The recently introduced ABGA instrument measures hematocrit simultaneously, of which difference correlate with those of other ABGA parameters. It is necessary to perform ABGA with hematocrit to assess whether samples have been mixed appropriately.

doi: 10.1016/j.cca.2022.04.583

T116

Performance evaluation of a point-of-care testing program operational at 30 sites with six sigma metrics – Experience from a tertiary care hospital in Pakistan

S. Shakeel, S. Ahmed, L. Jafri, S. Ahmed, A.H. Khan

Department of Pathology and Laboratory Medicine, The Aga Khan University Karachi, Pakistan

Background-aim

Point-of-care (POC), or bedside, testing is defined as medical testing at or near the site of patient care. Six Sigma concepts are adopted by clinical laboratories for the evaluation of their performance and to improve quality management. The aim of this study was to assess the performance of eight routine biochemical parameters available on POC testing based on a sigma scale by calculating the sigma metrics scores.

Methods

In total, 30 POC testing sites, working under the section of clinical chemistry, department of Pathology and Laboratory Medicine, the Aga Khan University Karachi were included in this study from January-July 2019. Average six Sigma metrics score were calculated for Glucose, ABG analysis (i.e. pCO2, pH, pO2), Potassium (K), lactate, Sodium (Na) and Chloride (Cl). Glucose and the other parameter evaluated were analyzed using ACCU CHEK INFORM II (Roche) and COBAS B221 (Roche), respectively. For comparison a target instruments was pre-selected as a gold standard meeting the analytical specifications against all POC testing instruments. Estimation of sigma metrics of each analyte was done using the formula i.e. Sigma Metrics = (Total Allowable error (TEa) - Systematic Error / Random Error

Random error was determined from cumulative SD, CV and mean of routine quality control data while systematic error was estimated from slope and intercept from linearity study done as per part of method validation. The sigma metrics of each analyte at various medical decision points were calculated using EP evaluator (version 10.3.0.556). A sigma metric of 6 or greater indicates a 'six sigma' process while 4-6, 3-4, 2-3 and below 2 sigma metric scores represents good, fair, marginal and unacceptable performances respectively.

Results

Average six sigma metrics score for Glucose, pCO2, pH, K and lactate were 8.2, 13.1, 8.3, 9.9, and 10.1 respectively indicative of 'six sigma'. Whereas, pO2, Na and Cl sigma metrics scores were between 4 and 6, which also indicates good performance although a chance of improvement exists.

Conclusions

Sigma metrics analysis provided a vital benchmark for internal quality control, address poor assay performances, and assess the efficiency of existing POC testing program.

doi: 10.1016/j.cca.2022.04.584

T117

Drug dosing using EGFR: Misclassification due to metamizole interference in a creatinine assay

L. Bojko, M.F. Moss, G.d.P. Ripka, C.L. Borges, E.A. Krum, L.M. Dionísio

Clinical Laboratory, Universidade Estadual de Ponta Grossa, Hospital Universitário Regional dos Campos Gerais, Brazil

Background-aim

To evaluate the impact in patient stratification by the estimated glomerular filtration rate for drug dosing of two antimicrobials.

Methods

An observational study was conducted in a cohort of 108 hospitalized patients who had metamizole prescription at fixed intervals of 6 hours. Serum creatinine was determined by enzymatic dry chemistry and Jaffé assays and, then, the estimated glomerular filtration rate was calculated using the CKD-EPI equation. The patients were stratified in different groups according to the estimated glomerular filtration rate for drug dosing of vancomycin and meropenem.

Results

Creatinine values were significantly lower in measurements performed using the dry chemistry enzymatic method when compared to Jaffé assay ($p < 0.0001$), suggesting a negative interference of metamizole. A significant bias (-40.3%) was observed between those two methods, with a mean difference exceeding the acceptable clinical limit (-3.4%). This variation led to a significant difference ($p < 0.0001$) in patient classification according to renal function using the estimated glomerular filtration rate for antimicrobials dosing.

Conclusions

For patients under metamizole treatment, stratification for drug dosing by the estimated glomerular filtration rate is not reliable when the creatinine measurement is realized by dry chemistry. This demonstrate the importance of cooperation between clinical and laboratory staff, seeking for solutions that could mitigate this interference and result in adequate pharmacotherapy.

doi: 10.1016/j.cca.2022.04.585

T118

Lactate and lactate clearance as predictors of adverse outcome in patients with sepsis and septic shock

P. Peneva [b], Y. Bocheva [b], S. Nikolova [a]

[a] *Department of Social Medicine and Healthcare Organization, Medical University – Varna Bulgaria*
[b] *UMHAT "St. Marina", Medical University – Varna, Bulgaria*

Background-aim

Septic is a life-threatening complication and the most common cause of death in patients with infections, especially when it is not identified and treated promptly. Early identification of elevated serum lactate levels can potentially lead to timely identification of patients who are

in danger of poor outcomes. The aim of this study is to explore and compare the prognostic accuracy of the lactate levels and lactate clearance at 0, 6, 24 hours for mortality in SEPSIS-3 defined patients with sepsis and septic shock.

Methods

Prospective single-center clinical follow-up was conducted. We enrolled 45 patients covering the sepsis criteria, according to SEPSIS-3 (2016). All participants have signed a written consent. About 26 of them were with sepsis and 19 patients were in septic shock during admission. Serum lactate levels and lactate clearance were measured at initial, at 6 hours and 24 hours from sepsis recognition on GEM 3000 Blood gas analyzer. Lactate clearance was calculated as [(initial lactate – 6-hr (or 24-hr) lactate)/initial lactate] × 100.

Results

A significant difference between the survivals and nonsurvivals was found at 6 and 24 hours lactate level ($t = -2.235$; $p < 0.05$ vs $t = -2.521$, $p < 0.016$). Our results showed higher mean values of lactate levels in sepsis schock patients with highes levest at 6-hours lactate (6.03 ± 2.91). About 62% of patients died (30-day mortality in sepsis patients was 28.9%; in sepsis schok patients mortality was 33.3%). Lactate clearance at 24-hours was a significant predictor for survival in both sepsis shock (($Exp(B) = 6.965$, $p = 0.014$) and sepsis patients ($Exp(B) = 4.603$, $p = 0.030$). Lactate at 0h –AUC 0.833 (95% CI 0.739 – 0.927, $p = 0.0001$), 6h – AUC 0.843 (95%, CI 0.758 – 0.928, $p = 0.0001$), and 24h– AUC 0.821 (95% CI 0.728 – 0.915, $p = 0.0001$) had a significant predictive value for mortality. Lactate at 24h between 2.45 and 2.60 mmol/L had the greatest sensitivity (79%). The prognostic values of lactate clearance had not significant values for mortality.

Conclusions

Our results show that lactate and lactate clearance are significant prognostic markers for mortality. The hour of lactate and lactate clearance can be used as effective and early lactate-guided resuscitation in tissue hypoperfusion sepsis.

doi: 10.1016/j.cca.2022.04.586

T119

Current status and future at GCMS immunodiagnostics platform (quantum dot)

J. Jeon, N. Ha, T. Kim, A. Song

Fluorescent Immuno Diagnostics Unit, R&D Center, GCMS, South Korea

Background-aim

The trend in recent POCT is not to determine the results by a naked eye, but to determine the results through measurement data provided using equipment.

Because equipment provides quantitative results, it is possible to obtain more objective test results than visual inspection.

Since quantitative results provide higher accuracy and precision than conventional qualitative results, various quantitative diagnostic devices have been launched in the market.

However, the released diagnostic devices have disadvantages such as difficulty in distribution (need a refrigerated storage), high test cost (high production cost than rapid kit, expensive equipment), and incapable of easy carrying (benchtop size).

Methods

The diagnostic accuracy and precision of the device depends on the performance of the signal generator and the measuring device.

Therefore, GCMS is developing a next-generation diagnostic platform with high diagnostic accuracy by introducing fluorescent nanoparticles that generate strong fluorescent signals and developing equipment to effectively measure them.

We applied a bead with a large amount of quantum dots to the surface to obtain a strong fluorescence signal to the lataeral flow immunoassay.

Since quantum dot beads are signal complexes, they are not only very strong fluorescent signals but also metal nanoparticles, which have the advantage of room temperature distribution.

To measure the generated fluorescent signals, a portable measuring device (filter simplification, LED light source) was manufactured and a signal algorithm was developed to distinguish the specific signal from a background signal.

Results

The diagnostic device under development at GCMS provides quantitative test results with high accuracy and precision within 15-20 minutes at the medical field.

Additionally, the test device can be stored and distributed at room temperature, and the measuring equipment can be supplied at a competitive price compared to other companies.

Conclusions

GCMS will apply the next generation fluorescent platform to various items (respiratory virus, inflammation level, mosquito-mediated virus, myocardial infarction), starting with the thyroid hormone measurement kit, and aims to provide more accurate and accurate test results.

doi: 10.1016/j.cca.2022.04.587

T120

Comparison between serum calcium levels measured using direct ion-selective electrodes and photometric method in automated analyzers

S. Datta [a], P. Chopra [b]

[a] *All India Institute of Medical Sciences, New Delhi, India*
[b] *Dr Lal Path Lab, NRL, New Delhi, India*

Background-aim

Serum calcium is measured by photometric methods or using ion selective electrodes (ISE). ISE measures free ionized Ca (FCa), which is not bound to proteins like albumin and is corrected using algorithms to calculate total calcium, TCa (TCa_calc). TCa obtained by photometry (TCa_meas) require correction for albumin and several equations are used to give corrected Ca (TCa_corr).

In this study we aim to find the agreement between total calcium levels calculated from direct ISE results (TCa_calc) and total calcium levels obtained by spectrophotometric methods after correction using formulae given in the literature (TCa_corr) at different levels of serum albumin.

Methods

In this study 332 serum samples were analyzed for TCa and albumin on Roche Modular P800 by ortho-cresolphthelein and bromocresol green methods respectively; and FCa by direct ISE on XI-921, Caretium and converted to TCa_calc. The results of TCa_calc and TCa_corr were compared using paired t-test.

Results

Significant difference was observed between TCa_calc (2.45 ± 0.34mmol/L) and TCa_meas (2.07 ± 0.27mmol/L). TCa_meas was corrected for albumin using several commonly used formulae. However, significant differences between TCa_calc and TCa_corr still existed. The cases were further subdivided into three groups on the basis of serum albumin however, significant differences were observed between TCa_calc and TCa_corr values in all subgroups.

Conclusions

With the infiltration of point of care (POC) devices in casualties and ICUs awareness needs to be raised amongst clinicians regarding the potential mis-interpretations of the tests involved. Caution should be exercised while interchangeable usage and interpretation of serum Calcium levels from direct ISE vis-a-vis photometric methods. Regulatory guidelines to the same effect may also be considered.

doi: 10.1016/j.cca.2022.04.588

T121

Evaluation of biochemystry markers for sepsis in newborns with asphyxia

A. Sofijanova, O. Jordanova , S. Naunova Timovska, M. Kimovska, H. Mangjukovska, T. Voinovska, A. Janchevska, S. Neskova

University Children hospital-Skopje

Background-aim

The aim of this study was to investigate the predictive values of biochemical parameters, including Procalcitonin (PCT), C reactive protein (CRP) as a early diagnostic and prognostic marker for sepsis in newborns with asphyxia.

Methods

In this study we included all risk neonates with severe asphyxia suspected of having early- or late-onset neonatal sepsis admitted to Pediatric Intensive Care Unit at the University Children Hospital in Skopje. (PCT) and CRP, one serum blood sample was obtained from each patient at the 24h at admission and day 3-6. Procalcitonin levels were measured by using a immunoassay system Vidas based on the Enzyme Linked Fluorescent Assay (ELFA) principles. CRP levels were measured by using immunoturbidimetric method Architect c4000 Abbott.

Results

Fifty five newborns were recruited. At first 24 hours of the admission . PCT levels were significantly increased in 16 asphyxiated preterm newborns (ε10 ng/mL) Increased PCT levels significantly correlated with positive blood culture. PCT levels were increased in 39 term newborns (ε2 ng/mL), 20 with positive blood culture an 19 with negative blood culture. The values of C-reactive protein gradually increase after 12hours at admission in all 55 newborns. Preterm newborns was the most common predisposing factor for severe sepsis and septic shock. The second measurement after 3-6 days is a parameter, who show us whether an appropriate antibiotic for the treatment is used, and develop of antibiotic resistants.

Conclusions

The levels of PCT and CRP has important clinical significance in predicting the prognosis of asphyxed newborns with sepsis, to prevent the development of severe sepsis and septic shock.

doi: 10.1016/j.cca.2022.04.589

T122

Better quality management and greater operating efficiency with GEM® PREMIER™ 5000 with intelligent quality management 2 (IQM®2) at Yokosuka General Hospital, Uwamachi (Kanagawa, Japan)

S. Yamazaki

Yokosuka General Hospital, Uwamachi, Japan

Background-aim

Uwamachi Hospital is one of the main hospital in this area where providing acute care medicine with 417 beds. Its blood gas testing program manages approximately 20,000 patient tests annually. For many years, these tests were run on three traditional benchtop analyzers located in the Central Laboratory as well as in several point of care (POC) locations.

The Laboratory team at Uwamachi Hospital aimed to improve quality management and testing efficiency in POC blood gas testing by transitioning from traditional, bench-top analyzers to the GEM Premier 5000 with iQM2 system. After implementing the new systems in POC, the team aimed to assess improvement metrics for quality and efficiency.

Methods

Data was collected for 3 months on both methodologies—GEM Premier 5000 (3 analyzers) and traditional blood gas systems (2 analyzers). The results were assessed and annualized for maintenance, troubleshooting and service interventions required as well as quality management performance. Instrument activity logs and user testimonials were collected and analyzed to quantify the usability impact after implementation of GEM Premier 5000 with iQM2 systems.

Results

Estimated savings in terms of component replacement demonstrated a reduction 212 to 60 annually; downtime for manual maintenance, troubleshooting, and service were reduced from 150 to 7 hours—nearly 97%.

Additionally, iQM2 automatically detected, corrected, and documented 47 sample-specific or systemic errors out of 1,867 patient samples during the period of study, representing a 2.52% error detection rate that traditional systems could have missed.

Conclusions

Introducing the new GEM Premier 5000 with iQM2 systems has enabled Uwamachi Hospital to realize a significant reduction in analyzer

maintenance, including instrument/component replacement, troubleshooting, hands-on system management and analyzer downtime.

This has enabled the Laboratory team to spend more time on value-added initiatives for the hospital and better support our clinical teams by delivering timely, high-quality results at the POC and Central Laboratory.

doi: 10.1016/j.cca.2022.04.590

T123

Verification Nova Stat Strip Xpress2 glucose meter in Tartu ambulance foundation

A. Ivanov [b], I. Pastarus [a], A. Kõrgvee [a]

[a] *Anesthesiology and Intensive Care Clinic, Tartu University Hospital, Estonia*
[b] *United Laboratories, Tartu University Hospital, Estonia*

Background-aim

Ambulance Foundation (AF) are often focused on rapid diagnostic and treatment of patients with acute and urgent illnesses and injuries. The objective of this study was to verify professional StatStripXpress2 glucose meter (Nova Biomedical, USA) of Tartu AF. The POCT12 guideline states that 98% of glucose meter results should be within 0,83 mmol/L of the result for glucose $<4,2$ mmol/L and within 20% for glucose ε4,2 mmol/L. The FDA guidance (Blood Glucose Monitoring Test Systems for Prescription Point-of-Care Use) states, that 100% of the results should be within 0,83 mmol/L of the result for glucose $<3,88$ mmol/L and within 20% for glucose ε3,88 mmol/L. We assessed the performance of glucose meter by using the spreadsheet program for estimating the bias between two methods using patient samples. The verification of the precision performed by using control samples (Nova Biomedical, USA).

Methods

Rapidpoint 500e (Siemens, USA) as a comparative method were used for the study. According to EP09 protocol, 40 whole blood heparinized samples were analyzes by duplicate. Precision of glucose meter estimated by measuring of three levels of quality control samples measured in five replicates during 5 days (EP15).

Results

The linear regression analysis of comparison demonstrated a slope and intercept of 0,96 and 0,20, respectively. The results correlated well (R2 = 0,992) and demonstrated that StatStrip Xpress2 and Rapidpoint 500e had no significant bias (p-0,315). The range of glucose was from 0,6 to 16,4 mmol/L, mean glucose concentration was 6,59 mmol/L and the mean bias was -0,06 mmol/L (-0,3%). Absolute maximum difference of results $<4,2/3,88$ mmol/L was 0,3 mmol/L and relative maximum difference of results ε 4,2/3,88 mmol/L was 10%. The intralaboratory imprecision of glucose across three levels (3,4/6,4/16,7 mmol/L) was 3,8/3,0/1,9 %. The glucose meter results corresponded to a confidence level of 95% was 3,4 $\pm$0,23 mmol/L, 6,3 $\pm$ 0,28 mmol/L and 16,3 $\pm$0,60 mmol/L.

Conclusions

Stat StripXpress2 glucose meter met POCT12 and FDA performance criteria and demonstrated a close correlation to the laboratory method. Precision of meter was within manufacture claims. StatStripXpress2 glucose meter is suitable for use in Tartu Ambulance Foundation.

doi: 10.1016/j.cca.2022.04.591

T124

Hormonal biomarkers associated with in-hospital mortality in therapeutic hypothermia after cardiac arrest: A case series

D. Morell-Garcia [c], J. Rodriguez-Pilar [a], M. Ferreruela-Serlavos [a], M.A. Ballesteros-Vizoso [c], P. Argente Del Castillo [c], J.M. Bauça [b], B. Barceló [b], J. Colomina-Climent [a], A. Barceló [c]

[a] *Critical Care Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[b] *Health Research Institute of Balearic Islands, Palma de Mallorca, Spain*
[c] *Laboratory Medicine Department, Son Espases University Hospital, Palma de Mallorca, Spain*

Background-aim

Some studies have reported lower mortality in cardiac arrest patients treated with therapeutic hypothermia (TH), patient's core temperature with a target between 32 and 36°C, compared to those not receiving such treatment. In addition, typical for critical illnesses are substantial alterations within the hypothalamic–anterior pituitary–peripheral hormonal axes that are proportionate to the risk of poor outcome.

The aim of this study is to detect those hormones that are associated with mortality in patients with recovered cardiac arrest (RCA) who have undergone TH.

Methods

A retrospective observational study, in a tertiary care hospital, between March 2013 and May 2015 was done. We recruited adult RCA patients admitted consecutively into Intensive Care Unit (ICU) and subjected to TH protocol, consisting of a drop in the first 2 hours after RCA at 33°C, and maintaining that temperature until after 24 hours, where it recovers 1°C every 3 hours until euthermia. Serum samples were collected at the basal time and at each temperature change. Hormonal biomarkers measures were full thyroid profile, prolactin, cortisol, testosterone (Architect i2000 SR, Abbott Diagnostics,US), and adrenocorticotropin (ACTH) (Immulite 2000 XPi, Siemens, Germany). A univariate analysis was performed using U Mann-Whitney test, considering a statistical significance of 5%.

Results

A total of 49 patients were included (78% males, mean age of 54 $\pm$ 12 years old), no age nor gender differences found between subgroups. Five patients died during hospital admission, 60% men, which represents 10.2%. Of those, only one patient was alive at the end of TH. We found, at 26 hours after TH starting, significant differences between non-survivors vs survivors group in free thyroxine (fT4) levels [0.87 vs 1.03 ng/dL;p = 0.04], free triiodothyronine (fT3) [1.35 vs 1.94 pg/mL; p < 0.001]. No differences were found in the rest of the hormones studied, especially in TSH levels [1.11 vs 1.05 μUI/mL; p = 0.936] probably due to the delayed kinetics of changes in this hormone.

Conclusions

For recovered cardiac arrest patients treated with therapeutic hypothermia at the ICU, decrease in circulating fT3 and fT4 levels, at

the end of the target temperature time, could be useful as prognostic factors of in-hospital mortality.

doi: 10.1016/j.cca.2022.04.592

T125

impact of the Covid-19 crisis on the point-of-care blood gases management in an ISO 22870 accredited laboratory in Paris (France)

M. Vaubourdolle, B. Beneteau-Burnat, C. Aussel, P. Pernet

Department of Biochemistry, DMU BioGeM, Hospital Saint Antoine, AP-HP. SU, Paris, France

Background-aim

Our laboratory is accredited for point-of-care (POC) blood gases activities according to the ISO 22870 standard. When, in March 2020, the Covid-19 crisis hit France, risk assessment was done to adapt POC management to the setting up of 3 new dedicated Covid-19 intensive care units.

Methods

We used our change management procedure based on risk assessment management (CLSI EP-23) and prioritization of risks (criticality scale) to reveal new risks to take into account:

Material: - Insufficient number of blood gases devices. - Shortage of reagents requiring anticipating the potential peak of analysis (units full of ventilated patients).

Manpower: - Newly trained-empowered people recruited for the Covid-19 crisis requiring to derogate from the usual training procedure to increase the rate of operational users. - Staffing shortage in the clinical units or in the laboratory due to illness.

Methods: - Reduced initial analyzer performance check for quick commissioning. - Necessity of indicators to monitor the impact of derogations to usual procedures and to verify the adequacy with the clinicians needs.

Environment: - Potential impact of SARS-CoV-2 on analyzers management (device contamination, waste, protection of users).

Results

Material: One GEM 4000 was put back into service and 2 additional GEM 5000 (Werfen) were ordered and put into service the day of reception 9 days later. Reagent orders were tripled to avoid shortage due to potential future manufacturer's deficiency. Eventually, POC blood gases activity increased +300% at the peak of the crisis in April without any particular problem. Manpower: 35 new users were trained-empowered with a quick-training procedure. A punctual lack of trained user never happened and daily monitoring of rejected analysis (new indicator) showed even better results than expected. Methods: Indicators allowed to verify that specific requirements of ISO 22870 were still achieved. Environment: No special procedure was required for the analyzer itself aside the general procedure for COVID-19 clinical unit management.

Conclusions

Our change management procedure allowed our ISO 22870 accredited laboratory to add these new locations/POC analyzers to our scope of accreditation during the peak of the COVID-19 crisis.

doi: 10.1016/j.cca.2022.04.593

T126

Prospective comparison of point-of-care INR versus plasma INR in supra-therapeutic INR values

M. Molina-Arrebola [c], J. Ruiz-Cara [b], S. Guardia-Alés [b], I. Portel-Rigo [b], Á. Alba-Sosa [c], R. Pérez-Moyano [c], M. Giménez-López [c], C. Avivar-Oyonarte [a]

[a] *Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

[b] *Clinical Analysis Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

[c] *Haematology and Haemotherapy Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

Background-aim

Point-of-care testing (POCT or bedside testing) provides faster results than those obtained in hospital laboratory analyzers, but it should not compromise quality and safety. Compared to arterial and venous blood, capillary blood can easily be collected from the fingertip and does not require a skilled worker. Therefore, it has excellent potential as an ideal blood source for disease diagnosis and health monitoring. In coagulation, these systems are very useful for obtaining the INR test to monitor vitamin K antagonist (VKA) oral anticoagulant therapy. Xprecia XtrideTM(Siemens®) is a POC system with an ergonomic design, touch screen and intuitive software that, after performing an automatic calibration and two quality control checks on each test strip, provides the INR from analyzing a 6 l sample of capillary blood, reporting data between 0.8 and 8. It uses Innovin® reagent with ISI 1. Protocol dictates that a POC device should be assessed for reproducibility against a central laboratory analyzer since INR is essential for proper oral anticoagulation management. Regarding the NCCLS guidelines, there is an statistical equivalence of INRs obtained from POC systems - within 0.4 for a target INR of 2.5 and within 0.7 for a target INR of 3.5. Generally, results with an INR exceeding 5.0 have reduced trueness, precision, and linearity, both in POC and laboratory-based testing.

Aims: This is a comparative study of INRs obtained using a POC system (POC-INR) compared to INRs obtained using plasma from venous samples collected in citrate tubes (P-INR), both taken from the same patient at the same time. In previous studies undertaken at our hospital, we found linearity between both methods and no statistically significant differences. In this study, our intention was to demonstrate if such a correlation existed exclusively in supra-therapeutic INR results.

Methods

POC-INR was obtained from a capillary sample analyzed with POC Xprecia XtrideTM(Siemens®). If the INR was above 3.0, a venous citrated sample was taken and a P-INR was obtained from an ACL-TOP500 hospital analyzer (Werfen®), using the reagent Hemosil Recombiplastin® ISI 1. The results were compared with the Medcalc statistical package to calculate the Pearson correlation coefficient and non-parametric Passing-Bablok regression analysis, suitable for method comparison studies.

Results

We took 215 POC-INR samples that were higher than 3.0, and also took P-INR from the same patients at the same time. A correlation coefficient of $r = 0.7698$with a 95% confidence interval (0.7091 to 0.8192) was obtained, with the regression equation $y = 0.160 + 0.967$ X, where

y = P-INR, x = POC-INR: a = 0.160 CI 95% (-0.4238 to 0.6581), b = 0.9667 CI 95% (0.8762 to 1.0692) with a value of p = 0.73; this determined that there were no significant differences regarding linearity deviation between the two techniques.

Conclusions

The POCT Xprecia XtrideTMsystem (Siemens®) offers INR results that are statistically comparable to those obtained from plasma in a laboratory analyzer, even with supra-therapeutic INR values.

POCT allows patient accessibility for the monitoring of VKA oral anticoagulant therapy. Being able to transmit the results via the web facilitates control decentralization in the health centers and allows validation and dosage to be overseen from the hospital.

The system offers a valid alternative to the historical pattern of testing being mostly confined to the medical laboratory and provides continuous improvement in patient care quality.

doi: 10.1016/j.cca.2022.04.594

T127

Gasometric and hematological chances in use gentamic 20 MG and ampicilin 500 mg by patients in a neonatal intense care

V.C. Parreira [a], L.D.d. Peder [b]

[a] *Academic in Centro Universitário Assis Gurgz, Brazil*
[b] *Doctor teacher in Centro Universitário Assis Gurgcaz, Brazil*

Background-aim

Background and Objectives: This study aimed to define the main changes in arterial blood gases and hemogram tests in patients who used ampicillin 500 mg associated with gentamicin 20 mg, in a Neonatal Intensive Care Unit of a private hospital in Cascavel, Paraná, in the period from July to October 2020, classifying the acid-basic disorder of blood gases and changes in the blood count. Methods: Using the Devenport diagram, the Winter equation and the Handerson-Hasselbalch equation, the primary disorder and the secondary compensation in arterial blood gas tests were classified. In the hemogram, the alterations were classified according to the literature and laboratory values. Results: From a total of 34 patients analyzed, 27 made the association of aminoglycosides and penicillins, these being represented by gentamicin and ampicillin, respectively. Of those. 11 had arterial blood gases and blood counts collected either on the same day or within a maximum of 3 days between exams. A total of 19 blood gases and 21 blood counts were analyzed. Of the blood gases that had a primary acid disorder, 6 corresponded to mixed acidosis, 5 were metabolic acidosis with adequate respiratory compensation, and 1 metabolic acidosis associated with respiratory alkalosis. Of the alkalosis as the primary disorder, there were 2 mixed alkalosis, 4 respiratory alkalosis with adequate metabolic compensation, and 1 respiratory alkalosis associated with metabolic acidosis. Of the hemograms, 17 had anisocytosis, 12 had macrocytosis, 12 had hyperchromia. Conclusion: The present study suggests that patients who used the association of 500 mg ampicillin and 20 mg gentamicin present changes in blood gas analysis and blood count that relate.

Keywords: Antimicrobials. Arterial Gasometric. Blood Count. Intensive Care Unit

Methods

The study was conducted in a private hospital located in the municipality of Cascavel - PR. Data collection was performed in July, August and September 2021. The project was approved by the Ethics Committee on Human Research of the Assis Gurgacz Foundation University Center (FAG), under protocol number CAAE: 44966921.0.0000.5219, opinion number 4.779.475, approved on June 14, 2021.

A quantitative descriptive research was carried out, starting with the printing of a report of antibiotic consumption in the neonatal ICU during the period from July to October 2020, showing which antibiotic was released, the quantity, patient and care, provided by the Tasy® health management system. The information was then tabulated in Microsoft Office Excel ® 2013. After the tabulation, the report was compared with the quantity in the patients' electronic prescriptions, and each visit was accessed to confirm the quantity dispensed.

In Tasy®, the "samy" was accessed to access the blood gas and blood count tests of the patients. Only patients who used the combination of ampicillin 500 mg and gentamicin 20 mg were selected. To delimit the study, only patients who had blood gas and CBC either collected on the same day or at a maximum interval of 3 days were selected.

For the interpretation of the CBC, during the use of the antimicrobial, the laboratory reference values for erythrogammogram, leukogram and platelets were considered, scoring possible changes such as thrombocytopenia, anisocytosis, leukocytosis, anemia, neutrophilia, and if possible analyzing the clinical evolution of patients during the therapeutic cycle of the antimicrobial.

For arterial blood gas analysis, the laboratory reference values for Pco2 (partial pressure of co2) and HCO3 - (bicarbonate) were considered, and the results were interpreted using the Davenport diagram, the Handerson-Hasselbalch equation, and the Winter equation, which allow us to assess the primary disturbance and analyze secondary compensation.

Metabolic acidoses as the primary disorder: Expected Pco2 = (1.5* patient's HCO3 - + 8) ± 2, where HCO3 - is given in mEq/L and Pco2 is given in mmHg. The equation returns an expected value for the patient's Pco2. After, the actual value is compared with the expected value, one of the following situations occurs:

The two values are similar: Adequate respiratory compensation.

Actual Pco2 is higher than expected: Associated respiratory acidosis.

Actual Pco2 is lower than expected: associated respiratory alkalosis.

In metabolic alkalosis as the primary disorder for the secondary disorder evaluation, the following will be used: The expected value of the patient's Pco2 is Expected Pco2 = (patient's HCO3 - + 15 ± 2) and follow the same classifications of metabolic acidoses.

The classification of respiratory acidoses as a primary disorder, it is observed that for every 10 that the Pco2 goes up the HCO3 - goes up 4, if the midpoint of the Pco2 is 40 in the laboratory reference value and the patient's was 60, he varied 20 above the midpoint. What would be the expected value of the HCO3 -, transcribing it would be:

10 Pco2 ———————— 4 HCO3

20 Pco2 ———————— X

X = 8 which added to the midpoint of the laboratory 24 reference value of HCO3 - we would have the patient's expected hco3 of 32 ± 2, thus defining the secondary disorder with a classification of:

The two values are similar: Adequate metabolic compensation.

HCO3 - actual is higher than expected: Associated metabolic alkalemia.

HCO3 - actual is lower than expected: associated metabolic acidemia.

For respiratory alkalosis as a primary disorder, at the end of the rule of 3 of the above example, the result will be decreased by the midpoint of the reference values of hCO3, leaving 16 ± 2 for the previous example, following the same classifications to define the secondary disorder.

Results

The municipality of Cascavel - PR is located in the western region of Paraná, with a territorial area of 2,101.074 km^2 and an estimated population of approximately 328,454 people, according to data from the Brazilian Institute of Geography and Statistics. 5

From July to October 2020, 615 vials/ampoules of antibiotics were released to the neonatal ICU, being 237 vials of ampicillin 500 mg, 91 ampoules of gentamicin 20mg, 46 vials of Vancomycin 500 mg, 46 bags of Linezolides 600 mg, 44 ampoules of Sulfamethoxazole 400 mg + Trimetropine 80 mg, 40 vials of Meropenems 1 g, 34 vials of Oxacillins 500 mg, 32 vials of Cefepimes 1g, 20 vials of Cefotaximes sodium 1g, 10 vials Polymyxin B 500. 000 IU, 8 vials Piperacillins sodium 4 g + Tazobactams 0.5 g, 6 vials Garamicins 20 mg, and 1 vial Cefazolin sodium 1 g.

Of the 80 cycles performed with the antimicrobial, the association of aminoglycosides and penicillins accounted for a total of 68 %, equal to 54 therapeutic cycles. This therapeutic cycle is a control of the HICC sector (Hospital Infection Control Center), where the physicians who prescribe antimicrobial therapy communicate the sector through a form containing the patient's information, which antibiotic will be used, the dosage, and the number of days that the therapy will last. The combination of gentamicin 20 mg and ampicillin 500 mg showed more metabolic or mixed acid disturbances, when compared to those classified first as respiratory. No metabolic alkalosis was classified. Suggesting that the association of these drugs benefits the non-retention of Pco2 by the lungs preventing respiratory acidosis in the primary disorder, it is also suggested, that it prevents the increase of HCO3 - resulting in metabolic alkalosis in the primary classification of the gasometry, and that despite the fluctuations in the gasometry, we obtained 1 death of the patients analyzed.

Of the acidoses defined as primary disturbance, of the mixed ones, the first patient with weight of 2819 g and gestational age of 34 weeks and 6 days, had his CBC collected 3 days after the gasometry, and presented macrocytosis, hyperchromia, anisocytosis, leukocytosis, eosinocytosis and monocytosis that evolved in 2 days, with the same alterations plus a lymphocytosis. The second, weighing 2690 g and gestational age of 35 weeks and 2 days, had his CBC collected 2 days before the blood gas analysis and presented erythrocytosis, macrocytosis, hyperchromia, neutrophilia, anisocytosis, and lymphocytosis. The third patient, weighing 4120 g and with a gestational age of 40 weeks and 2 days, had a complete blood count (CBC) collected on the same day as the blood gas analysis and showed macrocytosis, hyperchromia, anisocytosis, leukocytosis, neutrophilia, and thrombocytopenia, and within two days evolved to macrocytic anemia, leukocytosis with left deviation, thrombocytopenia, toxic granulations 2+, and mild polychromasia.

Of the metabolic acidoses with adequate respiratory compensation, 4 patients were analyzed. The first with a weight of 1975 g and gestational age of 37 weeks and 2 days, had his CBC collected on the same day as the blood gas and presented an anisocytosis, lymphocytosis, and a monocytosis, which evolved in 2 days to a macrocytosis, hyperchromia, anisocytosis, evolving in 4 days to a macrocytosis, hyperchromia, anisocytosis, leukocytosis, eosinocytosis, and lymphocytosis. The second with a weight of 3280 g and gestational age of 39 weeks and 5 days, collected the CBC on the same day as the blood gas and presented with a leukocytosis, macrocytosis and had rod present on blood extension slide. That progressed the next day to anisocytosis and leukocytosis. The third with a weight of 3370 g and gestational age of 39 semans and 5 days, collected blood gas 2 days after the blood gas and had a macrocytosis, hyperchromia and eosinocytosis. The fourth patient will be described as the first patient of the mixed alkaloses, as he had many fluctuations in the basic acid disturbance during therapy.

The single metabolic acidosis with respiratory alkalosis closes 11 of the 12 primary disorders classified as acidemia. The patient with a weight of 1925 g and gestational age of 33 weeks and 2 days had his CBC collected on the same day as the blood gas and showed one erythrocytosis and one leukocytosis.

Of the alkalosis as a primary disorder, 2 patients had mixed alkalosis as a primary disorder , the first with a weight of 1320 g and gestational age of 32 weeks, which by fluctuation in blood gas will be portrayed here by the disorder of his first blood gas, which presented a mixed alkalosis and had his CBC collected on the same day presented with an erythrocytosis, After 3 days he had metabolic acidosis with adequate respiratory compensation without a CBC on this date and after 37 days he had respiratory alkalosis with adequate metabolic compensation and had his CBC collected on the same day as the blood gas and showed macrocytosis, hyperchromia, anisocytosis, and lymphocytosis. The second, weighing 1915 g and with a gestational age of 35 weeks and 2 days, had his CBC collected on the same day as the blood gas analysis, which had no fluctuations and showed mixed alkalosis. The CBC showed a macrocytosis, hyperchromia, anisocytosis, thrombocytopenia, and a lymphocytosis.

Of the respiratory alkalosis with adequate metabolic compensation, there were 2 patients, where the first, weighing 3600 g and gestational age of 40 weeks and 2 days, presented respiratory alkalosis associated with metabolic acidosis and in the hemogram collected on the same day of the gasometry with an erythropenia, anisocytosis, leukocytosis, neutrophilia and was also observed erythoblasts 3% with a discrete polychromasia. That evolved after 3 days to an erythrocytosis and an anisocytosis with a respiratory alkalosis blood gas with adequate metabolic compensation. The second with weight of 2365 g and gestational age of 35 weeks and 2 days, presented a respiratory alkalosis with adequate metabolic compensation and had the hemogram collected on the same day of the gasometry, presenting an anisocytosis, eosinocytosis and a lymphocytosis that after 3 days presented a leukocytosis maintaining the same basic acid disturbance. In the mixed disorders, the patients that presented mixed acidosis all presented a leukocytosis in the CBC and one of them evolved to an anemic picture with macrocytosis, deviation to the left, thrombocytopenia and toxic granulations and died after 2 days. Of the mixed alkalosis patients did not present leukocytosis, but presented a lymphocytosis that evolved with thrombocytopenia.

Of the patients who had respiratory alkalosis with adequate metabolic compensation, all had lymphocytosis.

Of the 11 patients analyzed, 6 had gestational age less than 37 weeks, categorizing them as premature. They mainly presented with early sepsis. We cannot affirm that the use of antibiotics caused the alterations described in the study, because the patients already presented pathological clinical conditions that alter the analyzed exams.

It is suggested then, that the mixed disorder causes more alterations in the hemogram, being the mixed acidosis the one with the greatest alterations, followed by the alkalosis. Finally, the present study opens discussion for the influence of the basic acid disturbance in the alteration of the hemogram

Conclusions

Despite the small group of patients analyzed, the present study described all the changes in the CBC and blood gas analysis during the use of Ampicillin 500 mg and Gentamicin 20 mg, suggesting that the acid-base disorder has an influence on the changes presented in the CBC. The limitation of the research did not allow calculation of the anion gap in a disorder primarily classified as metabolic acidosis. This suggests a metabolic acidosis with elevated anion gap due to the increased positive charges present in the aminoglycosides. It was also not possible to perform the correction of the gap anion for albumin in those patients with gestational age less than 37 weeks.

doi: 10.1016/j.cca.2022.04.595

T128

POCT determination of neonatal capillary bilirubinemia using Bilistick®: Analytical performances and clinical value

A. Mailloux [a], F. Khettab [a], S. Huguet-Jacquot [a], H. Delaby [a], J. Beaud [a], C. Toly-Ndour [a], M. Vaubourdolle [b]

[a] *Centre National de Référence en Hémobiologie Périnatale (CNRHP), Hospital Saint-Antoine, AP-HP, Paris, France*
[b] *Départment of Biochemistry, DMU BioGeM, Hospital Saint-Antoine, AP-HP, Paris, France*

Background-aim

In pediatrics, accurate measurement of total serum bilirubin (TSB) is of major importance for reliable diagnosis and appropriate management of neonatal jaundice. This TSB assay should be available quickly for a safe management of newborn jaundice. Today equipment for point-of-care and laboratory testing perform bilirubin measurement. The follow-up of jaundice is done in real-time either using non-invasive transcutaneous bilirubin meters (BTc) or capillary blood POCT devices (involving CO-Oximetry or photometry). Several studies evidenced poor comparability of results obtained with the different methods. To progress, we assess the analytical performances of Bilistick®, a POCT device using photometric estimation of bilirubin on capillary blood, and we compared results both to central lab expected values and to thorax-measured BTc.

Methods

50 consecutive samples, addressed to the CNRHP lab for follow-up of neonatal jaundice, were measured either with diazo reaction method on an Indiko® analyzer (plasma), or using Bilistick® (Bilimetrix, Italy) device (total blood and plasma). Two control samples (200 and 270 μmol/L bilirubin concentrations) were used for imprecision studies. Bilistick® performances (repetability, accuracy) were estimated using CLSI-based protocols. Practicability was assessed for use in POCT situation.

Results

Analytical acceptance limits at high bilirubin levels (>150 μmol/L) were for imprecision a maximum CV 8 % and for accuracy 15 % limit bias. These limits integrate the guidelines for diagnosis and monitoring of neonatal jaundice. Results show that Bilistick® gave acceptable results for imprecision studies. Compared to expected central lab results, a mean bias of -15 μmol/L was found when blood sample were tested. This bias was reduced in plasma samples. A major positive bias was found in case of hemolysis but the device was able to detect this hemolysis in 5/6 cases. We compared the results between plasma and serum and between BIlistick® and BTc. These data confirmed the underestimation of BIlistick® in blood, probably linked to a light diffusion with RBC not corrected by a recalibration in silico. Practicability study (easy-to-use, small, cheap) showed that Bilistick® can be used in POCT situation for phototherapy monitoring in clinical settings where neither BTc device nor POCT CO-oximeter is available.

Conclusions

Our study allowed us to increase our knowledge about analytical performances of routinely used bilirubin tests, to progress in harmonization process for neonatal bilirubin. The added value of the clinical lab will be an adequate interpretation of results using published bilirubin nomograms for identification and monitoring of neonatal hyperbilirubinemia.

doi: 10.1016/j.cca.2022.04.596

T129

Stability of arterial blood gases (ABG)

M. Boudaya, N. Aissaoui, F. Gargouri, S. Fendri, F. Ayedi, R. Marrekchi, K. Jamoussi

Laboratory of biochemistry Sfax university hospital, Tunisia

Background-aim

The arterial blood gases (ABG) test is an emergency examination usually analyzed as soon as the sample is received. Sometimes this assay is deferred for technical reasons or a delay in delivery. Our aim was to study the stability of pH and partial pressures of O2 and CO2 (PO2 , PCO2) on samples collected in plastic syringes and stored at room temperature and in ice.

Methods

From 33arterial blood gases (ABG) samples collected on plastic syringes (total safety®) preflushed with liquid sodium heparinate, we determined the pH and the partial pressures of O2 and CO2 (PO2 , PCO2) on the ABL80 FLEX ® blood gas analyzer of Radiometer. Measurements made upon receipt of the samples served as reference results (T0). Samples stored at room temperature (22°c) and in ice were analyzed at 10min; 20min; 30 min ; 60min and 120 min respectively. We used Student's t test for comparison of means of paired series. The limit of acceptable clinical variability was estimated to be 3 CVa (analytical coefficient of variation).

Results

Over time we observed an increase in PO2 and a decrease in pH and PCO2. These differences were clinically significant at room temperature for the pH at the different measurement times (31.25%; 73.33%, 100%) and for the PO2 and PCO2 from 20 min onwards respectively (12.5%; 43.7%) and (6.25%; 12.5%).

On the other hand Ice helped preserve the PCO2 values and partially that of pH values (23%; 58.82%; 100%). However, it aggravated the increases in PO2 (31.25%, 62.5%, and 81.25%).

Conclusions

GDS samples on plastic syringes (total safety®) can be kept on ice for the first two hours for the determination of PCO2 but not for pH and PO2.

doi: 10.1016/j.cca.2022.04.597

T130

Accuracy of a point of care compact device for hematocrit testing in capillary blood samples

N. Apiratmateekul [b], W. Duanginta [a], P. Chuaychoo [c], K. Kongros [b], W. Treebuphachatsakul [d]

[a] *Biomedical Science Program, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*
[b] *Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan university, Phitsanulok, Thailand*
[c] *Medical Device Research Laboratory, Division of Research and Innovation, Naresuan University, Phitsanulok, Thailand*
[d] *Reference Material and Medical Laboratory Innovation Research Unit, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, Thailand*

Background-aim

Hematocrit (Hct), or the packed red cell volume by microcentrifuge method, is widely used as point of care (POC) test for anemia screening at primary care units in Thailand. Several factors influence the quality of Hct testing, including the use of uncalibrated centrifuges and poor user skills. This study verified the accuracy of Hct tests obtained from a POC compact device using capillary fresh blood samples.

Methods

Capillary blood samples were collected in EDTA microcentrifuge tubes from 95 subjects and used for Hct determinations by a hematocrit centrifuge and a POC compact device. A hematocrit centrifuge was based on a microcentrifuge method which is approved for calibration at a centrifuge speed of 12,000 rpm. The POC compact device was based on a low-speed centrifugation principle that displays Hct results as a percentage. Commercial whole blood quality control materials at two levels of Hct were used for quality control. The Hct measurements were performed by professional medical technologists. The paired t-test was used for data analysis.

Results

The Hct results obtained from EDTA capillary blood samples from 95 subjects ranged from 26%-49%. The mean and standard deviation of the Hct was 38.63% ± 4.76% by a hematocrit centrifuge, and 38.75% ± 4.72% by a POC compact device. There was no significant difference ($p > 0.05$) between the Hct results obtained from the hematocrit centrifuge and the compact POC device.

Conclusions

The POC compact device used for Hct testing of capillary blood samples was accurate with no significant differences compared to the hematocrit centrifuge used as a reference.

doi: 10.1016/j.cca.2022.04.598

Clinica Chimica Acta 530 (2022) S113–S117

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Education, Training

M067

Strategies used to introduce workplace based assessment in chemical pathology residency program

L. Jafri, A. Habib, H. Majid, M. Tariq, I. Siddiqui

Aga Khan University, Karachi, Pakistan

Background-aim

The principle of workplace based assessment (WBA) is to assess trainees at work with feedback integrated into the program at the same time. Objective was to introduce a student driven WBA model and perception evaluation of this teaching method was done subsequently by taking feedback from the faculty as well as the postgraduate trainees (PGs).

Methods

Descriptive qualitative & quantitative study was conducted. A WBA program was designed for PGs in Chemical Pathology on virtual learning environment using MOODLE and forms utilized were case-based discussion (CbD) on professionalism, data interpretation and management;direct observation of practical skills (DOPS) for laboratory procedures and test analysis and evaluation of clinical events (ECE) which included audits, consultations and complaints. After approval from institute's ethical review committee, consented faculty and PG were trained on conducting WBA through a workshop. Pretest to assess the knowledge of PGs was conducted before initiating WBA.Structured feedback was given by faculty to PG. Every time a WBA form was filled, perception of PGs and faculty towards WBA, time taken to conduct single WBA, feedback and discussion was recorded. Mean test scores were calculated and qualitative and quantitative evaluation of student and faculty feedback on perception of this WBA was done using frequency tables.

Results

Six eligible PGs and 17 faculty members participated in WBA over a two months period. Total 53 CBD (faculty n = 7 and PG n = 6), 11 ECE's (faculty n = 6 and PG n = 5), and 10 DOPS (faculty n = 4 and PG n = 5), were successfully recorded. PGs average pretest score was 55.6%.Overall mean time taken to evaluate PG's was 12.6 ± 9.9 min and feedback time 9.2 ± 7.4 min. Mean WBA process satisfaction of faculty and PGs on likert's scale of 1 to 10 was 8 ± 1 and 8.3 ± 0.8 respectively.

Conclusions

Both faculty and residents were satisfied with introduction and implementation of WBA. It gave the residents opportunity to interact with faculty more often and learn from their rich experience. WBA purports to provide essential information to both PGs and to their training and assessing bodies, and is often reported to support educational impact and shape clinical learning. The same model may also be transferred to other postgraduate training clinical specialties.

doi: 10.1016/j.cca.2022.04.600

M068

An audit on the awareness of Westgard rules as quality control monitoring tool among medical laboratory technologists of a tertiary care hospital

I. Siddiqui, A. Habib Khan, S. Imran, L. Jafri

Aga Khan University Hospital, Pakistan

Background-aim

About 70% of medical decisions are based on laboratory results and good quality practices are necessary for accurate results generation. Effective quality system implementation depends upon the knowledge and practices of laboratory personnel. Aim of this study was to assess the knowledge of Medical Laboratory Technologists (MLTs) regarding the Westgard rules as quality control (QC) monitoring tool.

Methods

This audit was conducted at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University (AKU) from January to March, 2015. All MLTs working in the section were included. A pre-test was conducted using a questionnaire designed to monitor MLTs knowledge and concepts regarding Westgard rules, their application and interpretation. After identifying the deficiencies from this audit, sessions on QC tools applications and interpretation were conducted. A post-test was conducted using the same questionnaire to close the audit cycle.

Results

The study population consists of 52% male and 48% female participants. The mean age of participant MLTs was 34.20 ± 7.16 (range 25-59) years. The mean working experience of the MLTs was 8.5 ± 5.9 (range 1-30) years. Response rate was 94%. Total 47 out of 50 MLTs responding in both pre and post-tests were included in final analysis. Mean results were 48.54 ± 19.45 (range 6.6-86.6) and 68 ± 22.7 (range 46-100) for Pre-test and Post-test respectively (p-value < 0.001).

Conclusions

The results showed a significant increase in the post-test scores compared to pre-test. This emphasizes the need for regular academic sessions, refresher courses, workshops, seminars for MLTs on QC monitoring strategies.

doi: 10.1016/j.cca.2022.04.601

M069

A preliminary study on the learning effectiveness of applying multiple teaching strategies

P. Chen, C.H. Cheng, C.W. Hung, J.X. Chuang, C.f. Chen, C.J. Chen, Y.c. Hsein

Department of Laboratory Medicin, National Taiwan University Hospital Yun-Lin Branch, Taiwan

Background-aim

The World Health Organization mentioned in 2010 that medical services could strengthen care capacity, provide optimum medical services, and improve health care outcomes through education and cross-team cooperation. However, the effective application or connection of professional knowledge to clinical practice is difficult for traditional teaching approaches. In addition to developing professional knowledge, attitudes, and skills, current education and training should also improve the capacity of learners for problem solving, critical thinking, clinical decision-making, and the integration of information. Using multiple teaching approaches will stimulate the interaction between teachers and students and peer learning, encourage potential ability of learners, and demonstrate the learning achievements of multiple intelligences. Hence, providing medical staff with perfect education and training can save patients from fear, reduce medical staff errors, reduce life-safety threats to patients, and reduce medical disputes to improve the relationship between doctors and patients, thus improve the quality of medical services.

Methods

1. Revise the medical examiner education and training program based on the ADDIE model.

2. Development and Implementation of Action Learning App: Keller proposes “ARCS Motivation Design Pattern” to develop and implement “Parasite Garden” and “Blood Primary School” action learning app, so that learners are not limited by time and space. Besides promoting the motivation and fun of learners through games, the design also uses competition to increase the satisfaction of learners.

3. Construct situational simulation teaching: Using Gagne’s “cognitive teaching mode” to create a biosynthesis obstacle situation simulation teaching, with the Dropbox application platform, each device can receive and transmit the same data in real-time through cloud synchronization. In the external environment, the stimulus is transformed into a stage of information processing from which new knowledge and skill can be learned and learning can be instantaneous.

4. Establish a blood bank case teaching material: Collect the blood bank clinical case with the Harvard Flip-Case-participatory Teaching Method. Through the formation of the situational simulation, the learner can think about the problem in the context of the situation, and learn and learn through the participation and discussion process. Problem solving ability and thinking ability.

Results

1. Teaching satisfaction increased from 56% before improvement to 84.8%, and the target achievement rate was 120%.

2. Using paired samples t verification to explore the difference between the medical examiner’s satisfaction with the improvement of teaching quality, the quality of teaching 22 questions

The items were significantly different ($P < 0.01$), and the satisfaction after improvement was significantly higher than before the improvement. The following twenty-two teaching

The overall situation of improving the satisfaction before and after the quality measurement is described:

(1) The top five items with the highest satisfaction after improvement are “for the internal curriculum, which can stimulate the interest of learners” (4.45 Points), “Feeling the teaching of the case in the department” (4.41 points), “The enthusiasm for the lecturer’s teaching “To” and “I feel that the results of the study after the end of the course” (both 4.36), “For the departmental course “Feeling the benefits of bed work” and “feeling about the situational simulation equipment provided by the department” (both 4.32).

(2) The top five items with the largest difference value are “I feel that the auxiliary teaching materials can trigger learning motivation” (1.91 points), “Yes In the departmental course, it is possible to arouse the interest of learners” (1.88 points), “I feel that I am interested in the design of teaching materials”(1.86 points), “I feel that there is no space and time limit for learning” (1.84 points), “For the case within the Ministry”For example, we will explore the feelings of teaching and “feeling about the situational simulation equipment provided by the Ministry” (all 1.82), and the quality of teaching will be changed.The aftercare examiner has the highest degree of satisfaction with the five indicators.

Conclusions

The initial stage of improving the quality of inspection teaching has reached the short-term goal, and the satisfaction of colleagues in diversified interactive teaching is significantly higher than that of traditional teaching mode ($P \geq 0.01$); but the activity is not over, and the medical examiner can be seen from the satisfaction survey results. We still have high expectations for the demand for and quality of teaching quality. Therefore, we will continue to expand to the various specialist groups of the Department of Laboratory Medicine in the future to continuously improve the quality of teaching and cultivate more outstanding professionals.

doi: 10.1016/j.cca.2022.04.602

M070

Urine sediment external quality assessment program in Brazil reveals the need for more education on epithelial cells morphology and clinical significance

J. Poloni [b], C. Dos Santos [a], A. Simundic [d], L. Rotta [c]

[a] *Universidade do Vale do Rio dos Sinos, Brazil*
[b] *Universidade do Vale do Rio dos Sinos and Controllab, Brazil*
[c] *Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil*
[d] *University Hospital "Sveti Duh" Zagreb and University of Zagreb, Croatia*

Background-aim

Urinary epithelial cells (EC) are divided in three categories: squamous, transitional or renal tubular (RTEC), with the later always linked to pathological conditions. Despite the clinical relevance of this finding RTEC challenges a lot of laboratory professionals due to the difficulty to properly identify this type of EC. The objective was to understand how Brazilian clinical analysis microscopists reports EC and how they judge the clinical relevance of this finding to help the diagnosis of kidney and urinary tract diseases.

Methods

A survey with four questions (1-How do you perform the urine sediment analysis?; 2- In what type of situation do you inform the different subtypes of EC?; 3- In your opinion inform the 3 subtypes of EC is usefull to the assistent doctor?; 4- Does your laboratory is interested in evaluate the performance of the correct identification of the different cells subtypes by an EQAP?) was submitted to the participants of the Urinalysis EQAP from Controllab. Laboratories were divided in 3 different categories according to the way they report EC : (1) differentiating EC subtypes as "squamous", "transitional" and "RTEC"; (2) differentiating EC subtypes "squamous" or "non-squamous" cells; (3) without subtype identification, as "EC".

Results

1336 laboratories answered the survey with 1144 (85.6%) reporting EC without discrimination between the subtypes while >71% of the laboratories declared to perform the analysis of all urine samples by microscopy. From laboratories that reports EC subtypes, 133 (95%) always report (according to the standard laboratory procedure); 652 (96%) and 782 (91.8%) of laboratories that don't differentiate EC subtypes, believe it isn't useful for the assistant doctor and have no interest in being evaluated on the identification of different EC subtypes, through an EQA program.

Conclusions

While laboratories that already report different EC subtypes want to be evaluated by EQAP, those that don't differentiate EC subtypes aren't aware of the usefulness of this information to the clinicians and aren't interested to be evaluated by EQAP. Our study reveals the need for more education on urine EC morphology and its clinical significance.

doi: 10.1016/j.cca.2022.04.603

M071

Laboratory diagnostics in internal medicine: developing learning texts for medical students

G. Kovac

Institute for Laboratory Medicine, Medical Faculty, Slovak Medical University, Slovakia

Background-aim

At the „Slovak Medical University "in Bratislava Slovakia the education in laboratory diagnostics for medical students was introduced. This new discipline is set in the fourth year of the study in duration of 1 semester. For this purpose we worked out learning texts in slovak and english version.

Methods

As no additional resources for this increased workload were dedicated, we were exposed to work in „fuel efficient" regimen: the first author made excerptions from the approved resources (Navratil: Internal Medicine and Fischbach: Laboratory Diagnostics), the second author modified the excerptions, and the third author corrected the contingent errors. We used brief comments focusing the substance of the described topic. Particular emphasis was devoted to the balanced equillibrium between the laboratory and clinical context of the discussed problem. The problem of teaching laboratory diagnostics in the fourth year is, that though the students are familiar with the pathophysiological aspects of the internal diseases, the clinical experience which is the prerequisit of the rational use of the clinical laboratory is lacking. We tried to bridge this gap in that we divided the textbook in two parts.

Results

The „General Part" covers all important fields of internal medicine and includes 14 chapters. In each chapter we tried to characterize the main pathophysiological and clinical features as well as the main laboratory investigation of the given disease with the help of brief comments. At the end of each chapter we inserted a short summary of the main aspects of the laboratory diagnostics. We appointed the students to focus this summary and use it as the base and starting point in the process of the selection of necessary laboratory investigations. The „Special Part" contains up to 100 most frequently used laboratory investigations in the internal medicine. Each laboratory parameter is characterized by it's definition, the indication (situations in which ought to be used), the reference value, the interpretation.

Conclusions

At the exam we focus this summary and the description of the selected laboratory investigations. At the poster session the issue of textbook is at the disposal.

doi: 10.1016/j.cca.2022.04.604

M072

Educational intervention improves performance indicators for faecal elastase manual ELISA testing at an academic core laboratory

R. Punchoo [b], J. Havinga [d], S. Bhoora [a], T. Pillay [c]

[a] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa*
[b] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa, National Health Laboratory Service, Tshwane Academic Division, Pretoria, South Africa*
[c] *Department of Chemical Pathology, Faculty of Health Sciences, University of Pretoria, South Africa, National Health Laboratory Service, Tshwane Academic Division, South Africa, Division of Chemical Pathology, University of Cape Town, South Africa*

[d] *National Health Laboratory Service, Tshwane Academic Division, Pretoria, South Africa*

Background-aim

The analytical phase of the total test process contributes to approximately 7-15 % of total errors in laboratory medicine. The manual ELISA assay, with its multiple sequence of steps, is prone to random and systematic errors. Pancreatic elastase 1, a useful stool biomarker of exocrine pancreatic insufficiency, can be analysed by commercial manual ELISA. This study investigated the effect of laboratory education on quality test indicators in faecal elastase 1 (FE1) testing.

Methods

An interventional study was conducted from June 2018 to March 2019 at National Health Laboratory Services (NHLS) Tshwane Academic Division (TAD) Core Laboratory, South Africa on FE1 analysed on ScheBo PE1™. Pre- and post-interventional test groups were defined based on the administration of a quality improvement education intervention. The intervention was formulated by laboratory management from root-cause-analysis of test failures in the pre-interventional study period. The target education intervention consisted of retraining, shadow-training and intermittent observation of all staff performing FE1 testing. Data were extracted from the NHLS database and manual test logs. Data included quality indicators for all test failures viz. total number of test failures, total cost of non-billable test failures and total test-turn-around-time of test failures. All data were analysed using descriptive statistics and Fisher's exact test was performed with statistical significance determined at $p < 0.05$.

Results

Root cause analysis identified sub-optimal staff knowledge and skills owing to prolonged rotational intervals on specials-bench, which resulted in failed calibration, failed internal quality controls and repeated testing of patient specimens. Post-intervention identified statistically significant improvement in test failure rates, 91% decrease in loss of billable test revenue per 100 tests and 37% decrease in turn-around-time indicators.

Conclusions

Root cause analysis of FE1 test failures enabled focused target intervention of education of all staff performing manual FE1 ELISA tests at NHLS TAD. Post-intervention testing demonstrated statistically improved indicators of FE1 test performance.

doi: 10.1016/j.cca.2022.04.605

M073

Entrepreneurial spirit in health promotion

Z. Chellouai, S. Chellouai

Faculté de Médecine, Université Oran 1, B.P 1510 El Menaouer 31000 - Oran, Algeria

Background-aim

The objective of this paper is to expose the interest of entrepreneurship in health education.

Methods

A search was conducted on the electronic Medline database (via PubMed) and published for the last ten years. The key words were "Entrepreneurship -health education - health promoting"

Results

Entrepreneurship for education and health promotion is still fertile ground that deserves to be exploited in order to help find innovative solutions to health problems. Especially when it comes to primary prevention of chronic diseases or therapeutic education for example. These business projects will have a direct or indirect impact through the creation of wealth and jobs with high added value. These companies can reach a large number of people in their workplaces in all sectors, and intervene in health promotion. It is very important that the promoters of these projects are well prepared to engage in this field, and this can be ensured via adequate support and an integration of the entrepreneurial spirit among students, new graduates and researchers in medical sciences. Several business opportunities for health educators will therefore continue to develop, citing "communication-information", nutrition, psychology for well-being, sport and others. To do this, a set of approaches must be adopted to help them take new entrepreneurial initiatives and successfully launch their business projects.

Conclusions

The health sector is a dynamic market, education and health promotion is an integral part of it. Encouraging entrepreneurial initiatives will go a long way in improving the quality of health care in the country.

doi: 10.1016/j.cca.2022.04.606

M074

Education of medical laboratory technicians in the Czech Republic

M. Bunesova, R. Prusa

Dept. of Medical Chemistry and Clinical Biochemistry, Charles University 2 nd Faculty of Medicin and University Hospital Motol, Prague, Czech Republic

Background-aim

Education evaluation of medical laboratory technicians, the staff in clinical laboratories

Methods

Higher requirements on education are put on the laboratory staff as the technologies of the laboratories grow more complex, the financial resources are limited and the population grows older. High quality of work is the most efficient mean of protecting the patient from possible risks. Observing, collecting a study on the types of education of medical laboratory technicians in the Czech Republic during the years 2010 – 2020 the context of the European standards of quality management and the process of accreditation. (ČSN ISO 15189, JCHO/JCI, ISO 9001)

Results

Statistical data of inhabitants in previous years were as follows:
Year 2000 = 10.260.000. 14% residents are older 65 years,
Year 2018 = 10.650.000, 20% residents are older 65 years.

Number of schools educating medical laboratory technicians:

(secondary nursing school = 86), number of hospitals (incl. outpatient care from year 2020 to 2012 are 188), number of clinical laboratories (324 at year 2010, 289 at year 2012). A summary of requirements on knowledge and skills of medical laboratory technicians. The state of the undergraduate education and its essential flaws. Meeting the need of new staff on clinical laboratories and the problems of professional competences. The postgraduate education has a better structure, though it is essential to add digitalisation in laboratories as another part of the education.

Conclusions

The main goal of laboratory medicine is the good of the patient. Quality work of clinical laboratories can only be achieved with well-educated medical laboratory technicians. The complexity of problems that medicine deals with, requires reduction of risks in healthcare. The education of medical laboratory technicians and their moral integrity is therefore an essential part of medicine. The current not very good state of undergraduate studies and the unmodernised themes of study on the postgraduate contradicts this fact.

doi: 10.1016/j.cca.2022.04.607

M075

An audit of the investigatory processes and documentation of proficiency testing failures at a cap accredited laboratory in Pakistan

S. Ahmed, F. Jahan

Department of Pathology and Laboratory Medicine, The Aga Khan University Karachi Pakistan

Background-aim

Participation in Proficiency testing (PT) is integral to the College of American Pathologists (CAP) accreditation program. The unsatisfactory PT results often suggest potential problems of which we might not be aware in routine work. Additionally, appropriate identification of the type of error, documentation and follow up using a structured root cause analysis plan of errors provided an opportunity for the continuous improvement of laboratory services.

Methods

The Clinical Chemistry section, Department of Pathology & Laboratory Medicine, AKU, Karachi avidly participated in external proficiency testing program of CAP. During a CAP inspection, various short comings were identified in the investigation process alongside lacking of a comprehensive documentation. Based on these findings, the audit team consisting of a Consultant Chemical Pathologist, Quality Control officer and Lab Manager analyzed the PT failure data of the year 2017 and implemented a sectional educational plan for all the technologists involved in PT testing. The teaching strategy used was electronic circulation of reading material, lectures and short group discussions. An investigation checklist was developed and circulated among the staff to improve and streamline the process of root cause analysis. A re-audit was done and the PT failure record of the year 2018 was analyzed to overview the improvements expected after the above undertaken measures. Analysis was done by using MS-excel.

Results

Out of a total of 204 analytes, PT surveys for 27 were found unacceptable in 2017 and 24 in 2018. On comparison, before the measures taken by the audit team in 2017, 22 PT results were not documented with complete record of investigations contrary to only 14 in 2018. While in 2017 (n = 4) and 2018 (n = 3) analytes were found to be having incomplete conclusion summary. A significant improvement was noted as in 2018, 7 PT surveys had detailed investigations with complete documentation matched to only 1 in 2017.

Conclusions

The audit of the investigatory processes and documentation demonstrated a trend of improvement from 2017 to 2018 after appropriate staff educational and training actions. Furthermore, appropriate review of causes and discrepancies provided an opportunity for the continuous improvement of laboratory services.

doi: 10.1016/j.cca.2022.04.608

Clinica Chimica Acta 530 (2022) S118–S149

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Endocrinology

T131

Insulin-mimetic role of kaempferitrin in glucose homeostasis: A dietary flavonoid exhibits anti-diabetic complications and promotes glucose uptake

K. Patel, D.K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

Herbal drugs and their derived phytochemicals are valuable for human being as a vital source of food material and drugs. Flavonoids are naturally occurring phytochemical having numerous biological activities. Kaempferitrin is a natural flavonoidal compound present in the edible plants apples, broccoli, strawberries, beans and grapefruit. It is also present in medicinal plants Aloe vera, Ginkgo biloba, Rosmarinus officinalis, Crocus sativus and Hypericum perforatum. Kaempferitrin have anti-diabetic, anti-oxidant, anti-inflammatory, anti-apoptotic, cardio-protective and anti-cancer activities.

Methods

Various literature databases have been searched to collect all the scientific information of kaempferitrin and evaluated for their hypoglycemic activity in in-vivo and in-silico model. All the collected data were further analyzed to know the anti-diabetic potential of kaempferitrin. Furthermore, molecular docking studies of kaempferitrin on GLUT, protein tyrosine phosphatase, ⟨-amylase, ⟨-glucosidase and aldose reductae enzymes were also performed to know their interaction, energy and scores.

Results

From the present data, it was concluded that kaempferitrin has positive activity in diabetic rats, maltase enzyme and adipocytes. Kaempferitrin significantly decreased malondialdehyde levels, increased superoxide dismutase activity and suppressed reactive oxygen species (ROS) generation. Further long-term effect of kaempferitrin on glycaemia in diabetic rats also revealed significant result. Molecular docking studies showed that kaempferitrin binds directly to GLUT4 and molecular docking simulation demonstrated negative binding energies. Kaempferitrin were able to bind to the active sites of ⟨-amylase, ⟨-glucosidase and aldose reductase which are main targets of anti-diabetes drugs. The docking calculations of the insulin receptor also have significant result which could be used for the treatment of diabetes and related complications.

Conclusions

This work will be valuable to justify the importance of kaempferitrin in scientific field. The molecular docking showed that kaempferitrin is a potent hypoglycemic molecule which will be used for the treatment of diabetes and diabetes induced disorders in the future.

doi: 10.1016/j.cca.2022.04.610

T132

Retrospective study of the incidence of hyperprolactinemia

R. Rubio Sánchez, E. Lepe Balsalobre, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville, Spain

Background-aim

Hyperprolactinemia is a frequent finding in clinical practice. Prolactin levels may increase during pregnancy, as a result of nipple stimulation during breastfeeding, and in stressful situations. Some medications can also cause an increase in this hormone, among which are neuroleptics, psychotropics, tricyclic antidepressants, oral contraceptives, antihypertensives and prokinetics. Among the pathological causes that can cause an increase in prolactin levels, hyperplasia or adenoma of lactotropic cells (prolactinoma) stands out.

Methods

An extraction of the results of all prolactin determinations requested during 2018 was performed from the laboratory computer system (SIL). Those patients with prolactin values ε 100 ng/mL were selected and a statistical analysis of the data was performed.

Results

In 2018, 7012 prolactin determinations were performed in the biochemistry laboratory of our hospital. The prolactin concentration was normal (<100 ng/mL) in 6912 cases (98.57%) and pathological (ε 100 ng/mL) in 100 cases (1.43%).

The etiology of the pathological prolactins were the following: 60% secondary to drugs, 25% due to pregnancy/lactation, 9% due to prolactinomas and 6% due to other causes (stress, polycystic ovary syndrome, etc.). 93% of patients were women, while only 7% were men. 83% of prolactin requests came from Primary Care, while 17% came from the Endocrinology Service.

During the study period, in 100% of the cases it was the laboratory that gave the alarm about possible cases of prolactinoma and the one that launched the rest of the confirmation tests.

Conclusions

The role of the laboratory is fundamental in the early diagnosis of a pathology with clinical implications as serious as prolactinoma, a prolactin-producing CNS tumor.

doi: 10.1016/j.cca.2022.04.611

T133

Circulating levels of miR-24-3p and miR-198 in type 2 diabetes mellitus

A. Abhilasha [b], P. Mitra [b], S. Suri [b], I. Saxena [a], R.K. Shukla [c], P. Sharma [b]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Gorakhpur, India*
[b] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India*
[c] *Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, Jodhpur, India*

Background-aim

Type 2 Diabetes mellitus (T2DM) is a metabolic disease, associated with genetic, lifestyle, and environmental factors. It is characterised by hyperglycaemia, mainly due to insulin resistance. From recent studies, microRNAs have been implicated in regulation of post transcriptional gene expression mainly by repressing protein production. Dysregulated miRNA in type 2 diabetes interrupts insulin signalling cascade and multiple physiological processes leading to disease progression. miRNAs are released from cells in circulation and due to their stable nature, they are now considered as a new class of biomarkers. Database analysis have predicted association of miR-24-3p & miR-198 in Type 2 Diabetes, but their roles remain unclear. The aim of this study was to compare miR-24-3p and miR-198 expression levels in newly diagnosed Type 2 Diabetes Mellitus patients and healthy controls

Methods

30 newly diagnosed type 2 diabetic cases and 30 healthy controls were recruited after obtaining due informed consent. Venous blood was obtained under aseptic conditions. Biochemical parameters were analysed using autoanalyzer. Expression levels of miR-24-3p and miR-198 was performed using RT-PCR by Taqman Advanced miRNA assay. miR-16-5p was used as internal control.

Results

The mean ± SD of fasting blood sugar among cases and controls were 163.84 ± 51.27 mg/dl and 91.35 ± 6.20 mg/dl respectively. The mean ± SD of glycated hemoglobin among cases and controls were 8.25 ± 1.39 and 5.5 ± 0.27 gm% respectively. The difference between circulating levels of miR-24-3p and miR-198 were statistically significant among study group. miR-24-3p showed a fold change of 0.46 and miR-198 showed a fold change of 0.48.

Conclusions

Findings of our study suggests that expression of miR-24-3p and miR-198 are downregulated in newly diagnosed Type 2 Diabetes Mellitus.

doi: 10.1016/j.cca.2022.04.612

T134

Establishment of algorithms and threshold of thyroid stimulating immunoglobulin for diagnosis of Graves' disease by data mining: A Chinese multicenter study

M. Chaochao [a], H. Yingying [a], C. Xinqi [a], D. Zuoliang [b], Z. Zuncheng [c], Q. Ling [a]

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*
[b] *Department of Clinical Laboratory, Tianjin Medical University General Hospital, Tianjin, China*
[c] *Nuclear medicine department, The Second Hospital of Tianjin Medical University, TianJin, China*

Background-aim

Thyroid stimulating immunoglobulin (TSI) is diagnostic hallmarks of Graves' disease (GD). The aim of this study is to establish different algorithms and corresponding threshold of TSI for diagnosis of GD based on Chinese population by data mining.

Methods

Sera were evaluated from 1,013 subjects from three centers representing a variety of conditions: 100 subjects with untreated GD, 200 with treated GD, 62 with autoimmune thyroid disease, 216 with other thyroid diseases, 214 with non-thyroid autoimmune diseases, 191 had other diseases and 120 health subjects. First, Tukey method was used to identify outliers based on the results of thyrotrophin receptor antibody (TRAb). Then ROC, percentile algorithm, parallel and serial algorithms for TSI were established to diagnose GD. The corresponding threshold, sensitivity, specificity, positive value and likelihood ratio were also calculated.

Results

Using treated GD or untreated GD for ROC analysis, 0.23 IU/L and 0.31IU/L of thresholds of TSI for diagnosis of GD were obtained, which were both lower than that of the manufacturer (0.55IU/L). The threshold for diagnosis of GD was 0.35 IU/L and 1.29 IU/L for females and males, respectively. The fifth quantile of TSI distribution in 300 GD patients was 0.47 IU/L in quantile algorithm,whicht had a good diagnostic performance as ROC analysis.

Conclusions

Sex-specific threshold of TSI for diagnosis of GD based on the fully automated stimulating TSH receptor autoantibody immunoassay was established in Chinese population, which could improve the diagnostic accuracy of GD.

doi: 10.1016/j.cca.2022.04.613

T135

Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantification of serum perfluorooctanoic acid and perfluorooctane sulfonate

S. Kim [b], H. Nam [a], J. Han [a], E. Lee [a]

[a] *Department of Laboratory Medicine, Green Gross Laboratories, Yongin, Gyeonggi, Republic of Korea*
[b] *Department of Laboratory Medicine, Shinwon Medical Foundation, Gwangmyeong, Gyeonggi, Republic of Korea*

Background-aim

Perfluorooctanoic acid (PFOA) and Perfluorooctane Sulfonate (PFOS) are part of a larger group of chemicals called per- and polyfluoroalkyl substances (PFASs). PFASs produced and used worldwide as industrial products (e.g. food packaging materials, fire-fighting foams and textiles) due to their heat-resistant and oil- and water-repellent properties. They are known to be a representative endocrine disruptor and carcinogenic in human. We developed and validated a rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based method for quantifying serum PFOA and PFOS.

Methods

Primary PFOS and PFOA standards were obtained from Wellington Laboratories (Guelph, Ontario). A standard stock solution, prepared in methanol by dilution of the primary standard solutions, was further diluted using a 50:50 (vol:vol) methanol/water to make calibration standards ranging from 1 to 200 ng/mL in PFAS-free polypropylene (PP) containers. Sample spiked with isotopically-labelled internal standards was prepared based on protein precipitation and liquid-liquid extraction with 0.1M formic acid in ACN, followed by reconstitution with ammonium acetate, and LC-MS/MS analysis in ESI negative mode. Separation was achieved using a Unicon UK 3 lm C18 2.0 x 50 mm (Imtakt Crop, Japan) column with a gradient of 2 mM Ammonium acetate in water and in ACN mobile phase. Mass spectrometry was performed in multiple reaction monitoring mode, which was performed through the transitions from the precursor to the product ions (m/z 412.99 to m/z 368.9 for PFOA, m/z 416.99 to m/z 371.8 for PFOS, m/z 499.0 to m/z 80.0 for PFOA IS, and m/z 502.98 to m/z 79.7 PFOS IS, respectively). Retention times of PFOA and PFOA IS were both 3.6 min and that of PFOS and PFOS IS were both 6.9 min in a 15 min analysis. Linearity, accuracy, precision, carry over, matrix effect, and stability were evaluated to validate the method. Serum PFOA and PFOS concentrations from 172 samples were determined to set the reference interval of normal population.

Results

The LC-MS/MS method yielded a linear response from 0.50 to 80.00 ng/mL ($R2 = 0.9989$). Lower limit of detection of the method was 0.004 ng/mL. The mean bias with NIST standard reference material was less than 5% at 4 concentrations within the analytical measurement range (AMR). The average within-batch and total coefficients of variation were below 3%. Carry-over was found to be 0.34%. Ion suppression or enhancement was not observed in blank and 6 patient samples. The stability of PFOA and PFOS in refrigeration and freezing were less than 15% for 28 days. The reference interval was 1.14 – 15.74 ng/mL and 1.46 – 18.34 ng/mL for PFOA and PFOS, respectively.

Conclusions

The LC-MS/MS PFOA and PFOS assay showed an adequate accuracy, precision, sensitivity, AMR, and good specimen stability. Hence, it is suitable for routine clinical use. To the best of our knowledge, this is the first report describing the reference interval for normal Korean population for clinical setting. Our method will help to measure the level of exposure to common endocrine disruptor in the general population.

doi: 10.1016/j.cca.2022.04.614

T136

The role of fibroblast growth factor-23 in primary hyperparathyreoidism

K. Kotaska, M. Valkus, L. Hanouskova, J. Cepova, R. Prusa

Department of Medical Chemistry and Clinical Biochemistry, 2nd Medical Faculty, Charles University, University Hospital Motol, Prague, Czech Republic

Background-aim

Fibroblast growth factor-23 (FGF23), a regulator of secretion of parathyroid hormone (PTH), is implicated in phosphate-wasting disorders. The role of FGF23 in primary hyperparathyroidism (pHPT) is unclear. The aim of the study was to investigate the role of FGF23 in patients with pHPT.

Methods

Study involving 23 patients (18 females, aged from 24 to 77 years and 5 males, aged from 38 to 75 years) with primary hyperparthyreoidism submitted to parathyreoidectomy. PTH was investigated at admission and 10 minutes after parathyreoid adenoma excision, cFGF23, iFGF23, phosphorus, eGFR (CKD-EPI), Cl/P index, eGFR (cystatin C) and P1NP were investigated perioperatively and next day after the surgery.

Results

iFGF23 levels were elevated above the reference values with initial mean values of 221 + 119 pg/ml. cFGF23 levels were at the upper limit of the reference values (initial mean values of 145 + 56 RU/ml). We observed significant decrease in postoperative cFGF23 values (118.2 + 56 RU/ml, $p = 0.0009$) with significant elevations of phosphate values ($p = 0.02$) and decrease in eGFR (CKD-EPI) ($p = 0.01$) and eGFR (cystatin C) values ($p = 0.047$). We evaluated positive correlation of perioperative PTHi levels with iFGF23 ($r = 0.30$, $p = 0.02$). We also proved significant negative correlation of iFGF23 and cFGF23 with eGFR (CKD-EPI) ($r = -0.64$ with $p < 0.0001$ and $r = -0.51$ with $p = 0.0006$) and positive correlation of iFGF23 and cFGF23 with P1NP($r = 0.41$ with $p = 0.0082$ and $r = 0.82$ with $p < 0.0001$).

Conclusions

Results confirm the significant role of iFGF23 and cFGF23 in phosphate metabolism in patients with primary hyperparathyreoidism. Intact FGF23 and c-terminal FGF23 seem to have importance as a markers of bone and renal metabolic impairment in patients with primary hyperparathyreoidism.

doi: 10.1016/j.cca.2022.04.615

T137

To assess the levels of Vitamin D in males with low testesterone levels (total & free)

S. Bembey, R. Verma, E. Belho, H. Mahajan

Mahajan Imaging Pvt Ltd, Sir Ganga Ram Hospital, Delhi, India

Background-aim

The aim was to correlate the levels of Vitamin D in males with low Testosterone levels as compared to normal Testosterone levels.

Low levels of vitamin D are significantly and independently associated with low levels of Testosterone in otherwise healthy middle-aged men as seen in this study.

Methods

A retrospective study was done of 300 patients who had undergone testing for Testosterone (Total & Free) and Vitamin D levels using Chemiluminescence Method(CLIA).

150 patients with normal levels and 150 with low levels of Testosterone(Total& Free)

Normal Range:

Testosterone:

Total: 250-850 ng/ml ; Free: 8.69-54.69 pg/ml

Vitamin D:

Deficient: >20ng/ml ; Insufficient:21 -29 ng/ml ; Sufficient: >30 ng/ml ; Toxic: > 150 ng/ml

Results

On analyzing the data of 300 Males with normal and low testosterone levels with the Vitamin D levels we found a significant correlation between the two parameters with a Pearson score of 1 for Free and Total Testosterone levels and a CI of 97%.

There was 79% incidence of Low to very Low levels of Vitamin D in Patients with low Testosterone levels.

Conclusions

Such a high correlation of low levels of Vitamin D in males with low Testosterone levels indicate a need for an initial screen of all patients with low levels of Testosterone followed by a need based Vitamin D supplementation programme as part of the treatment.

doi: 10.1016/j.cca.2022.04.616

T138

IL-2 and IL-18 levels in newly diagnosed type-2 diabetes mellitus

S. Suri[a], P. Mitra[a], A. Abhilasha[a], I. Saxena[a], M. Garg[b], G. Bohra[b], P. Sharma[a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India*

[b] *Department of General Medicine, All India Institute of Medical Sciences, Jodhpur, India*

Background-aim

Diabetes mellitus (DM) is a serious chronic multifactorial metabolic disorder prevalent worldwide caused by defective insulin production, secretion and signalling. Type 2 diabetes mellitus (T2DM) is a type of diabetes, characterised by insulin resistance condition, wherein the body cannot effectively use the insulin produced. It is often considered as a state of chronic low grade inflammation which is associated with altered levels of many inflammatory markers. Among these inflammatory markers, cytokines are one of the major influencing elements. Inflammatory cytokines influence lipid profile levels in blood plasma by modulating hepatic secretion of TG, HDL, LDL eventually modulating pathophysiology, leading to T2DM. Various interleukins have been studied in context to their association with T2DM. Due to paucity of data in relation to IL-2 and IL-18 in newly diagnosed diabetes subjects, this study was planned to compare their levels in newly diagnosed T2DM cases and healthy controls.

Methods

30 T2DM cases, and 30 age and sex matched healthy controls were recruited after obtaining due informed consent. Biochemical parameters such as fasting plasma glucose (FBS), glycated haemoglobin (HbA1c), high sensitivity C reactive protein (hsCRP) and lipid profile were estimated in Beckman Coulter (AU680) auto analyser. Serum IL-2 and IL-18 levels were estimated by commercial ELISA kit.

Results

The levels of FBS and HbA1c were significantly different among cases and control ($p < 0.0001$). The mean ± SE of IL-2 in cases (63.67 ± 121.8 pg/ml) were significantly lower ($p < 0.05$) then in controls (108.3 ± 132.6 pg/ml). On the other hand, IL-18 levels showed a significant increase ($p < 0.01$) in diabetic cases (724.1 ± 267.3 pg/ml) than non-diabetic control (513.8 ±152.9 pg/ml).

Conclusions

Our study is the first to correlate anti-inflammatory interleukin IL-2 and pro-inflammatory interleukin IL-18 in newly diagnosed T2DM patients. Findings from this study do highlight the pro-inflammatory role of IL-18 and anti-inflammatory role of IL-2 in T2DM.

doi: 10.1016/j.cca.2022.04.617

T139

Klinefelter syndrome because of a breast pain

S. Bilbao De La Vega

Osakidetza, OSI-Barrualde, Hospital Galdakao-Usansolo, Spain

Background-aim

Gynecomastia is the enlargement of male breast glandular tissue caused by an imbalance in the action of estrogens and androgens. It can appear during childhood, puberty and adulthood and manifests with swollen breast tissue and pain to palpation. It is usually a benign process that disappears alone, but sometimes it is necessary to resort to hormonal therapy and/or surgery. The factors that can alter the hormonal balance are the natural hormonal changes themselves and the drugs, but sometimes it is the result of the expression of an underlying disorder (hypogonadism, tumor processes, renal failure, liver failure ...), so it is essential to rule out any pathology that could be causing it.

We reported the case of a 31-year-old male with pain in his left breast and a “puncture” sensation for 15 days. The physical examination was normal except pain on palpation. He has hypogonadal appearance, little hair and little beard, he was born of normal pregnancy, cryptorchidism with 8 years, onset of puberty with 13-14 years and no

mumps. Ultrasound, hormonal analysis and karyotype were carried out looking for an underlying pathology.

Methods

The hormonal analysis was carried out using a Cobas e 601 module (Roche Diagnostics), including: luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and estradiol (E2). The cobas e 601 module is a fully automated analyzer that uses a patented electrochemiluminescence (ECL) technology for immunoassay analysis. It is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications (including anemia; bone, cardiac and tumor markers; critical care; fertility/hormones; maternal care; and infectious diseases).

Fot he identification of the chromosomes (karyotype), the G banding technique was used (GTG bands). The metaphase chromosomes are treated with trypsin (to partially digest them) and stained with Giemsa stain. Heterochromatic DNA regions stain more darkly, are rich in adenine and thymine and relatively gene-poor. In contrast, euchromatin, which is less condensed chromatin, rich in guanine and cytosine and more transcriptionally active, appear as light bands in G-banding.

Results

In the ultrasound 15mm gynecomastia was observed, without secretion and bilateral lipomastia.

The hormonal analysis revealed the following results: LH 18 U/L [2.4-9.4 U/L], FSH 24.6 U/L [0.9-15 U/L]), testosterone 0.79 ng/mL [2.6-13 ng/mL], and E2 9ng/mL [10-52 ng/mL]. Low levels of testosterone with elevated levels of LH and FSH suggest primary hypogonadism. After analysis of 50 metaphases with GTG bands, 47 metaphases with an extra X chromosome, 2 metaphases with two extra X chromosomes and a normal diploid metaphase (47XXY [47] / 48XXXY [2] / 46XY [1]) were observed; karyotype that clinically corresponds to Klinefelter syndrome.

Treatment with tamoxifen and testogel is started. After 2 months, he shows normalized hormone levels, LH 11.5 U/L, FSH 13.6 U/L, E2 49 ng/mL, testosterone 681 ng/mL and almost complete disappearance of gynecomastia.

Conclusions

Gynecomastia, caused by a hormonal imbalance between testosterone and estrogen levels (hormones that control the development of sexual characteristics), is a sign that sometimes can be seen in Klinefelter syndrome. This syndrome is a form of primary hypogonadism, characterized by the presence of an extra X chromosome (47XXY), small testicles, dysgenesis of the seminiferous tubules, elevated gonadotropin levels, low serum testosterone levels, underdeveloped secondary sexual characters and male infertility. It is the most common cause of male hypogonadism with an incidence of 1 in 1000 live births. Some men have no symptoms and do not know what they suffer until adulthood when infertility occurs. It is mainly caused by an error in the disjunction during meiosis, or by an error during the mitotic divisions of the zygote, producing cases of mosaicism.

In this case, the importance of teamwork between different services to obtain an accurate diagnosis is highlighted. Breast pain revealed gynecomastia, the abnormal hormonal values confirmed an underlying pathology that was identified with the study of the karyotype. It's important to establish the early diagnosis of this syndrome, in order to treat and offer the appropriate genetic counseling.

doi: 10.1016/j.cca.2022.04.618

T140

Comparison of BRAHMS TRAK human radioimmunoassay and Siemens Immulite 2000 TSI for measurement of TSH-T antibodies

S.K. Lim, J. Guéchot, A. Pilon

Laboratoire d'Hormonologie-RIA-Immunoanalyse, DMU Biologie et Génomique Médicales, France

Background-aim

The measurement of thyroid-stimulating hormone receptor (TSH-R) antibodies is widely used for diagnosis and follow-up of Graves' disease (GD). These antibodies may be stimulatory (TSAb), inhibitory (TBAb) or functionally neutral. Classically only time-consuming bioassays can measure functional activities of TSHR antibodies (TSH-R-Ab). Since 2016, Siemens commercializes an automated immunoassay supposed to measure specifically TSAb. The aim of the study was to compare the performance of BRAHMS TRAK radioimmunoassay (RIA) and Siemens Immulite 2000 TSI and to analyse the reasons for discrepancies

Methods

All sera referred for TSHR-Ab measurement between November 2016 and October 2019 (n = 2554) were analysed by BRAHMS TRAK RIA (TRAK) and Immulite 2000 TSI (TSI). The positivity cut-offs were ε 1.5UI/L and ε0.55UI/L respectively.

The data were analysed using GraphPadPRISM.

Results

Spearman's coefficient of correlation was 0.767. Deeming's regression equation showed y = 0.899x-0.522 (y = TRAK, x = TSI). Negative agreement (TRAK < 1.5UI/L and TSI < 0.5UI/L) was 81.7% and positive agreement (TRAKε1.5UI/L and TSIε0.55UI/L) was 14.6%. Thus, total agreement was 96.4%. Disagreement for which TRAKε1.5UI/L and TSI < 0.55UI/L was 1.1% of which 82% were treated GD patients or new-borns of treated mothers. None of the TRAK values was above 6UI/L. Disagreement for which TRAK < 1.5 UI/L and TSIε0.55UI/L was 2.5% of which 75% were treated GD patients or new-borns of treated mothers. A major discrepancy was observed in one patient with TRAK < 0.5UI/L and TSI > 20UI/L. She had a history of GD but thyroid function was normal during the course of the study.

The two TSH-R measurement methods were well correlated ($p < 0.0001$). Agreement for positivity or negativity between the two methods reached 96%. The majority of disagreement was observed in treated patients and in new-borns of treated mothers, which suggests that in treated patients the decay kinetics of TSH-Ab measured by BRAHMS TRAK and Siemens TSI are different. The observation of high TSI levels without active GD argues against the specificity of TSI for TSAbs.

Conclusions

Immulite TSI test could be used for diagnosis and follow-up of GD patients. However, the manufacturer's claims to measure only TSAb should be reconsidered.

doi: 10.1016/j.cca.2022.04.619

T141

9 am cortisol as a predictor of short synacthen test in adrenal insufficiency and value of 60min cortisol measurement

G.P.N. Abeynayake, C. Meegama

Department of Clinical Biochemistry, National Hospital of Sri Lanka, Colombo 10, Sri Lanka

Background-aim

The short synacthen test (SST) is the dynamic function test most widely used to assess pituitary adrenal axis. It is possible that a single basal cortisol value can predict the response of this dynamic test. Furthermore there is no clear consensus on sampling times which could best assess the adrenal reserve. Our aim was to determine a morning baseline cortisol value that could predict SST response and value in measuring 60 min cortisol following synacthen administration

Methods

Observational, retrospective cross sectional study at Radio Immunoassay laboratory in National Hospital of Sri Lanka.

Retrospective analysis of results of short synacthen test with Advia Centaur XP from May 2017 to October 2017. The SST was considered to have an inadequate response when 30 min cortisol value was below 500 nmol/L. ROC curve was generated to determine a predictive value of basal morning cortisol for failing SST. Data was analyzed with mCNemar's test using SPSS version 20 to see the diagnostic disagreement between both sampling times.

Results

Of the 108 patients 33 passed the SST while 75 had failed the test. Using receiver operator curve (ROC) analysis baseline cortisol level predicting failing the SST with 100 percent specificity was 132 nmol/L (sensitivity 36%). A basal cortisol level < 437 nmol/L had 100% sensitivity and 27% specificity for failing test. Out of 98 matched pairs 28 had normal responses while 46 patients had inadequate response at both 60 min and 30 min. Twenty three cases (23.5%) detected as insufficient at 30 min were found to have a normal response at 60 min and the p value was < 0.001.

Conclusions

9 am cortisol can be a useful tool in initial assessment of adrenal function and restricting SST to patients with basal cortisol in between 132 and 437 would have prevented 37% of SST resulting in significant cost benefit. If 60 min cortisol is not measured a significant proportion of people undergoing SST could be inappropriately diagnosed as having adrenal insufficiency.

doi: 10.1016/j.cca.2022.04.620

T142

Association of 25-(OH)-Vitamin D and Vitamin B12 status with poor glycaemic control type II dibetes patient among Nepalese population

A. Chaudhary, S. Pokhrel, R. Pokhrel, N. Pokhrel

Modern Diagnostic Laboratory and Research Center, India

Background-aim

Vitamin D deficiency leads to impaired glucose tolerance, and Vitamin D affects insulin sensitivity and insulin secretion. Hence, this study was carried out to establish the relationship of Vitamin D status with poor glycaemic control of diabetes mellitus.

Methods

This cross-sectional study was conducted among 100 already diagnosed diabetes patients attended in Modern Diagnostic Center. Sociodemographic data and anthropometric measurements were recorded using a standard questionnaire. Fasting Plasma glucose, HbA1c and Vitamin D3 &Vitamin B12 were estimated by Dimension RxL Max Chemistry Analyser, Lefetronic-H9 Hemoglobin Analyser and Advia Centaur Xp Immunoassay. Student's t-test and Chi-square test were used for comparison between different groups and the correlation was established by Spearman's correlation

Results

The mean serum level of 25-(OH)-Vitamin D and Vitamin B12 of study population was 21.51 ± 11.34 ng/ml and 509.08 ± 510.84 ng/L respectively. Lower level of Vitamin D3 was significantly associated with higher level HbA1c, duration of diabetes and Fasting Plasma glucose level ($p < 0.05$). Higher level of HbA1c shows significantly negative correlation with serum vitamin D3 ($r = -0.395$, $p < 0.01$).

Conclusions

The poor glycaemic control in our study population was associated with Vitamin D3. We recommend evaluating the Vitamin D3 in diabetic patients, and supplementation of Vitamin D may improve the poor control of diabetes and their comorbidities.

doi: 10.1016/j.cca.2022.04.621

T143

The effect of thyroid status on HbA1c

E. Urrechaga[a], C. Valladares[c], A. Martinez[b], M. Merino[c], G. Muguerza[c]

[a] *Biocruces Bizkaia Research Institute, Baracaldo, Spain*
[b] *Facultad de Ciencia y Tecnología, Universidad de País Vasco, Leioa, Spain*
[c] *Laboratorio, Hospital Galdakao – Usansolo, Galdakao, Spain*

Background-aim

HbA1c is a marker of long-term glycemic control and a diagnostic tool for diabetes. Several factors other than glycemic status can influence HbA1c levels, including altered red blood cell (RBC) lifespan. Thyroid function status can affect the erythropoiesis, RBC turnover is increased in thyrotoxic states whereas hypothyroidism has the opposite effect. Our aim was to determine the effects of altered thyroid status on HbA1c in non-diabetic individuals and with hyper or hypothyroidism, and to verify the reverse effect after achieving euthyroid state.

Methods

55 hypothyroid, 43 hyperthyroid and 50 healthy subjects were recruited. Anemic patients and hemoglobinopathies carriers were excluded. HbA1c, complete blood with reticulocyte counts, p-glucose

were analyzed in all groups. When euthyroidism was restored the analysis were repeated and HbA1c compared with the baseline value. HbA1c was measured with Arkray 8180V analyzer; p-glucose using Roche reagent for Cobas 8000 modular analyzer and CBC with Sysmex XN 20 counter.

Differences among groups were studied using ANOVA and post-hoc tests. Changes after treatment were compared using t Student and Mann–Whitney tests considering $P < 0.05$ to be significant.

Results

None of the groups had significantly different fasting p-glucose values at baseline and posttreatment, $P = 0.001$. Baseline reticulocyte count did not differ significantly between hyperthyroid (1.15 ± 0.49 109/L) and controls (1.1 ± 0.34 109/L) while was lower in hypothyroid patients (0.81 ± 0.64 109/L) ($P = 0.03$). In hypothyroid group HbA1c was significantly higher (39 ± 2 mmol/mol) than controls (33 ± 1 mmol/mol), $P < 0.001$). HbA1c in hyperthyroid (34 ± 5) mmol/mol were not significantly different from controls $P = 0.193$). Reticulocyte count increased significantly following treatment in hypothyroid patients,1.25 ± 0.89 109/L, $P < 0.001$. After treatment HbA1c reduction was significant in hypothyroid (35 ± 7 mmol/mol) and the difference with pretreatment values significant $P < 0.001$.

HbA1c did not change significantly following treatment in hyperthyroid group, 35 ± 7 mmol/mol, $P = 0.133$.

Conclusions

Interpretation of HbA1c values is complex, erythropoiesis status and RBC turnover can affect the results. In patients with hypothyroidism HbA1c values tend to be higher, so data should be interpreted with caution in patients with thyroid dysfunction.

doi: 10.1016/j.cca.2022.04.622

T144

Catecholamine secreting cervical mass in a patient with hypotensive episodes

A. Ortiz Temprado, C. Montero Dominguez, L. Martinez Figueras, M.L. Fernandez Ruano

Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain

Background-aim

Paragangliomas (PGLs) are rare neuroendocrine tumors that can be derived from either the parasympathetic or sympathetic nervous system. They typically occur in the second and third decades of life. PGLs of the head and neck (HNPGLs) are characteristically located at the carotid bifurcation, the vagus nerve, the jugular foramen and the middle ear space. Their location in close proximity to cranial nerves and vasculature may result in considerable morbidity due to compression or infiltration of the adjacent structures.

Methods

Urinary normetaneprhine (NMN), metanephrine (MN), and 3-methoxytyramine (3-MT) were measured by HPLC (Agilent® 1100 series, BIO-RAD®).

Results

A morbid obese 69-year-old man was admitted to our hospital with a history of constitutional syndrome, hypotensive episodes and fever. Neck physical examination revealed a right sided cervical mass with no palpable lymphadenopathy. During his hospital stay he presented episodes of hypotension likely resulted from irritation of the carotid sinus. 24-hour biochemical tests showed raised NMN: 3643 nmol/24h (885-2880 nmol/24h), with normal MN and 3-MT. A contrast computed tomography scan showed a hypervascularized mass on the bifurcation of the right common carotid artery; magnetic resonance imaging confirmed a tumor of 3.1 x 8.5 x 6.4 cm (Shamblin III).

The patient was treated with alpha-1 adrenergic blocking agents (Doxazosin) but treatment was ceased due to exacerbation of the hypotensive crisis. Preoperative embolization was performed before excision of the carotid tumor. The patient was referred for genetic testing. He had no early postoperative complications.

Conclusions

Clinical presentation of PGLs varies greatly due to different patterns of catecholamine secretion or tumor mass effect. However, in many cases there is a lack of signs and symptoms and, if present, these can be very similar to those observed in other conditions. HNPGLs are rare, hence a high level of suspicion is necessary for early diagnosis. Only a small proportion of HNPGLs are hyperfunctional. Detection of these tumors is possible by biochemical measurement of NMN, MN and 3-MT in 24h-urine samples. As rapid discovery and treatment of PGLs is potentially lifesaving, biochemical testing of catecholamines and their metabolites is essential for the evaluation of patients with cervical masses.

doi: 10.1016/j.cca.2022.04.623

T145

Assessment of urine and serum iodine status in the population of Tibet, China: No longer an iodine-deficient region

Z. Yutong

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing 100730, China

Background-aim

Iodine is critical for synthesis of thyroid-related hormones, and either low or high iodine status can lead to thyroid dysfunction. This study aimed to evaluate the status of the Tibetan population.

Methods

From September 2016 to August 2018, 1,499 healthy adults from 3 areas of varying altitude in Tibet were enrolled in the study. Iodine concentrations were measured using inductively coupled plasma mass spectrometry. The urine iodine concentration (UIC) was adjusted by the urine creatinine concentration, and the ratio of serum iodine concentration (SIC) to UIC was calculated.

Results

Respectively, the median concentration of UIC, adjusted UIC and SIC was 137.9 μg/L, 118.4 μg/g and 58.3 μg/L. And 30.4%, 63.0%, and 9.6% of the participants were considered as iodine deficiency, iodine sufficiency, and iodine excessive, according to the recommendation from WHO. The correlation between UIC, adjusted UIC and SIC was good ($r > 0.6$, $p < 0.01$). The SIC was more stable than the UIC, and was not

associated with age or sex. The SIC/UIC ratio and thyroid-stimulating hormone (TSH) increased with age (SIC/UIC: 0.39 vs 0.45; and TSH: 2.65 vs 3.05 mIU/L, in participants aged <50 and >50 years, respectively).

Conclusions

Human iodine status of adults in Tibet were evaluated and considered adequate, based on this multicenter cross-sectional study.

doi: 10.1016/j.cca.2022.04.624

T146

Comparative study on the effect of Implanon, Jadelle and Depo-Provera on Apo A1 and Apo B concentrations on females attending a tertiary hospital in south-south Nigeria

S. Meludu [b], O. Bamigbowu [c], E. Dioka [b], C. Obi-Ezeani [a]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Nnamdi Azikiwe University, Awka, Anambra State, NIGERIA*
[c] *University of Port Harcourt, Rivers State, Nigeria*

Background-aim

Apolipoproteins function as structural components of lipoprotein particles, cofactors for enzymes and ligands for cell-surface receptors. Apo A1 a major component of high density lipoprotein, while ApoB is a primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL, when in reference to both heart and vascular diseases indictment, helps in the transportation of fat molecules (lipids): cholesterol inclusive around the body. The study was carried out to compare Apo A1 and ApoB levels in women on Implanon, Jadelle and Depo-Provera- the three most popular steroidal contraceptives used in south-south part of Nigeria.

Methods

Ninety (90) female subjects in three groups of 30 each were recruited for Implanon, Jadelle and Depo-provera. Blood samples were randomly collected from all subjects at baseline, 3rd, 6th, 9th and 12th month and their ApoA1 and ApoB concentrations were determined using turbidimetric method.

Results

The result of the study showed that there was significant difference in Apo A1 concentration of females on Implanon, Jadelle and Depo-provera at 3rd, 6th, 9th and 12th months compared with the baseline. Also, a significant difference ($P < 0.05$) was observed at the 3rd, 6th, 9th and 12th month in ApoB when compared with the baseline. A monthly dependent increase in the three contraceptives studied was observed, a pattern which is also observed in Apo A1 except in implanon.

Conclusions

The study showed that there were changes in Apo A1 and ApoB concentrations in female subjects taking Implanon, Jadelle and Depo-provera at different months.

doi: 10.1016/j.cca.2022.04.625

T147

Catecholamine-secreting jugular paraganglioma presenting as vocal cord palsy

C. Montero Dominguez, A. Ortiz Temprado, L. Martinez Figueras, A. Mata Fernandez, J. Ubeda Arades

Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain

Background-aim

Paragangliomas (PGLs) are rare tumors which arise from paraganglionic tissue derived from neural crest cells. Genetic testing for germline mutations has been recommended in patients with PGLs. In the head and neck region, PGLs may be located in the carotid bifurcation, the jugular bulb, the tympanic plexus and the vagal ganglia.

Jugular foramen paragangliomas (JPs) are slow-growing, encapsulated, hypervascular tumors that arise from jugular foramen of temporal bone. Pulsatile tinnitus is the most common presenting symptom. Lower cranial neuropathies are less common. JPs are locally aggressive and involve important neurovascular structures, like the internal carotid artery, the jugular bulb, the facial nerve and the lower cranial nerves. Complete surgical resection is the gold-standard management.

Methods

Urinary dopamine (DA), norepinephrine (NA), epinephrine (A), 3-methoxytyramine (3-MT), normetanephrine (NMN) and metanephrine were measured by HPLC (Agilent® 1100 series, BIO-RAD®).

Results

A 68-year-old women presented with a 2-year history of dysphonia. Without a history of neck mass, hearing loss or pulsatile tinnitus. On examination, there was right vocal cord palsy and dysphagia. The biochemical analysis revealed an increase in NMN: 3650 nmol/24h (885–2880), 3-MT: 3484 nmol/24h (565–2390), DA: 567 ↑g/24h (190–450), NA: 217 ↑g/24h (12–85) and A: 147 ↑g/24h (2–22). Computed Tomography and Magnetic Resonance Imaging examination was performed, which showed evidence of an expansile, well-defined mass lesion in the region of the right jugular fossa of approximately 16 mm. After tumor embolization, the patient underwent a subtotal resection of the lesion through the type B infratemporal fossa approach, without transposing the facial nerve. After mass-resection, 24-h urine NMN and 3-MT were normalized at 5 months. Hereditary PGL genetic testing was requested.

Conclusions

PGLs present a clinical challenge due to their variable clinical presentation and the high proportion of clinically silent tumors. Early diagnosis is based on recognition of clinical symptoms and maintaining a high index of suspicion for this disease. Though head and neck PGLs are rarely secretors of catecholamines (<4%), screening catecholamine metabolites should be included in the diagnostic workup to identify the rare secreting PGLs as well as the follow-up of these patients.

doi: 10.1016/j.cca.2022.04.626

T148

There is something growing inside of me: A case of Giant Cystic Pheochromocytoma (GPCCS)

S. Ximbi, A. Rampul

Steve Biko Core Lab NHLS, University of Pretoria, South Africa

Background-aim

Giant Cystic Pheochromocytomas are rare neuroendocrine tumours that arise from the chromaffin cells of the renal medulla or in ectopic tissue sites. These may or may not secrete catecholamines making their diagnosis much more difficult to make. Patients with these tumours may present with a clinical tetrad of palpitations, hypertension, headaches and abdominal symptoms.

Methods

A 47-year-old female presented to Tshwane academic Hospital with intermittent diarrhoea, left sided abdominal pain, occasional dyspepsia and significant weight loss over a period of two years. The patient also had menorrhagia and iron deficiency anaemia which was treated with supplements. Her physical examination showed that she had pallor and a high blood pressure on two separate occasions. The abdominal examination revealed a large palpable left upper quadrant mass

Results

Her haematological investigations revealed a mild hypochromic microcytic anaemia. The biochemical investigations showed an elevated serum chromogranin of 5952 ng/ml (reference range 0-84 ng/ml), elevated urine normetanephrines of 5715 nmol/24hours (reference range 480 – 2424) and an elevated homovanillic acid of 408,6 μmol/24hours (reference range 8 – 48). A computerized tomography scan was then done and it showed a large left adrenal mass >7cm in size. The diagnosis of Giant Cystic Pheochromocytoma was made and the patient is being worked up for surgical excision of the adrenal mass.

Conclusions

Giant Cystic Pheochromocytomas are rare and when they do occur according to literature, they rarely secrete both dopamine and normetanephrines. By definition GPCCS are tumours that are more than 7cm in size. Biochemical and physical findings are usually diagnostic, while CT scans serve as adjuncts for the confirmation of the diagnosis. The secretory capacity of catecholamines will help the surgical and anaesthetic teams pre-operatively pre-empt an intra-operative hypertensive crisis by administering alpha or beta blockers.

The diagnosis of GPCCS serves to bring attention to a surgical and medical condition with little literature of its description to our health care professionals as the vague symptomatology is not very useful in helping them make the diagnosis. This case will hopefully help yield a high index of suspicion when a patient presents with vague abdominal symptoms.

doi: 10.1016/j.cca.2022.04.627

T149

Effect of depo provera on female hormones in child bearing aged women attending a tertiary hospital in southern Nigeria

O.E. Bamigbowu[c], S.E. Meludu[d], E.C. Dioka[b], A.O. Adegoke[e], B.O. Onyema-Iloh[a]

[a] *Department of Chemical Pathology, Nnamdi Azikwe University Teaching Hospital Nnewi, Anambra, Nigeria*
[b] *Department of Chemical Pathology, Nnamdi Azikwe University, Awka, Nigeria*
[c] *Department of Chemical Pathology, University of Portharcourt, Rivers, Nigeria*
[d] *Department of Human Biochemistry, Nnamdi Azikwe University, Awka, Nigeria*
[e] *Department of Medical Laboratory Science, Madonna University, Elele, Nigeria*

Background-aim

Depo provera is a popular hormonal medication of progestin type popularly used in the southern part of Nigeria. It is used as a method of birth control and also a part of menopausal hormone therapy. It is an injectable known as depot medroxyprogesterone acetate. It stops pregnancy by preventing ovulation and thickening the cervical mucus. Though having some noticeable side effects like acne, dizziness, nausea, sleeplessness and irregular menstrual period and weight gain after a period of use. Depo is usually administered as a shot of 150mg/ml injection which last for 12weeks. Advantageously, it is very easy to administer and it helps women against the monotony and forgetfulness in taking daily pills. This study is aimed at ascertaining the effect depo has on the hormones- gonadotropins and gonadal

Methods

Two hundred (200) women attending a tertiary hospital in southern part of Nigeria, who has not been on any previous contraceptive, had 5millilitre of their blood collected at baseline, 3rd, 6th, 9th and 12th month after quarterly administration of depo provera. LH, FSH, Prolactin, Progesterone and Estradiol were determined using Enzyme Linked Immuno Assay method kit. The data obtained were subjected to statistical analysis using statistical package for social science (SPSS) version 21

Results

There is a significant decrease in LH concentration, while there is a significant increase in prolactin and progesterone from baseline to the 12th month. There was no significant difference in FSH and Estradiol concentrations. Also, there was significant difference ($P < 0.05$) in LH concentration at different age groups, while no significant difference ($P > 0.05$) in FSH, prolactin, progesterone and estradiol at different age groups.

Conclusions

The result of the study showed that Depo provera usage caused a decreased in LH with increase in prolactin and progesterone; an endocrinology condition which supports anovulatory cycle.

doi: 10.1016/j.cca.2022.04.628

T150

The relationship of serum dehydroepiandrosterone levels with ejection fractions in heart failure patients

R.H. Chrisnanda [b], M.R. Fuadi [b], S.P. Edijanto [b], M. Yusuf [a]

[a] *Department of Cardiology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Hospital, Surabaya, Indonesia*
[b] *Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Hospital, Surabaya, Indonesia*

Background-aim

Cardiovascular disease is still a serious healthcare problem in the world. Life expectancy after being diagnosed with heart failure is 50% and 10% for 5 and 10 years. Heart failure is characterized by a decrease in heart ejection fraction. Steroid hormones such as dehydroepiandrosteron (DHEAS) have cardioprotective effects by inhibiting the formation of atherosclerotic plaque, pulmonary artery vasodilators and protecting cardiomyocytes. DHEAS levels decrease with age. DHEAS is expected to be an alternative laboratory examination to help diagnose heart failure quickly

Methods

A sample of 34 people aged > 30 years were diagnosed with heart failure by a cardiologist. Ejection fraction data obtained from echocardiographic examination. DHEAS levels were taken from venous blood and examined using the CLEIA method with an Immulite tool (Siemens Healthineers, Germany). Statistical analysis was performed with the Spearman correlation test, with a significance level of $p < 0.05$.

Results

From 34 research subjects, found that 13 people had an ejection fraction <40% (heart failure with reduced ejection fraction / HFrEF), 12 people had an ejection fraction 41-49% (borderline) and 9 people had an ejection fraction ε 50% (heart failure with preserved ejection fraction / HFpEF). Spearman correlation test results showed a significant relationship between DHEAS and ejection fraction with $r = 0.357$ and $p = 0.038$. A decrease in DHEAS has a positive correlation with a decrease in the heart ejection fraction.

Conclusions

Decreased serum DHEAS levels have a significant relationship with ejection fractions in patients with heart failure even though the correlation value is low. The lower the DHEAS level in the serum, the lower the ejection fraction.

doi: 10.1016/j.cca.2022.04.629

T151

Hormone profile in benign breast disorder

A. Yadav [b], A. Chopra [a], S. Thomas [a], M. Jain [a], R. Anand [a]

[a] *Lady Hardinge Medical College and Assoc. Hospital, New Delhi, India*
[b] *vardhaman Mahavir Medical College and safdarjang Hospital, New Delhi, India*

Background-aim

Background: The prevalence of benign breast disorders (BBD) has been ranged from 16-50% in various reports. These disorders are often a cause of anxiety to the patient as well as the clinician as they are often misdiagnosed and misinterpreted as malignancy. These disorders are an established risk factor for breast cancer, the risk gradually increasing from proliferation without atypia having a 2-fold risk, to proliferation with atypia with a risk of more than 4-fold.There are various studies done that show a relation between various hormones and the incidence as well as progression of benign breast diseases.

Aim :To study the serum levels of Estrogen, Progesterone, Leutinising hormone (LH), Follicle stimulating hormone (FSH), Testosterone, Thyroid stimulating hormone (TSH), Prostate specific antigen (PSA), Prolactin in patients with BBD.

Methods

Cross-sectional study with total of 134 patients with non- inflammatory benign breast diseases attending the surgery OPD at a tertiary care hospital were included the study. All the females with infective or inflammatory diseases of breast, pregnant or lactating, taking hormonal treatment, or with obvious features suggestive of malignancy were excluded from the study. A written and informed consent was taken from all the patients included in the study. Estrogen, progesterone, LH, FSH, Testosterone, Prolactin ,TSH and PSA were measured using chemiluminiscence immunoassay

Results

The study patients were between 17-59 years of age, with an average age of 26.9 years. 129 (96.26%) patients were in the reproductive phase, 2 were post-menopausal and 3 were post-hysterectomy. In the study there were Non-proliferative BBD (n = 23), Proliferative BBD (n = 67), Fibroadenoma (n = 49) and Mastalgia (n = 44) patients.We found estrogen (21.04-856pg/ml), Progesterone (0.105-4.32ng/ml),LH (1.57-42.71IU/L), FSH(1.86-66.45IU/L), Prolactin(1.86-59.29ng/ml),Testosterone (2.5- 67.71ng/dl),TSH(0.09-26.94 ⌈ IU/ml),PSA (0-0.11ng/ml). In our study , Estrogen (9.7%) , LH (6.7%) , Progesterone (2.98%) , FSH (8.95%) , prolactin (8.95%) levels were raised in repective patients while 1 patient had decreased LH levels and 2 had decreased prolactin levels. 28 (20.89%) patients in our study population, showed less than normal testosterone levels. 69 (51.49%) patients had significantly detectable s.PSA levels. Our results may be explained by findings that suggest that in premenopausal women, circulating testosterone and estradiol levels peak at midcycle, but in transition to the luteal phase (during breast epithelial proliferation), testosterone levels go down and estradiol levels increase further. However, it has also been shown that testosterone has the potential to be metabolized within breast tissue to 17®-estradiol (E2), the most potent natural ER⟨ ligand, or DHT, the most potent natural AR ligand, via the activity of aromatase and 5⟨-reductase enzymes, respectively.

Conclusions

Considering the association of various hormones with benign breast diseases, a raised serum levels of all the 7 hormones (estrogen, progesterone, LH, FSH, prolactin, thyroid, PSA), most prominent with PSA, estrogen, prolactin and FSH, and a decrease in testosterone may be associated with the occurrence of BBD. The change in hormone profile of the patient may be the hint to look for the benign disorders of breast and may help in the treatment.

doi: 10.1016/j.cca.2022.04.630

T152

Falsely evaluated ACTH due to presence of heterophile antibody in serum: A case report

Y. Yin, L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing, China

Background-aim

The accurate measurement of plasma ACTH has important significance to the etiologic diagnosis of Cushing syndrome. Falsely evaluated ACTH due to serum heterophile antibody would mislead the decision of clinicians. A case of falsely evaluated ACTH in our laboratory was presented.

Methods

Herein, a case was described that a 15 years old Chinese boy with Cushing syndrome, was found to have a moderate ACTH level of 23.6pg/mL (Siemens Immulite) and low dose dexamethasone inhibition test showed serum cortisol was not inhibited. Biochemical evidences point to ACTH-dependent Cushing. However, imaging test and experience of clinician prompted physician to doubt the accuracy of ACTH. A series of tests for interference identification were conducted, which included replacing the test platform, sample dilution, PEG precipitation and using of blocking antibodies.

Results

Results indicated the presence of heterophilic antibodies led to false positive ACTH performed on Siemens Immulite. The final diagnosis of the boy was a rare type of ACTH-independent Cushing syndrome, primary pigmented nodular adrenocortical disease.

Conclusions

Falsely evaluated ACTH lead to the deviation of clinical thought, increasing treatment time and unnecessary tests. Physician and laboratory should increase communication and raise concern of interference cases. Laboratory should establish their anti-interference workflow.

doi: 10.1016/j.cca.2022.04.631

T153

Correlation analysis of serum GDF-15, miR 21 and SMAD7 in obese pre-diabetics and type 2 diabetics at a tertiary care centre of India

P. Purohit [a], D. Roy [a], M. Khokar [a], A. Modi [a], R. Shukla [b], P. Sharma [a]

[a] *Department of Biochemistry, AIIMS Jodhpur, India*
[b] *Department of Endocrinology & Metabolism, AIIMS Jodhpur, India*

Background-aim

Despite having lower overweight and obesity rates, Indians have a higher prevalence of diabetes compared to the western population. miR-21 is known to be raised in obesity. Growth differentiation factor15 (GDF15) is identified as a novel marker of IR. However, there is a lack of Indian data in obese- pre-diabetics and Type 2 diabetics for circulating levels of miR-21, GDF-15 and their association with potential regulatory molecule SMAD7.

The current study was designed for analyzing the association of circulating miR-21 with serum GDF-15 and SMAD7 in obese-pre-diabetics and Type 2 diabetics.

Methods

The study included 30 obese pre-diabetics, 73 obese Type 2 diabetics, and 53 healthy controls. Data collection included demographic and anthropometric information along with basic biochemical profile (including Fasting blood sugar, serum insulin, HOMA-IR, lipid profile and GDF-15). Gene expression of SMAD7 and hsa-miR-21-5p was done by real-time PCR using reagents and primers by Qaigen. Data were analysed using one way ANOVA for appreciating the difference across the study groups for biochemical parameters. Fold change expression was done using delta-delta Ct method for miRNA and mRNA and normalization was done using internal controls as RNU6 and GAPDH for miRNA and mRNA respectively.

Results

The one way ANOVA of BMI showed a significant difference across the three study groups ($p < 0.0001$). Biochemically, there was a significant difference across the three study groups for FBS, HbA1c, lipid profile (Total Cholesterol, Triglycerides) HOMA-IR but not Insulin. The fold change expression of hsa-miR-21-5p was 1.10 fold upregulated in obese pre-diabetics and 7.6 fold upregulated in Type 2 Diabetics. Circulating miR-21 showed a positive association with GDF-15 ($p = 0.030$) and SMAD7 ($p < 0.0001$) and GDF-15 showed a negative and significant association with SMAD7 ($p < 0.0001$). ROC curve of GDF-15 showed an AUC of 0.868 ie 86.8% sensitivity.

Conclusions

The above study findings help to conclude that serum GDF-15 and miR-21 show a gradual increase in obese individuals as they progress from pre-diabetics to Type 2 diabetic state. Further, miR-21 over-expression possibly promotes insulin resistance via SMAD7 and GDF-15. Thus, miR-21 and GDF-15 maybe potential new biomarkers of pre-diabetes and type -2 diabetes mellitus.

doi: 10.1016/j.cca.2022.04.632

T154

Functional paraaortic paraganglioma in a 28-years-old woman

C. Montero Dominguez, J. Gorrin Ramos, A. Mata Fernandez, A. Ortiz Temprado, L. Martinez Figueras, P. Blanco Soto

Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain

Background-aim

Paragangliomas (PGLs) are a rare type of non-adrenal neuroendocrine tumor derived from chromafine cells of the neural crest, with the ability to secrete catecholamines. PGLs can be divided into sympathetic and parasympathetic tumors; sympathetic PGLs are located in the thorax (12%), abdomen (85%) and pelvis. PGLs had a low incidence (2-8 cases per million) and an equal sex distribution. The most common clinical presentation is hypertension, headache and sympathetic hyper-

activity. At least 30% of PGLs are known to be hereditary, a proportion that has increase with the discovery of new susceptibility genes.

Methods

Urinary methanephrine (MN), normetanephrine (NMN) and 3-methoxythyramine (3-MT) were measured by HPLC (Agilent® 1100 series, BIO-RAD®). The genotyping study was performed on an Illumina® (MiSeq) platform and analyzed by SOPHiA® platform.

Results

A 28-years-old female was admitted to emergency department with rectal bleeding. She had a previous history of hypertensive crisis and oppressive chest pain unrelated with other symptoms or stressful situations. During the radiological examination by ultrasonography was observed a solid mass measuring approximately 3x2.7x2.5 cm in left paraaortic region. The previous findings were confirmed by Magnetic resonance imaging and ^{123}I-metaiodobenzylguanidine scan. Biochemical results were: MN 378 nmol/24h (325-1530); NMN 9680 nmol/24h (885-2880) and 3-MT 1779 nmol/24h (565-2390). Blood pressure control was achieved with combined ⟨-adrenergic and ®-adrenergic blockers. There was intraoperative hypertensive crisis and arrhythmias due to manipulation of the tumor. Postsurgical catecholamines levels were in normal range. Genetic test of the patient demonstrated a heterozygous germinal variant of uncertain significance (VUS) in the SDHB gene (c.622G > C (p.Gly208Arg)).

Conclusions

PGLs are rare tumors associated with high morbidity secondary to mass effect and high circulating catecholamine. The high complexity requires: a criterial clinical evaluation, establishing the correct differential diagnosis and a multidisciplinary team to improve outcomes. In our case, we assessed the utility of urinary 3-MT, NMN and MN for the differential diagnosis of PGLs. Genotyping study is important to perform an adequate genetic counselling.

doi: 10.1016/j.cca.2022.04.633

T155

Serum anti-Mullerian hormone levels and insulin resistance in polycystic ovary syndrome

R. Makhlouf, K. Chaabouni, A. Charfi, D. Jallouli, A. Elleuch, F. Ayedi

Research Unit of Molecular Bases of Human Diseases/Medicine School of Sfax, Clinical Chemistry Departement/Habib Bourguiba University Hospital of Sfax, Tunisia

Background-aim

Polycystic ovary syndrome (PCOS) is a prevalent disease affecting women of reproductive age. The anti-Mullerian hormone (AMH) is increased in PCOS and is related to the degree of severity of PCOS. The mechanisms resulting in increased AMH are poorly understood. Insulin resistance (IR) is a feature of PCOS. IR is also associated with the severity of PCOS. However, there are no clear correlation between levels of AMH with the incidence of IR in PCOS women.

Aim:

To analyse the serum AMH levels in PCOS with and without IR.

Methods

This Cross-sectional study was conducted on women with PCOS. The diagnosis of PCOS was based on the Rotterdam criteria. PCOS women were categorized into four different PCOS phenotypes according to the Rotterdam criteria. Assessment of serum AMH and insulin were performed using an electrochemiluminescence immunoassay on a Cobas 6000® automated assay system (Roche Diagnostics GmbH, Germany). IR was estimated via the Homeostatic model assessment insulin resistant (HOMA-IR) formula. Women with PCOS were grouped according to the presence of IR (HOMA-IR ε2.5). Serum AMH were compared between this two groups. Further, HOMA-IR and AMH values were compared among different PCOS phenotypes. Data analysis was done using Statistical Package for the Social Sciences version 20.0(SPSS®). A $p < 0.05$ was regarded as statistically significant.

Results

A total of 40 women with PCOS were selected and divided two groups: 11 women with IR; 29 women without IR. AMH levels in PCOS patients with and without IR do not differ significantly ($p = 0.887$). No correlation was found between AMH and insulin ($p = 0.625$) and HOMA-IR ($p = 0.97$). HOMA-IR and AMH were not correlated with different PCOS phenotypes ($p = 0.58$).

Conclusions

No correlation between serum AMH and IR could be established in Tunisian PCOS women and among the different phenotype groups. The mechanism underlying the relation between AMH and IR is not clear yet and further larger studies are needed.

doi: 10.1016/j.cca.2022.04.634

T156

Salivary cortisol and immunoglobulin A amongst healthcare workers in a Nigerian tertiary hospital; A biochemical study of job strain model of occupational stress

A.S. Aleke [a], J.M. Olisekodiaka [a], C.C. Ibeh [b], J.A. Onuegbu [a], C.E. Onah [c]

[a] *Department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria*

[b] *Department of Community Medicine, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria*

[c] *Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria*

Background-aim

Healthcare workers are associated with high job stress and psychological distress. Measurement of physiological response to stress and its biochemical correlates could add objective evidence to subjective perception of job stress. This study was designed to compare salivary cortisol and immunoglobulin A amongst 84 apparently healthy male healthcare workers consisting of Medical doctors (15), Nurses (11) Medical Laboratory Scientists (MLS) (18), Radiographers (16), Pharmacists (7) and non-clinical staff (17). Grouped based on job demand (JD): high JD (34) and low JD (50). Grouped based on job control (JC): high JC (39) and low JC (45). Grouped based on job strain: no job strain (62) and high job strain (22).

Methods

Salivary cortisol and immunoglobulin A concentrations were measured by ELISA. Saliva volume was measured by gravimetric method. Saliva flow rate and immunoglobulin A secretion rate were computed. Serum albumin, fasting plasma glucose concentrations were determined by spectrophotometric methods.

Systolic and diastolic blood pressures were measured in a sitting position and after 5 minutes rest using a standardized automatic blood pressure monitor (Omron HEM 7113), body weight and height were measured and body mass index (BMI) computed.

Results

Results from this study showed that radiographers (14.56 ± 3.24) had significantly higher JD than MLS (11.06 ± 2.38); BMI was lower in low JC (24.67 ± 3.20) than high JC (26.17 ± 3.57). Salivary cortisol was higher in low JC (10.95 ± 3.20) than high JC (7.89 ± 4.20). salivary cortisol and immunoglobulin A concentrations were significantly higher in high job strain (11.65 ± 5.76 and 120.6 ± 23.5) than in no job strain (8.48 ± 4.12 and 105.7 ± 30.5) respectively. JC had significant negative Pearson's correlation coefficient (r = -0.268, p = 0.014) with salivary cortisol.

Conclusions

Low job control may stimulate hypothalamus-pituitary-adrenal system. In addition, acute exposure to job stress may lead to stimulating effect on the mucosal immunity.

doi: 10.1016/j.cca.2022.04.635

T157

The application of additional medical information obtained from HbA1c results by capillary electrophoresis method

A. Thongkomon

Chemistry Unit, Buddhasothorn hospital, Chachoensao, Thailand

Background-aim

There are factors that interfere HbA1c measurement which generate inaccurate HbA1c results that do not correspond to the patients' blood glucose, in particular the patient with thalassemia and hemoglobinopathies. This study aims to evaluate the usefulness of additional medical information obtained from HbA1c results by SEBIA capillary electrophoresis method in order to detect thalassemia and hemoglobinopathies.

Methods

6,148 diabetes patients from out patients department at Buddhasothorn hospital, Thailand during 1 July 2019 - 31 August 2019 have been run HbA1c analysis by SEBIA Capillarys 2 Flex Piercing system. The samples have shown that hemoglobin disorders will be run hemoglobin analysis by SEBIA Minicap System.

Results

There are 4,890 normal hemoglobin patients (A2A with or without alpha thalassemia trait) or 79.54%, heterozygous Hb E (EA) 927 samples or 15.08 %, Blood transfusion 190 samples or 3.09% , homozygous Hb E (EE) 57 samples or 0.93%, Beta thalassemia trait 29 samples or 0.47%, heterozygous Hb CS 28 samples or 0.46%, Hb H disease 17 samples or 0.28%, EA Bart's disease 6 samples or 0.10%, Beta thalassemia-Hb E disease 3 samples or 0.05% and Elevated Hb F (A2A with elevated Hb F) 1 or 0.02%.

Conclusions

There are a lot of thalassemia and hemoglobinopathies among diabetic patients which may affect the accuracy of HbA1c results. The additional medical information obtained from HbA1c results by SEBIA capillary electrophoresis is useful, as medical laboratory can use this information to alert the physician to use the HbA1c result with caution.

doi: 10.1016/j.cca.2022.04.636

T158

Effects of methadone maintenance therapy in plasma thyroxin levels

D. Janicevic Ivanovska [a], A. Spasovska Trajanovska [c], S. Domazetovska [b], V. Koloska [b]

[a] *Institute of Clinical Biochemistry, University Clinical Skopje, UGD Medical Faculty Stip, The Former Yugolav Republic of Macedonia*
[b] *Institute of Clinical Biochemistry, University Clinical Skopje, The Former Yugolav Republic of Macedonia*
[c] *Psychiatric Hospital Skopje, The Former Yugolav Republic of Macedonia*

Background-aim

Although endocrine abnormalities are recognized in opiates users and methadone maintenance patients, very little is known about the range of hormones affected, their path physiology and their clinical relevance. Various endocrine abnormalities have been reported in these patients with included increased levels thyroxin (T4). Pathophysiological mechanism postulated does explain these findings included a direct action of heroin or methadone at the hypothalamic or pityitary level. The AIM of this study was to explore the effects of methadone maintenance therapy and heroin abuse on hypothalamic-pityitary-gonad axis. To determinate the differences of plasma thyroxin levels between examination groups.(group of heroin addicts and in the patients of MMT (methadone maintenance treatment)).

Methods

The patients of this study were examined in the Psychiatric Hospital Skopje . The study was cross section. We evaluated two groups. The first group A consisted of 60 patients of MMT in Day hospital on methadone treatment of heroin addicts and second group B consisted of 60 heroin addicts evaluation to the clinic for outpatients (ambulance) in both medical institution. In the study was using these methods: medical documentation, CLIA methods for determinate the serum thyroxin levels..The results of this study were determined by statistical program SPSS for Window 13,0 with: descriptive methods, Chi-square test, Mann-Whiten U test, t-test for independent samples, Pearson coefficient of linear correlation. The p level of statistical significances results was p < 0,05 and p < 0,01.

Results

The plasma thyroxin levels in heroin addicts (65%) were higher than in MMT patients (33%) but the results were not statistical significances p = 0,081.

Conclusions

The results in this study show that that in group of heroin addicts we got higher plasma thyroxin levels then in group of MMT patients but the correlation between two examinations groups was not significantly. So maybe treatment heroin addicts with methadone maintenance therapy will be normalization plasma thyroxin levels.

doi: 10.1016/j.cca.2022.04.637

T159

Comparison of thyroid function test results determined by two analyzers: Alinity I and Beckman Coulter UniCell DxI

S. Mandic [a], I. Lukic [b], T. Rolic [b], V. Horvat [b], M. Lukic [c], K. Mijatovic [a], D. Mandic [b], V. Seric [b]

[a] *Clinical Institute of Nuclear Medicine and Radiation Protection, Osijek University Hospital, Osijek, Croatia*
[b] *Department for Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Osijek, Osijek, Croatia*
[c] *Institute of Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia*

Background-aim

The objective of this study was to determine the comparability of thyroid stimulating hormone (TSH) and thyroid hormones (TH) results obtained by two different platforms. Accurate measurement of TSH and TH is critical for thyroid disease management. Various immunoassay methods have been used for their measurement. Comparability of test results is particularly important when two instruments are being used simultaneously in order to examine the possibility of their interchangeable usage.

Methods

This cross-sectional study was performed on 116 serum samples obtained from randomly selected patients, median age 46 (18-79), admitted to the Clinical Institute of Nuclear Medicine and Radiation Protection, Osijek University Hospital. TSH, free thyroxin (FT4) and free triiodothyronine (FT3) levels were determined on both Alinity I (Abbott Laboratories, Lake Forest, USA) and Beckman Coulter UniCell DxI (Beckman Coulter, Brea, USA) analyzers, and both by chemiluminescent immunoassay methods. Statistical analysis was performed using MedCalc for Windows, version 12.4.0.0. (MedCalc Software, Marikerke, Belgium). Passing-Bablok regression analysis was performed for methods comparison. $P = 0.05$ was considered statistically significant.

Results

Passing-Bablok regression analysis showed proportional and constant difference obtained by both methods for TSH [y = 0.01(95%CI 0.004-0.028) + 0.77(95%CI 0.747-0.781)x] and FT4 [y = 1.90(95%CI 1.032-2.806) + 0.91(95%CI 0.825-0.986)x] and constant but no proportional difference for FT3 [y = -1.83(95%CI -2.689- -0.894) + 1.08(95%CI 0.906-1.230)x]. Cusum test for linearity reveled no significant deviation from linearity for FT3 ($P = 0.98$) and FT4 ($P = 0.24$), contrary to TSH where significant deviation from linearity ($P = 0.01$) exist.

Conclusions

Comparison study showed that Alinity i and Beckman Coulter UniCell DxI analyzers can not be used interchangeably for thyroid function test measurement.

doi: 10.1016/j.cca.2022.04.638

T160

Addison disease in a child: A case report

S. Mandic [a], T. Rolic [a], I. Lukic [a], I. Marijanac [c], M. Lukic [b], V. Horvat [a], D. Mandic [a], V. Seric [a]

[a] *Department for Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, University of Osijek, Osijek, Croatia*
[b] *Institute of Clinical Laboratory Diagnostics, Osijek University Hospital, Osijek, Croatia*
[c] *Paediatric Clinic, Osijek University Hospital, Osijek, Croatia*

Background-aim

Primary adrenal insufficiency, also known as Addison disease, is a rare disorder resulting from progressive destruction or dysfunction of the adrenal gland. Hereby we present a case of a 12-year old boy admitted to the Pediatric clinic due to vomiting and weakness. The symptoms were present for a month. Laboratory findings showed severe hyponatremia and hypochloremia, along with prerenal azotemia and liver lesion. Due to persistent weakness and abdominal pain accompanied by nausea and vomiting he visited the doctor twice more. After parenteral rehydration, he was discharged home. Last time he was admitted due to muscle weakness associated with previously listed symptoms. He was afebrile, dehydrated with a low blood pressure 90/45 mmHg and a high pulse rate of 117/min. The folds on his feet and palms were hyperpigmented.

Methods

Extensive clinical and laboratory evaluation was performed. Common biochemistry parameters were measured using Beckman Coulter AU 480 analyzer (Beckman Coulter, Brea, USA). Electrolytes were determined by indirect potentiometry, while urea, creatinine and liver enzymes were measured with spectrophotometric methods. Cortisol was measured using the Alinity i (Abbott Diagnostics, Lake Forest, USA) and ACTH using the Cobas e 601 (Roche Diagnostics, Mannheim, Germany) analyzer, both with chemiluminescent immunoassays.

Results

Common laboratory findings revealed decreased potassium (114 mmol/L) and chloride (80 mmol/L), hyperkalemia (5,6 mmol/L) and elevated urea, creatinine and liver enzymes. Endocrine laboratory tests showed hypocortisolism (79 nmol/L) and elevated adrenocorticotropic hormone (ACTH) level (430 pmol/L). These findings raised suspicion of Addison disease. After hydrocortisone therapy, his condition has stabilized.

Conclusions

This presented case of Addison disease in a child due to inappropriate cortisol production caused a series of symptoms accompanied by electrolyte disturbances and low blood pressure. Such a state can be life-threatening and requires urgent medical attention in order to pre-

vent an adrenal crisis, a dangerous event leading to symptoms of shock or convulsion that can be fatal. Cortisol measurement can possibly lead to more appropriate tests and accelerate diagnosis establishment in patients with such symptoms and severe hyponatremia.

doi: 10.1016/j.cca.2022.04.639

T161

Neonatal presentation of Liddle's syndrome

B. Chale-Matsau [a], G. Van Biljon [b], T.S. Pillay [a]

[a] *Department of Chemical Pathology, University of Pretoria/National Health Laboratory Service, South Africa*
[b] *Department of Paediatric Nephrology, University of Pretoria/Steve Biko Academic Hospital, South Africa*

Background-aim

Liddle syndrome is an autosomal dominant disorder arising from mutations of the SCNN1A, SCNN1B and SCNN1G genes, which encode for the alpha, beta and gamma epithelial sodium channel (ENAC) respectively. This leads to refractory hypertension, hypokalaemia, metabolic alkalosis, hyporeninaemia and hypoaldosteronism.

We report on a 6 year old male who at the age of 10 days was admitted to the neonatal ICU with severe dehydration and acute renal failure. The renal impairment resolved following treatment and he was discharged. He subsequently developed uncontrolled hypertension requiring readmission and was managed on multiple antihypertensive drugs and potassium supplements.

Methods

Electrolytes were measured (ion selective electrodes, Abbott Architect ci8200) using serum samples. Adosterone and renin were tested with immunometric methods. Genetic testing for the c1815G > A (pR563Q) mutation of the SCNN1B gene was performed.

Results

Electrolyte measurements revealed potassium values ranging between 2.0 to 3.0 (3.7-5.9 mmol/L), sodium levels 159 to 171 (136-145mmol/L). Metabolic alkalosis was also present [HCO3- = 29 to 34 (23-29 mmol/L)]. In addition, it was found that both aldosterone and renin were suppressed [aldosterone < 27 (49-643pmol/L) and renin 0.5 (6.5 -36.2 miUL)].

As the c1815G > A (pR563Q) mutation was not detected, further testing involved sequencing of the distal part of exon 13 of SCNN1B gene. No mutations were detected. Further investigations are underway to evaluate other subunits of the ENAC gene.

Conclusions

The clinical and biochemical features are in keeping with Liddle syndrome, a rare disease that can be easily missed or overlooked in paediatric patients. To our knowledge this is the youngest patient diagnosed with Liddle syndrome. It should be considered in patients presenting with hypokalaemia, hypertension in the presence of low renin and aldosterone levels.

doi: 10.1016/j.cca.2022.04.640

T162

Evaluation of salivary cortisol and cortisone analysis for the diagnosis of adrenal insufficiency in decompensated liver cirrhosis patients using liquid-chromatography tandem mass spectrometry

D. Kim [a], M.Y. Jeon [c], J.S. Lee [c], S. Lee [a], C.R. Ku [b], J.Y. Park [c], S. Lee [a], J. Kim [a], K. Han [c]

[a] *Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea*
[b] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea*
[c] *Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea*

Background-aim

Adrenal insufficiency (AI) is usually diagnosed with serum cortisol assays. As liver cirrhosis (LC) patients usually showed hypoalbuminemia, total cortisol levels to assess AI by serum total cortisol could be potentially misleading, because AI is known to be determined by free cortisol. We evaluated the salivary cortisol, and cortisone, comparable to free cortisol, with serum total cortisol, in low dose adrenocorticotropin (ACTH) stimulation test for Korean LC patients.

Methods

This study was conducted in the Hepatology Unit at Severance Hospital in 25 LC patients. Serum total cortisol, salivary cortisol and cortisone concentrations were measured before (T0: between 8 to 9 am) and 60 min (T60) after an intravenous injection of 1 mg ACTH stimulation test. Serum cortisol was measured using electrochemiluminescence methods. Saliva samples were collected with Salivette cotton tubes, and salivary cortisol and cortisone concentration were determined using Chromsystem kit and LC-MS/MS, QTRAP 5500.

Results

Salivary cortisol increased exponentially ($R2 = 0.757$, $p < 0.001$), the increase in salivary cortisone was linear ($R2 = 0.425$, $p < 0.0001$) according to serum cortisol. Salivary cortisone tended towards a plateau at higher cortisol levels ($R2 = 0.637$, $p < 0.05$). The adjusted R-squared (R2) between peak salivary cortisol and cortisone with the peak serum total cortisol levels were 0.645 and 0.274, respectively. Correlations between serum cortisol and salivary cortisol assays for T0, T60 and Delta (T60-T0) were strong in patients with albumin > 25 g/L ($R2 = 0.759$, 0.744 and 0.636, $p < 0.001$, < 0.001 and < 0.005) whereas, in patients with albumin δ25 g/L, the correlation was poor at T60 and ⊗($R2 = 0.358$ and 0.204, $p > 0.05$). Peak salivary cortisol also had a largest Area Under Curve (1.000 ± 0.000) just as the peak serum cortisol (0.990 ± 0.015) from the receiver operating characteristic curve analysis.

Conclusions

Our study shows that salivary cortisol assays are comparable to serum cortisol assays to detect AI in the decompensated LC patients, although we could not confirm its superiority.

doi: 10.1016/j.cca.2022.04.641

T163

A pilot study: The relationship between serum bisphenol a and sex steroid hormone levels in maternal and child pairs in a South African population

V. Gounden [a], Z. Warasally [b], T. Magwai [b], R. Naidoo [d], A. Chuturgoon [c]

[a] *Department of Chemical Pathology, University of Kwa Zulu Natal, National Health Laboratory Services, Inkosi Albert Luthuli Central Hospital, South Africa*
[b] *Department of Chemical Pathology, National Health Laboratory Services, Inkosi Albert Luthuli Central Hospital, South Africa*
[c] *Department of Medical Biochemistry, University of KwaZulu Natal, Durban, South Africa*
[d] *Department of Occupational and Environmental Health, University of KwaZulu Natal, South Africa*

Background-aim

Exposure to Bisphenol A (BPA) during early development particularly in- utero has been linked to a wide range of pathology. Studies have associated BPA exposure to the development of cancers, polycystic ovarian syndrome, pregnancy-related complications and also with cardiovascular disease, obesity and type 2 diabetes mellitus. BPA exerts its varied effects by several different mechanisms. As part of a larger study examining the effect of BPA on maternal and child pairs our aim was to examine the relationship between BPA and its common metabolite BPA-glucuronide (BPA-G) levels and sex steroid hormone levels

Methods

Blood samples and data collected as part of the Mother and Child in the environment (MACE) birth cohort study were utilized for this study. The MACE study population consists of pregnant females recruited from antenatal clinics in Durban, South Africa. Third-trimester serum maternal samples and matching cord blood samples were analyzed for BPA and BPA-g using LC-MS/MS. The same maternal and cord blood pairs were then further analyzed for steroid hormone levels. The following sex steroid hormones were using LC-MS/MS methodology: estradiol, testosterone, 11-deoxycorticosterone, DHEA, androstenedione, 17-OH progesterone, dihydrotestosterone, and progesterone. A commercially available kit the MassChrom Steroid Panel 2 kit (Chromosystems Instruments and Chemicals GmbH, Germany) was utilized for analysis of the sex steroid hormones. Chromatographic separations were carried out using the AB Sciex 4500 triple quadrupole mass spectrometer equipped with an Agilent 1260 ultra-high-performance liquid chromatography (uHPLC) system.

Results

Twenty-five maternal and cord blood pairs were analysed for sex steroids and BPA and BPA-g levels. Maternal BPA levels and BPA-G levels median (range) were as follows: 0.95 ng/mL (0.4- 15.3) and 4.71 ng/mL (0.48-21.8). Median cord BPA levels 0.92 ng/mL (0.4-13.2) and BPA-G levels 4.21 ng/mL (0.4-26). Median maternal and cord blood estradiol levels were respectively 118 nmol/l (range 4-772) and 67 nmol/L (range 3-371). Median maternal and cord blood testosterone median(range) levels were respectively 2.3 nmol/L (0.34-17.7) and 0.66 nmol/L (0.16-14.9). Spearmans correlation showed poor correlation for both maternal BPA and BPA-G levels with cord estradiol ($r = 0.3$; $r = 0.25$) and cord testosterone levels ($r = 0.4$; $r = 0.04$) , respectively.

Conclusions

It is recommended that larger studies be performed to examine the relationship between BPA and its effect on neonatal steroid hormones.

doi: 10.1016/j.cca.2022.04.642

T164

False TSH level in ulcerative colitis: Case report

K. Chaabouni [d], H. Jmal [a], S. Maalej [a], R. Makhlouf [d], M. Boudayara [c], A. Amouri [b], F. Ayedi [d]

[a] *Clinical Chemistry Departement/Habib Bourguiba University Hospital of Sfax, Tunisia*
[b] *Department of Gastroenterology, Hedi Chaker Hospital, Sfax, Tunisia*
[c] *National Social Security Fund, Sfax, Tunisia*
[d] *Research laboratory of Molecular Bases of Human Diseases/Medicine School of Sfax, Clinical Chemistry Departement/Habib Bourguiba University Hospital of Sfax, Tunisia*

Background-aim

The quality of assay methods for thyroid stimulating hormone (TSH) assessment has importantly improved last decades. However, immunological interference remains of matter vigilance.

We describe a case of ulcerative colitis with discordant thyroid evaluation.

Methods

Thyroid function tests were made on Cobas 6000® of Roche and controlled with Architect® of Abbott®.

Results

A subtotal colectomy has been scheduled in a 52-years-old woman with a pancolitic ulcerative colitis. With a past medical history of secondary hypothyroidism post thyroidectomy and complete arrhythmia by atrial fibrillation treated with L-thyroxin, cordarone, and anti-vitamin k. Thyroid function tests requested en preoperative, were discordant. An immunological interference on TSH assay were shown highlighted by serial dilutions and polyethylene glycol test. This interference was resolved on postoperative.

Conclusions

Immunological interference on TSH assay with immuboglobulin having a short-half live, may explain preoperative levels. In fact IgA and IgG 3 have a short half-live and they are implicated in ulcerative colitis pathophysiology. Thus, we should bear in mind the possibility of immunological interference with thyroid function tests mainly when clinical findings and laboratory results show discrepancies or in case of discrepant results.

doi: 10.1016/j.cca.2022.04.643

T165

Diagnostic value of HbA1C and glycated albumin tests in obese and nonobese prediabetic subjects

A. Rovshan, L. Didem Kozaci

Department of Medical Biochemistry, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey

Background-aim

Diabetes mellitus (DM) is a chronic disease with high morbidity and mortality risks. Prediabetes indicates high blood glucose levels that aren't high enough to be diagnosed as diabetes. HbA1C measurement is one of the biochemical laboratory parameters used to diagnose prediabetes. Superiority of other glycosylated proteins (glycated albumin (GA), fructosamine) over HbA1C have been discussed in prediabetes diagnosis. Obesity, a risk factor for DM, influences the diagnostic and follow-up tests for prediabetes. In this study, we aimed to assess the value of GA and HbA1c values in obese and non-obese subjects in prediabetes diagnosis.

Methods

Individuals (n = 126; 72 females, 54 males) admitted to the Endocrinology Clinic were included in study. Prediabetes, diabetes and insulin resistance groups were sub-divided as obese and non-obese, according to body mass index (BMI). GA levels were determined spectrophotometrically (DIAZYME GSP). HbA1C measurements were performed with (1) ion exchange chromatographic HPLC (Agilent 1100 Series, NGSP-certified), (2) Abbott c8000 and (3) Roche Cobas 501 instruments.

Results

GA values were significantly higher in patients with diabetes than those with insulin resistance and those with prediabetes ($p < 0.05$). GA values were similar in between insulin resistance and prediabetes groups ($p > 0.05$).The class correlation coefficient (CCT) value was calculated to evaluate the correlation between ROCHE Cobas, ABOTT and HPLC methods. We found that the CCT value as 0.842 (95% CI:0.599; 0.923). This value indicated good agreement between our study methods ($p < 0.001$).There was a positive correlation between BMI and HbA1c (0.065; $p = 0.468$) and a negative correlation between BMI and glycosylated albumin (-0.129; $p = 0.149$). There was a statistically significant difference in the gender distribution of nonobese and obese individuals ($p < 0.001$).

Conclusions

In conclusion, GA levels have higher sensitivity and lower selectivity than HbA1c in detection of type 2 DM in nonobese subjects. GA has higher selectivity and sensitivity than HbA1c, independent of BMI in detection of prediabetic individuals. The combination of HbA1c and glycosylated albumin may improve diagnostic sensitivity and enable early detection of diabetes and preventive measures. However, increased sensitivity carries risk of increasing false positives.

doi: 10.1016/j.cca.2022.04.644

T166

Evaluation of plasma free metanephrine and normetanephrine laboratory request forms

K. Chaabouni[a], R. Makhlouf[a], M. Yaich[a], M. Messedi[b], A. Elleuch[a], F. Ayedi[a]

[a] *Research laboratory of Molecular Bases of Human Diseases/Medicine School of Sfax, Clinical Chemistry Departement/Habib Bourguiba University Hospital of Sfax, Tunisia*

[b] *Research Unit of Molecular Bases of Human Diseases/Medicine School of Sfax, Tunisia*

Background-aim

Metanephrines and normetanephrines (MN/NMN) are the respective O-methylated metabolites of epinephrine and norepinephrine. Measurements of plasma or urine MN/NMN provide a useful diagnostic test for phaeochromocytoma/ paraganglioma. Plasma free MN/NMN is a promising new biochemical test and is increasingly prescribed.

Aim : To evaluate plasma free MN/NMN requests transmitted to the clinical chemistry department by the clinicians .

Methods

The cross-sectional descriptive study was conducted during a six month period from June to December 2019 at the clinical chemistry department. Laboratory request forms of MN/NMN tests were collected. Each request was accompanied by a clinical information sheet including: age, sex, weight, size and reason. Patient preparation was carried out according to the recommendations of the endocrine society. The results were analyzed by SPSS 20.0 software.

Results

A total of 114 MN/NMN requests were collected. The average age was of 51.5 +/- 14.5 years [15; 74], with a sex ratio of 0.56 and an average BMI of 32kg / m2. High blood pressure was reported in 68% of the requests. It was particularly associated with adrenal incidentalomas. The common clinical justification for MN/NMN requests in descending order of frequency were: an adrenal incidentalomas, a Menard's triad, a syndromic pheochromocytoma's screening, an unresponsiveness of hypertension to normal treatment, a hypertensive young adults, and a labile hypertension.

Conclusions

Adult obese women seem to be the most concerned with the sreening for pheochromocytoma / paraganglioma even in the absence of high blood pressure. Secreting pheochromocytomas / paragangliomas can affect all age groups. The peak occurrence of this tumor is in the fifth and sixth decade. Both sexes are concerned with a female predominance according to certain studies. These tumors may not directly secrete catecholamines. Their diagnosis is based on measurements of plasma MN/NMN which are more sensitive and more specific.

doi: 10.1016/j.cca.2022.04.645

T167

Measurement of plasma allopregnanolone levels and the impact of solid phase extraction step on competitive immunoassay performance

C. Armitage, E. Braybrook, N. Anderson, D. Grammatopoulos

Institute of Precision Diagnostics and Translational Medicine, University Hospital Coventry and Warwickshire, Coventry & Warwickshire Pathology Service and Division of Translational Medicine, Warwick Medical School, United Kingdom

Background-aim

Allopregnanolone is a neuroactive steroid synthesized from progesterone. It acts to fine tune transmissions in the central nervous system via inhibitory action at the ©-aminobutyric acid A receptor and has been linked with neuronal plasticity, memory processes, and the modulation of hormonal responses to stress; it is increasingly recognised as a potential biomarker in the investigation of stress disorders. Allopregnanolone is usually measured by LC-MS and RIA methods, although more recently, ELISA kits have been developed and are commercially available to allow for rapid, sensitive and specific measurement that overcomes issues around need for specialist equipment or facilities. A major difference between analytical methodologies is the requirement for solid phase extraction step in some analytical protocols. However, the impact of this step on results or in fact assay performance has not been fully investigated and experience from other ELISA assays suggests that marked differences exist between manufactures in sample preparation, antibodies used and the performance characteristics. In this study we measured allopregnanolone levels in plasma isolated from pregnant women using two commercially available kits, utilising a competitive ELISA format and offered a suitable detection range, although one of the protocols required an additional extraction step.

Methods

EDTA samples from 24 pregnant women were collected around 12-14 weeks of gestation and were centrifuged at 4 °C for 30 min and split. Prior to analysis, one aliquot underwent a solid phase extraction step using ethyl acetate, as recommended by the manufacturer. Sample analysis was carried out in duplicate on both kits according to the manufacturer's specific instructions. In addition to comparison of sample results, the effect of freeze thaw on analyte concentrations was investigated, reproducibility across plates and the extraction technique was optimised through variance of volumes and repeated extraction cycles.

Results

A 1.3 x concentration was identified as optimal for the extraction process, producing results within the measuring range whilst using the smallest volume of clinical sample. However, significant variation in calculated allopregnanolone concentration from the same sample was seen as a result of the extraction process and there was no evidence that a second extraction step improved sensitivity. Results across plates for the same samples exhibited poor reproducibility. Results for the direct immuneassay fell within the middle of the standard curve, coefficient of variation was <15% for all samples and there was no drift evident between plates.

Conclusions

In conclusion, an immunoassay format that does not require solid phase extraction appears to provide more reliable results. Assays with enhance sensitivity and limit of detection can overcome the need for sample extraction, which can introduce variability to the data, even when volumes areoptimised. This pilot study can be used to support large cohort data investigating the role of allopregnanolone in stress related disorders.

doi: 10.1016/j.cca.2022.04.646

T168

Is serum PSA a predictor of male hypogonadism? Testing the hypothesis

I.K. Green[b], C. Amadi[a]

[a] *Department of Chemical Pathology, Rivers State University, Port Harcourt, Nigeria*
[b] *Department of Obstetrics and Gynecology, University of Port Harcourt, Port Harcourt, Nigeria*

Background-aim

Male hypogonadism (MH) is very common among infertile couples with limited biomarkers for its diagnosis. Recently, investigators had suggested the utility of total prostate-specific antigen (tPSA) to aid the diagnosis of MH mostly among middle-aged and elderly Caucasians. Hence, the present study aimed to evaluate the diagnostic potentials of both tPSA and free PSAto predict MH among relatively young Nigerian males.

Methods

This was prospective, cross-sectional and hospital-based study, conducted among 707 relatively young (35–45 years) male partners of infertile couples with tPSA level < 1.5μg/l presenting for infertility evaluation in a third-level care center in Nigeria. MH diagnosis was made using strict clinical (MH-related features and the Androgen Deficiency in the Aging Male test questionnaire) and laboratory (seminogran parameters, serum free and total testosterone levels) standard guidelines. Receiver operating characteristics (ROC) analysis was used to explore the MH diagnostic potentials of tPSA and fPSA to predict MH and MH-related clinical features.

Results

Male hypogonadism (MH + ve) presented in 29.7% (n = 210) of the study cohorts. Best fPSA cutoff value <0.25μg/l yielded higher accuracy (ROC area under the curve: 0.908 vs 0.866), sensitivity (87% vs 83%), specificity (42% vs 37%) for MH diagnosis compared to best tPSA cutoff value < 0.74μg/l, though both rendered poor specificities. Best fPSA cutoff value < 0.25 μg/l rendered higher sensitivity (72.7% vs 61.3%), specificity (91.2% vs 84.3%), PPV (80% vs 63.3%), and NPV (87.2% vs 83.1%) outperforming tPSA cutoff value <0.74 μg/l when correlated to the MH-diagnostic threshold applied in the present study. Adjusted for age, fPSA δ0.25μ g/l had greater likelihood of predicting MH-related reduced libido (OR: 2.728; p < 0.001) and erectile dysfunction (OR: 3.9825; p < 0.001) compared to tPSA δ0.74μg/l threshold among MH + ve cohorts.

Conclusions

Both fPSA and tPSA rendered good sensitivities but very poor specificities for MH diagnosis, making both molecules poor diagnostic parameters for MH among young males. However, fPSA had better MH diagnostic potential and association with MH-related clinical features

than tPSA when correlated with the strict diagnostic threshold applied in the present study. Hence, fPSA could compliment other biomarkers for MH diagnosis among young males.

doi: 10.1016/j.cca.2022.04.647

T169

Serum 21-deoxycortisol in adrenal adenoma patients

D. Mandic, S. Mandic, Z. Debeljak, I. Markovic, T. Pavosevic, V. Seric

Faculty of Medicine, University of Osijek, Osijek, Croatia
University Hospital Osijek, Osijek, Croatia

Background-aim

Aim of this study was to investigate occurrence and concentrations of 21-deoxycortisol in patients with non-secreting adrenal adenomas. 21-deoxycortisol is produced by oxidation of 17-hydroxyprogesterone with 11-ß hydroxylase in individuals with reduced 21-hydroxylase activity (usually in congenital adrenal hyperplasia due to 21-hydroxylase deficiency).

Methods

During routine steroid hormone analysis of 87 patients with non-secreting adrenal adenomas (confirmed with imaging techniques), full steroid profile of blood serum samples was done in order to establish concentration of 21-deoxycortisol and other steroid hormones and metabolites.

Steroid profiling was done using liquid chromatography tandem mass spectrometer (LC-MS/MS) Shimadzu LCMS-8050 (Shimadzu, Kyoto, Japan) with ClinMass® LC-MS/MS Steroids in Serum/Plasma (RECIPE GmbH, Munchen, Germany) commercial kit for 8 steroid hormones and metabolites (androstenedione, 21-deoxycortisol, cortisol, 17-hydroxyprogesterone, 11-deoxycorticosterone, 11-deoxycortisol, dehydroepiandrosterone-sulfate and testosterone).

Results

Steroid hormones and metabolites analyzed were inside reference range in all 87 patients, except for 21-deoxycortisol, which was detected in 16 patients. 21-deoxycortisol concentration in this patient group ranged from 0.203 to 40.697 nmol/l, with median concentration of 0.343 nmol/l.

Conclusions

21-deoxycortisol is not detectable in serum samples of individuals with normal 21-hydroxylase activity, but there are several reports of diminished 21-hydroxylase activity in adrenal adenoma patients that could lead to production of measurable levels of 21-deoxycortisol. Limit of quantitation for 21-deoxycortisol in our method is 0.173 nmol/l. Modifying the LC-MS/MS method for even lower limit of quantitation of 21-deoxycortisol could enable its detection and quantitation in greater proportion of adrenal adenoma patients, which could be used for diagnostic/monitoring purposes.

doi: 10.1016/j.cca.2022.04.648

T170

Three cases of endocrine immune-related adverse events caused by immune checkpoint inhibitor therapy

S. Braga, J. Barreto, A. Torgal, J. Pereira, A. Leão, N. Gonçalves, L. Araújo

Portuguese Institute of Oncology of Porto, Portugal

Background-aim

Immune Checkpoint Inhibitors (ICIs) induce several immune-related Adverse Events (irAEs). Among these, endocrinopathies are frequent and can affect the pituitary, thyroid, pancreas and adrenal glands, as well as other target organs. We report 3 cases of Endocrine irAEs in patients treated with ICIs.

Methods

1. Male, age 55, with metastatic melanoma treated with Nivolumab + Ipilimumab. After cycle 9 presented with vertigo and vomiting. Tests revealed ACTH of 376,7 pg/mL, morning Cortisol of 50,79 μg/dL and Prolactin of 35,20 ng/mL.

2. Female, age 44, with metastatic melanoma treated with Nivolumab. After cycle 2 presented with oedema of the face, neck and feet, along with paraesthesia of the hands. Tests revealed TSH of 310 μUI/mL, with FT4 and FT3 under the detection limit and positive thyroid antibodies – Anti-TG 471 UI/mL and Anti-TPO 172 UI/mL.

3. Female, age 72, with lung adenocarcinoma pT2N2M1a treated with Pembrolizumab. After cycle 3 presented with vomiting, myoclonus of superior limbs and decreased strength. Tests revealed hyperglycaemia (>658 mg/dL), ketoacidosis (pH < 7,0; HCO3- 5,2 mmol/L), autoimmune thyroiditis with hypothyroidism (TSH 11,2 μUI/mL; FT4 0,569 ng/dL; TT4 2,97 μg/dL; FT3 0,666 pg/mL; TT3 32,77 ng/dL; Anti-TPO 319 UI/mL).

All endocrine assays were performed on cobas e801 immunoassay analyser by electrochemiluminescence.

Results

These cases portray different irAEs with different degrees of severity.

Patient 1 had increased ACTH and Morning Cortisol suggesting a transient Cushing Syndrome. He was treated with methylprednisolone and immunotherapy was suspended until values became normal.

Patient 2 developed acute thyroiditis with hypothyroidism and suppression of thyroid hormone synthesis. As TSH values began to lower with levothyroxine supplementation, it was decided she would continue Nivolumab under continuous supplementation.

Patient 3 had life threatening multi-organic compromise, including diabetic ketoacidosis and thyroiditis, with neurological repercussions, and was admitted to the Intensive Care Unit.

Conclusions

Besides reporting side-effects, we hope these cases bring to light the importance of closely monitoring patients under treatment with ICIs, including regular evaluation of blood glucose, thyroid function and hypophysis-adrenal axis function.

doi: 10.1016/j.cca.2022.04.649

T171

Measurement of plasma allopregnanolone levels and the impact of solid phase extraction step on competitive immunoassay performance

C. Armitage [a], E. Braybrook [a], N. Anderson [a], D. Grammatopoulos [b]

[a] *Institute of Precision Diagnostics and Translational Medicine, University Hospital Coventry and Warwickshire, Coventry & Warwickshire Pathology Service, United Kingdom*
[b] *Institute of Precision Diagnostics and Translational Medicine, University Hospital Coventry and Warwickshire, Coventry & Warwickshire Pathology Service and Division of Translational Medicine, Warwick Medical School, United Kingdom*

Background-aim

Allopregnanolone is a neuroactive steroid synthesized from progesterone. It acts to fine tune transmissions in the central nervous system via inhibitory action at the ©-aminobutyric acid A receptor and has been linked with neuronal plasticity, memory processes, and the modulation of hormonal responses to stress; it is increasingly recognised as a potential biomarker in the investigation of stress disorders. Allopregnanolone is usually measured by LC-MS and RIA methods, although more recently, ELISA kits have been developed and are commercially available to allow for rapid, sensitive and specific measurement that overcomes issues around need for specialist equipment or facilities. A major difference between analytical methodologies is the requirement for solid phase extraction step in some analytical protocols. However, the impact of this step on results or in fact assay performance has not been fully investigated and experience from other ELISA assays suggests that marked differences exist between manufactures in sample preparation, antibodies used and the performance characteristics. In this study we measured allopregnanolone levels in plasma isolated from pregnant women using two commercially available kits, utilising a competitive ELISA format and offered a suitable detection range, although one of the protocols required an additional extraction step.

Methods

EDTA samples from 24 pregnant women were collected around 12-14 weeks of gestation and were centrifuged at 4 °C for 30min and split. Prior to analysis, one aliquot underwent a solid phase extraction step using ethyl acetate, as recommended by the manufacturer. Sample analysis was carried out in duplicate on both kits according to the manufacturer's specific instructions. In addition to comparison of sample results, the effect of freeze thaw on analyte concentrations was investigated, reproducibility across plates and the extraction technique was optimised through variance of volumes and repeated extraction cycles.

Results

A 1.3 x concentration was identified as optimal for the extraction process, producing results within the measuring range whilst using the smallest volume of clinical sample. However, significant variation in calculated allopregnanolone concentration from the same sample was seen as a result of the extraction process and there was no evidence that a second extraction step improved sensitivity. Results across plates for the same samples exhibited poor reproducibility. Results for the direct immuneassay fell within the middle of the standard curve, coefficient of variation was $<15\%$ for all samples and there was no drift evident between plates.

Conclusions

In conclusion, an immunoassay format that does not require solid phase extraction appears to provide more reliable results. Assays with enhance sensitivity and limit of detection can overcome the need for sample extraction, which can introduce variability to the data, even when volumes areoptimised. This pilot study can be used to support large cohort data investigating the role of allopregnanolone in stress related disorders.

doi: 10.1016/j.cca.2022.04.650

T172

Profile of CA-125 in ovarian pathology: A retrospective study at BP Koirala Memorial Cancer Hospital

S. Bastakoti

BP Koirala Memorial Cancer Hospital, Nepal

Background-aim

CA-125 is used as a tumor marker because CA-125 concentrations may be elevated in the blood of some patients with ovarian cancer, but also in some benign conditions. The aim of the study is the know the status of CA 125 in female patient visiting cancer hospital for ovarian pathology.

Methods

Fully Automated Roche ecobas 411 analyzer is being used to perform the test

Results

The concentration of CA 125 was defined in the blood serum of 150 patients with ovarian tumors: benign–40, borderline–5 and malignant–105. In 87% of benign tumors CA 125 concentrations did not exceed the normal level. In three from five patients with borderline tumors CA 125 concentration varied from 50 to 150 U/ml. High levels of CA 125 (from 150 to 10.000 U/ml and higher) were revealed in 87 from 105 patients with malignant ovarian tumors.

Conclusions

CA-125 is a useful tool for supporting ovarian malignancy however one should be aware that sometimes it may raised in benign pathology alike endometriosis.

doi: 10.1016/j.cca.2022.04.651

T173

Clinical presentation of mild hyperthyroidism and hyperthyroidism

N. Serdarevic [b], A. Redžepovac [a]

[a] *Faculty of Health Sciences Sarajevo, University of Sarajevo, Bosnia and Herzegovina*
[b] *Institute for Clinical Biochemistry and Imunology, Clinical Center University of Sarajevo, Faculty of Health Sciences, Bosnia and Herzegovina*

Background-aim

The aim of our study was to determine the serum concentration of TSH, FT3, and FT4 in patients with latent and expressed hyperthyroidism and compare the results to determine the differences between the mild hyperthyroidism and hyperthyroidism.

Methods

The study included 900 patients age (30-40) years, divided into three groups that are into a control group with healthy patients, groups with respondents who have hyperthyroidism and a group of patients with mild hyperthyroidism. The concentration of the hormone TSH, FT3 and FT4 were analyzed using IMMULITE 1 Siemens. The determination of hormones was based on the immunochemical method.

Results

The patients with hyperthyroidism had an average concentration of TSH 0.016 mIU/L, FT3 11.960 pg/mL, FT4 51.323 pmol/L. The group with mild hyperthyroidism had an average concentration of TSH 0.088 mIU/L, FT3 3.773 pg/mL, FT4 17.2767 pmol/L. Man Whitney's U test showed that the value of parameter TSH between our group investigated the existence of significant statistical differences as between the control group and the group with hyperthyroidism ($Z = 6.745$; $p < 0.001$) and between the control group and the group with mild hyperthyroidism ($Z = 6.655$; $p < 0.001$). For FT4, we completed a statistically significant difference between the control group and the group with hyperthyroidism ($Z = 6.625$; $p < 0.005$) and between groups with hyperthyroidism and mild hyperthyroidism ($Z = 1.747$; $p < 0.002$). In our study the significant difference between concentrations FT3 between the control group and the group with hyperthyroidism ($Z = 6.65$; $p < 0.001$) and between groups with hyperthyroidism and mild hyperthyroidism ($Z = 2.832$; $p < 0.005$). Sensitivity TSH hyperthyroidism group and the control group was 98.5 % with the specificity of 90.5 %, while the sensitivity was 98.3 FT3 with a specificity of 86.7 %, sensitivity was 99.3 % FT4 with a specificity of 92.3 %. AUC for TSH was 0.935, while for FT3 it totaled 0.847 and for FT4 was 0.856.

Conclusions

In patients with latent and expressed hyperthyroidism serum hormone TSH decreased while the concentration of FT3 and FT4 elevated in comparison to the control group of patients of the same age. The concentration of TSH is reduced in both hyperthyroidism and mild hyperthyroidism. Serum concentrations of FT3 and FT4 were elevated in hyperthyroidism, while in mild hyperthyroidism serum concentration of FT3 and FT4 were in the reference area.

doi: 10.1016/j.cca.2022.04.652

T174

Prevalence of haemoglobin variants and thyroid disorders in African blacks with type 2 diabetes mellitus

F.A. Mapayi [a], M. Ogunlakin [a], M.A. Charles-Davies [a], T.R. Kotila [c], J.O. Adeleye [d], F.R. Afolabi [b], E.O. Agbedana [a]

[a] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria*
[b] *Department of Epidemiology and Medical Statistics, College of Medicine, University of Ibadan, Nigeria*
[c] *Department of Haematology, College of Medicine, University of Ibadan, Nigeria*
[d] *Department of Medicine, College of Medicine, University of Ibadan, Nigeria*

Background-aim

Haemoglobinopathies are among the most prevalent genetic disorders in individuals with Type 2 diabetes mellitus (T2DM). Altered thyroid function has also been reported in persons with T2DM. The prevalence of thyroid dysfunction and haemoglobin variants in individuals with T2DM particularly in African blacks is uncertain.

The aim of this study is to determine the prevalence of thyroid dysfunction and haemoglobinopathies in African Blacks with T2DM in Ibadan, Nigeria.

Methods

This case control study consists of 204 individuals aged 25 – 80 years with T2DM (n = 100) and without T2DM (Control, n = 104), enrolled from a tertiary hospital, in Ibadan, Nigeria and environs. Blood (7mL) was obtained from each participant. Haemoglobin Variants (HbA2, HbA, HbC and HbS) and Glycated haemoglobin (HbA1c) were determined by HPLC (Bio-Rad Variant II) while serum thyroid stimulating hormone, free thyroxine, free triiodothyronine were estimated using ELISA. Fasting plasma glucose was analysed enzymatically. Data were analysed using descriptive statistics.

Results

34 (34%) and 17 (16.3%) in the T2DM and Control groups had haemoglobinopathies either singly or in combination with HbA2, respectively. 54 (54%), 20 (20%) and 26 (26%) of the T2DM group had euthyroidism, hypothyroidism and hyperthyroidism, respectively. Among the Control group, 50 (48.1%), 20 (19.2%) and 34 (32.7%) were euthyroid, hypothyroid and hyperthyroid, respectively.

Conclusions

The prevalence of haemoglobin variants appears high in African blacks with T2DM. This may be important in the use of HbA1c for diagnosis and monitoring of T2DM. Screening of the general population for altered thyroid function may be necessary.

doi: 10.1016/j.cca.2022.04.653

T175

MPV as an indicator of vascular complication in poor control diabetic population

S. Pokhrel [a], R. Pokhrel [b], N. Pokhrel [b], N.B. Khadka [b], B.D. Pardhe [a]

[a] *Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, Kathmandu, Nepal*
[b] *Department of Laboratory, Modern Diagnostic Laboratory and Research Center, Kathmandu, Nepal*

Background-aim

Increased platelet activity indicates the development of diabetic complications. This study aims to find out the association of mean platelet volume with diabetic dyslipidaemia and glycaemic control of diabetes. This study also aims to find the relationship between atherogenic variables and mean platelet volume among diabetes populations.

Methods

This is a cross-sectional study of 861 participants among which 592 were diabetes population attending in Modern Diagnostic Laboratory and Research Center Kathmandu, Nepal. Glycaemic control and dyslipidaemia were defined by the level of HbA1c and lipid profiles respectively. HbA1c and mean platelet volume were measured by Lifotronic-H9 hemoglobin analyser and Sysmex XN350 respectively while lipid profiles and Fasting plasma glucose was measured by Dimension RxL chemistry analyser. Shapiro-wilk test was used for the test of normality distribution. Independent kruskal wallis test and Mann-Whitney U test were used for the comparison between different groups. Risk factors associated with increased mean platelet volume were verified by binary logistic regression analysis while Correlation between Atherogenic variables and mean platelet volume was established by spearman correlation.

Results

The median (P25-P75) platelet volume of diabetic population was 12.2 (11-13.5) fl while non-diabetic population was 10.3 (9.6 – 11.05) fl. Higher mean platelet volume was significantly associated with poor diabetic control, Abnormal glucose metabolism, higher triglyceride and higher low-density lipoprotein cholesterol. Multivariable logistic regression analysis by adjusting the variables showed poor diabetic control (OR = 1.557), high triglyceride (OR = 2.33) and high LDL-C (OR = 1.53) were independent risk factor of increased mean platelet volume. Cardiac Risk ratio (r = 0.155), Atherogenic coefficient (r = 0.155) and Atherogenic Index Plasma (r = 0.250) shows significant correlation ($p < 0.001$) with mean platelet volume.

Conclusions

Our study showed that increased mean platelet volume is strongly and independently associated with poor control of diabetes and dyslipidaemia while atherogenic variables show strong positive correlation with mean platelet volume. Poor control of diabetes and dyslipidaemia prone to micro vascular and macro vascular complications.

doi: 10.1016/j.cca.2022.04.654

T176

Comparison of urinary amino acid pattern in patients of type 2 diabetes mellitus and healthy controls

S. Sankanagoudar [a], S. Bidi [b], P. Sharma [a]

[a] *Biochemistry, All India Institute of Medical Sciences, Jodhpur, India*
[b] *Biochemistry, Jawaharlal Nehru Medical College, Belagavi, India*

Background-aim

Glycosuria is commonly seen in uncontrolled diabetes and hyperglycemia has been proposed to be associated with changes in urinary amino acid pattern. Specific amino acids have been linked to regulation of insulin secretion from pancreatic ®-cells. The objective of the present study was to quantify and to compare the pattern of amino aciduria in diabetics and healthy controls and also to determine the association of amino aciduria with blood glucose levels.

Methods

Quantification of total urinary amino acids (UAA) was done spectrophotometrically and the patterns of amino acid excretion was elucidated by thin layer chromatography technique. Fasting blood samples were used for plasma glucose estimation by fully automated analyzer.

Results

The concentrations of UAA in subjects of T2DM in comparison to healthy controls were higher ($p < 0.0001$). The urinary levels of phenylalanine, arginine, tryptophan, tyrosine and cysteine were significantly higher in persons with T2DM than controls. There was also a strong positive correlation of UAA levels with blood glucose levels.

Conclusions

From this study an association between urinary amino acids in diabetes has been established and the observed specific pattern of aminoaciduria could serve as an inexpensive and non-invasive marker for type 2 diabetes mellitus.

doi: 10.1016/j.cca.2022.04.655

T177

Reference intervals of thyroid stimulating hormone for children under 2 years of age

S. Muneer

Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, Pakistan

Background-aim

Congenital Hypothyroidism is prevalent worldwide.

Reference Intervals (RI) of Thyroid Stimulating Hormone (TSH) are population, age and assay specific.

No formal RI for TSH is available in our population.

Methods

This cross-sectional study was conducted at Section of Chemical Pathology, Aga Khan University Hospital Karachi. Children under 2 years of age coming for vitamin D testing were included in this study after informed consent from their parents/guardians. Children with recent abnormal total leucocyte count in the medical record, history of any diagnosed disease or infection, congenital hypothyroidism or transient congenital hypothyroidism, history of hospitalization during the previous 4 weeks were excluded. Serum Free T4 (FT4) along with TSH was measured and children with low FT4 results were also excluded.

A total of 120 subjects were included, based on recommended target sample size by CLSI C28-A3 guidelines. Serum TSH and FT4 were measured on ADVIA Centaur (Siemens Diagnostics, US), performed using Chemiluminescence immunoassay following standard operating procedures.

Data was analyzed using EP evaluator version 10. Normality of the data was assessed and mean and two standard deviations were used to derive the RI.

Results

The mean age of the children was 13 ± 5 months with 59.8% boys.

The established reference intervals of TSH and FT4 for children under 2 years in our population were found to be 0.74-4.5 uIU/mL and 0.79-1.34 ng/dL respectively.

Conclusions

The thyroid hormones concentrations differ widely in different age groups, making it challenging to interpret measurements in children, especially infants. To address this issue we have established age-specific reference ranges for TSH and FT4 in children.

doi: 10.1016/j.cca.2022.04.656

T178

Adiponectin-Resistin Index in individuals with autoimmune thyroid diseases

R. Nwaelugo [a], F. Abbiyesuku [b], M. Charles-Davies [d], F. Bello Sani [c]

[a] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[b] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[c] *College of Medicine, Ahmadu Bello University, Zaria, Nigeria*
[d] *Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*

Background-aim

Background
Homeostatic model assessment of insulin resistance (HOMA-IR) measures insulin resistance (IR), implicated in individuals with autoimmune thyroid diseases. Adiponectin Resistin Index (AR) probably correlates with glycemic indices including HOMA-IR and may be a reliable diagnostic biomarker for insulin sensitivity. The association of AR with HOMA-IR in Graves' disease (GD) and Hashimoto's thyroiditis (HT) is uncertain.
Aim
The aim of this study is to find the association of AR index with glycemic indices in individuals with GD and HT.

Methods

230 adults { 100 and 30 with GD and HT, respectively, age matched with apparently healthy individuals without metabolic syndrome (Control)} were enrolled consecutively into this case-control study from the endocrine clinics of two tertiary hospitals in North Western Nigeria. Fasting blood (10mL) was obtained from each participant. Plasma glucose (FPG) was analysed enzymatically while serum insulin, adiponectin and resistin were determined by ELISA. HOMA-IR and AR were calculated. Data obtained were statistically significant at $p < 0.05$.

Results

AR index was significantly higher in GD compared with HT and controls while insulin, HOMA-IR were significantly lower in GD compared with controls ($p < 0.05$). Adiponectin was significantly higher while resistin and AR were significantly lower in HT than GD (($p < 0.05$). AR correlated with resistin in GD, HT and controls ($r = 0.827, 0.841, 0.799$ ($p < 0.001$). Additionally, FPG correlated with resistin and AR in HT ($r = 0.543$, $p = 002$, $r = 0.390$, $p = 0.033$)

Conclusions

Adiponectin Resistin Index does not appear as a reliable biomarker of insulin resistance in individuals with Graves' and Hashimoto's thyroiditis diseases.

doi: 10.1016/j.cca.2022.04.657

T179

Establishment of early pregnancy related thyroid hormones models and reference intervals for pregnant women in china based on real world data

C. Ma [b], X. Li [a], L. Liu [d], X. Cheng [a], L. Qiu [a], L. Juntao [c]

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*
[b] *Department of Laboratory Medicine, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*
[c] *Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, China*
[d] *Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China*

Background-aim

Thyroid hormone RIs are crucial for diagnosing and monitoring thyroid dysfunction during early pregnancy, and the dynamic change trend of thyroid hormones during pregnancy can assist clinicians to assess the thyroid function of pregnant women. This study aims to establish early pregnancy related thyroid hormones models and reference intervals (RIs) for pregnant women.

Methods

We established two derived databases: derived database* and derived database#. Reference individuals in database* were used to establish gestational age-specific RIs for thyroid hormones and early pregnancy related thyroid hormones models for pregnant women. Individuals in database# were apparently healthy non-pregnant women. The thyroid hormones levels of individuals in database# were compared with that of individuals in database* using nonparametric methods and the comparative confidence interval (CI) method.

Results

The differences in TSH and FT4 between pregnant and non-pregnant women were statistically significant ($P < 0.0001$). Results concerning TSH and FT4 RIs of early pregnancy are comparable with those from other studies using the same detection platform. Early pregnancy related thyroid hormones models showed various change patterns with gestational age for thyroid hormones.

Conclusions

Early pregnancy related thyroid hormones models and RIs for pregnant women were established. So as to provide accurate and reliable reference basis for the diagnosing and monitoring of maternal thyroid disfunction in early pregnancy.

doi: 10.1016/j.cca.2022.04.658

T180

Establishing healthy distribution for thyrotropin receptor antibodies, thyroid stimulating immunoglobulin and thyroid stimulating blocking antibody for individuals in Beijing, China

C. Ma [a], X. Cheng [a], Y. Hu [a], A. Song [a], X. Lian [b], L. Qiu [a]

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*
[b] *Department of Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*

Background-aim

Autoimmune thyroid disease involves thyrotropin receptor antibodies (TRAb), thyroid stimulating immunoglobulin (TSI) and thyroid stimulating blocking antibody (TSBAb), which are of great value in the diagnosis of disease. However, the distribution of the three antibodies in the health individuals has not been established. This study will establish a healthy distribution of the three antibodies in the Chinese population with suitable methods to provide theory basis for diagnosis of autoimmune thyroid disease

Methods

In total, 120 apparently healthy individuals were included in this study by questionnaire. Thyrotropin binding inhibitor immunoglobulin (TBII) assay was used for the measurements of TR-Ab, IMMULITE 2000 TSI assay for TSI, and enzyme linked immune sorbent assay (ELISA) for TSB-Ab.

Results

The baseline level of common biochemical analytes of individuals enrolled in the study were in the normal range. The distribution of TR-Ab in males was significantly higher than that in females ($P < 0.05$), while there was no statistical difference for TSB-Ab measurements between males and females. The healthy distribution of TRAb, TSI and TSBAb were established.

Conclusions

TRAb and TSBAb are dispersed in healthy individuals, while TSI cannot be quantified in healthy individuals due to methodological reasons. We suggest that TSBAb and TRAb health distribution be taken into account when diagnosing autoimmune thyroid disease.

doi: 10.1016/j.cca.2022.04.659

T181

Establishment of influence factors and ageing models for thyroid hormones in the elderly using real-world big data

C. Ma, X. Cheng, Y. Hu, L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China

Background-aim

An increasing amount of attention has been paid to the elderly in the medical and health care with the acceleration of aging society. However, there are few studies on the biological law and reference interval (RIs) of thyroid hormones in the elderly. Therefore, study aims to establish influence factors model and RIs of thyroid hormones for the elderly, and analyze the factors affecting thyroid in the elderly, explore the pattern of thyroid hormone changes with age by using polynomial composite model.

Methods

Two derivation sets was established. The first derivation set including 53191 individuals was to establish the age-related models for thyroid hormones. Furthermore, the second derivation set of the elderly was to build up the influence factors model and establish RIs of thyroid hormones for the elderly.

Results

The width of 90% confidence interval(CI) of RIs for thyroid hormones of the elderly were less than 0.2 multiply of width of RIs. Influence factors model indicated that in the elderly, FT3 significantly affected by sex (standardized regression coefficient: 0.205, $P < 0.01$) and age (standardized regression coefficient: -0.258, $P < 0.01$), and total triiodothyronine (TT3) decreased with age. (standardized regression coefficient: -0.171, $P < 0.01$). The age-related models showed a various change patterns with age for thyroid hormones.

Conclusions

The RIs for thyroid hormones of the elderly were established, which were different with RIs established in previous study. Influence factors model and age-related models are beneficial for physicians using thyroid hormones to monitor the status of various thyroid diseases for the elderly.

doi: 10.1016/j.cca.2022.04.660

T182

Assessment of some platelet parameters in patients with long-standing type 1 diabetes mellitus

G. Chausheva [a], Y. Bocheva [a], V. Iotova [b], S. Galcheva [b], M. Boyadzhieva [f], Y. Iotov [e], G. Valchev [c], N. Usheva [d]

[a] *Department of general medicine and clinical laboratory, Medical University- Varna, Bulgaria*
[b] *Department of pediatrics, Medical University- Varna, Bulgaria*
[c] *Department of radiology, Medical University- Varna, Bulgaria*
[d] *Department of social medicine and healthcare organization, Medical University- Varna, Bulgaria*
[e] *First department of internal medicine, Medical University- Varna, Bulgaria*
[f] *Second department of internal medicine, Medical University- Varna, Bulgaria*

Background-aim

Platelet hyper reactivity is a factor which contributes towards increased risk of cardiovascular events in diabetes mellitus (DM). We analyzed platelet indices in long-term DM type 1(T1DM) patients with

the aim to investigate whether platelets' morphology is altered, potentially predisposing them to cardiovascular events (CVE) in the future.

Methods

The study group consists of 83 patients with T1DM (male/female = 45/38; mean age = 41.23 ± 11.1 years without previous CVE; duration of diabetes 29.9 ± 9.7 years) and a control group of 32 age- and gender-matched healthy people (male/female = 13/19; mean age = 40.3 ± 9.7 years), studied in 2018-2020. Platelet parameters were derived from the results for complete blood count (Sysmex XN1000). Morphological analysis of the Romanowsky-stained blood smears was performed for all patients.

Results

We observed elevated platelet indices in the clinical group, comparing them with the control group: PLT- 290.7 × 10 9/ l ± 80 vs 256.18 × 10 9/ l ± 44; MPV-10.41fl ± 1.14 vs 9.98fl ± 1.34; P-LCR-29.903% ± 7.82 vs 29.086% ± 8.38; PCT- 0.307% ± 0.074 vs 0.239% ± 0.05; PDW- 12.697% ± 11.84 vs 1.58% ± 1.71. A significant positive correlation was found between MPV and PDW ($r = 0.94$; $p < 0.0001$) and a negative correlation between MPV and PLT ($r = -0.39$; $p = 0.0003$), PDW and PLT($r = -0.56$; $p = 0.001$); PLT and age ($r = -0.30$; $p = 0.03$) in the clinical group. For 54 (65%) of diabetic patients, the evidence for macroanisothrombocytosis was positive. These patients had a value for MPV > 10fl (mean = 11.08fl; CV = 0.47; SD = 0.68).

Conclusions

Platelets of patients with long-standing T1DM show morphological evidence of hyper reactivity, potentially contributing to increased cardiovascular risk. Macro-thrombocytes have larger biological activity. With advancing age, their proportion increases in contrast to the accelerated platelet consumption in order to maintain the constant functional platelet activity. Unlike other markers for assessment of platelet function, platelet indices do not require specialized laboratory equipment, which determines their financial efficacy in clinical and diagnostic terms.

doi: 10.1016/j.cca.2022.04.661

T183

A comparison study of the TRAB immunoassay between Snibe MAGLUMI and Roche Cobas

S. Cuesta De Juan, J. Garcia Ayuela, R.A. Torrado Carrion, A. Mata Fernadez, N. Soriano Balcazar, M. Perez Alonso, M. Santos-Ruiz Rodriguez-Arias, A. Siguin Gomez

Central Laboratory in Eurofins Megalab, Madrid, Spain

Background-aim

TRAbs (TSH receptor antibodies) are the antibodies of the thyroid membrane TSH receptor (TSHR). TRAbs are grouped depending on their effects on receptor signaling; activating antibodies (associated with hyperthyroidism), blocking antibodies (associated with thyroiditis) and neutral antibodies.

TRAbs of the stimulating variety are the hallmark of Graves' disease, it stimulates TSH receptor and induces hyperthyroidism. Neutral antibodies could be viewed as the ideal marker for the diagnosis and management of this autoimmune disease. TRAbs may be detected in other conditions, indicating the possible association of Graves' disease with other thyroid diseases.

The study aimed to evaluate the concordance between two chemiluminescence Immunoassay direct methods for the determination of TRAb (TSH receptor antibody)

Methods

A total of 182 serum samples were included in this laboratory comparison study.

The precision was evaluated using 3 serum samples (low, middle, and high level sample) and 1 control sample by assaying 20 replicates for each sample on MAGLUMI system.

The study compared results of 182 serum samples on MAGLUMI system with Cobas system. Snibe MAGLUMI adopts sandwich chemiluminescence immunoassay for its TRAb assay, and Roche Cobas uses sandwich electrochemiluminescence Immunoassay (ECLIA) for its Anti-TSHR assay. The results of Snibe Maglumi TRAb and Roche Cobas Anti-TSHR were analyzed by Passing-Bablok regression. The statistical test used was the XLSTAT 2018 Software Statistical the Microsoft Excel®

Results

The correlation between these 2 methods showed a coefficient of correlation $r = 0.97$ ($p < 0.001$), the 95% confidence interval for r was 0.9540 to 0.9741, the intercept -0.00315 and the slope 0.798 ($y = -0.00315 + 0.798x$).

The result of the assay for the pool serum 1 was SD 0.043 UI/L and CV 2.89%, for the pool serum 2 was a SD 0.150 UI/L and CV 5.31%, pool serum 3 showed a SD 0.483 UI/L and CV 5.00% and de pool control SD 0.148 UI/L and CV 3.72 %. The total precision of the assay was 2.89%-5.31%.

Conclusions

Based on above study, the results of TRAb exhibit a good correlation between Snibe Maglumi TRAb assay and Roche Cobas Anti-TSHR assay.

doi: 10.1016/j.cca.2022.04.662

T184

HbA1c and serum uric acid to creatinine ratio: A predictor of kidney diseases in association with estimated glomerular filtration rate in type 2 diabetes patients

S. Baral

Physician, Bir Hospital, National Academy of Medical Science, Kathmandu, Nepal

Background-aim

Diabetes mellitus is one of the major non-communicable and metabolic disorder recognized as chronic hyperglycemia associated with cardiovascular and renal complications if uncontrolled. Uric acid and HbA1c are used as an independent risk factor in diabetes. HbA1c is used for monitoring tool for measuring glycemic control in diabetic patients. It has shown that a 0.2% decrease of HbA1c level can lower the risk of CVD development by 10%. Serum uric acid is an indicator of glycol-metabolic disorder due to its relationship with the metabolism of glucose and have involved in atherosclerotic. Uric acid serves as an early indicator of renal complications in diabetes mellitus patients and cause a cardiovascular disease. Similarly, various study demonstrated that

baseline SUA/Cr ratio was independently and significantly associated with future renal function decline among diabetic patients. So this study is focused to find the correlation between glycated hemoglobin, Serum Uric acid to Creatinine ratio and estimated glomerular filtration rate (eGFR) among the diabetes patients to know the kidney function.

Methods

It is an observational, descriptive hospital-based, cross-sectional study. Patients with a diagnosis of diabetes were selected. This study designed to correlate Glycated hemoglobin (HbA1c), serum Uric acid to creatinine ratio (SUA: Sr Cr) with eGFR and its relationship with other biochemical parameters. The data were analyzed by SPSS version 20. Mean values of different variables, standard deviations and p-values were calculated.

Results

The mean age in the study subjects was 54.16 ± 10.36 years predominated by age group of 41 – 50 years (35%). Similarly, the mean HbA1c was 9.73 ± 1.98 and were increased in 77.5%(HbA1c > 8%) and 22.5% had less than 8%. There was strong positive correlation between HbA1c with Fasting blood sugar (r = 0.644, p = 0.000) and Post prandial blood sugar (r = 0.669, p = 000). Mean serum uric acid: Serum Creatinine ratio (SUA/S. Cr) was 6.09 ± 1.71 and elevated among 49.1%. Mean blood urea and serum creatinine levels were 28.0 ± 10.72 and 1.01 ± 0.18, respectively. There was a significant positive correlation between eGFR with HBA1c (r = 0.223, p = 0.015) and SUA /S. Cr (r = 0.246, p = 0.007) in this study.

Conclusions

In this study, HbA1c and serum uric acid are significantly correlated with eGFR thus can be considered as an independent risk factor. Similarly, SUA/S. Cr ratio was positively correlated with eGFR. Hence high HbA1c and SUA/S. Cr can be used as the predictor of early identification of kidney diseases in diabetes patient with preserved renal function.

doi: 10.1016/j.cca.2022.04.663

T185

Clinical usefulness of ultra-performance liquid chromatography-tandem mass spectrometry method for low serum testosterone measurement

S. Cho [a], J. Han [a], J. Park [a], E. Park [a], G.Y. Kim [a], J.H. Lee [a], A. Yi [a], S. G. Lee [a], E.H. Lee [a], Y. Yun [b]

[a] *Department of Laboratory Medicine, GCLabs, Gyeonggi-do, Republic of Korea*
[b] *Department of Laboratory Medicine, Konkuk University Medical Center, Konkuk University School of Medicine, Seoul, Republic of Korea*

Background-aim

Mass spectrometric methods exhibit higher accuracy and lower variability than immonoassays, especially at low testosterone concentrations. We developed and validated an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay for quantitation of serum testosterone.

Methods

We used ExionLC UPLC (SCIEX, USA) system and a SCIEX Triple Quad 6500 (SCIEX, USA) MS/MS in electrospray ionization and positive ion modes with multiple reaction monitoring transitions to evaluate precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ), carryover, ion suppression, stability, and reference intervals. For method comparisons, we measured serum testosterone concentrations by this method in 40 subjects whose testosterone concentrations were from 0.04 to 16.00 ng/mL by Architect i2000 (Abbott Diagnostics, USA) immunoassay, additionally measured testosterone concentrations in 40 men, 40 women, 40 boys, and 40 girls' sera of which testosterone concentrations were less than 0.481 ng/mL. We compared them with those from immunoassay.

Results

The intra-, inter-run precisions and accuracy were within the acceptable range. The linearity range was 0.008-52.155 ng/mL. The LOD and LOQ were 2.43 pg/mL and 0.008 ng/mL, respectively. No significant carryover and ion suppression were observed. The serum was stable in several conditions. The reference intervals were successfully validated. The overall correlation was fairly good (R = 0.989, Slope 0.995) with Architect, but Architect had positive percent biases at low testosterone concentrations.

Conclusions

The UPLC-MS/MS assay showed acceptable performance with lower LOD and LOQ compared with those from immunoassay. This method will enable the quantitation of low testosterone concentrations.

doi: 10.1016/j.cca.2022.04.664

T186

Possible role of prolactin in dysfunctional uterine bleeding

S. Eltabakh [a], I. Lotfy [b], H. Eldamarawy [b], T. Abdelsalm [b], S. Shoeir [b], N. Eldib [b]

[a] *Department ofMedical Biochemistry, Alexandria University, Alexandria, Egypt*
[b] *Department of OB/GYN, Alexandria University ,Alexandria, Egypt*

Background-aim

Regulation of menstrual cycle depends not only on proper FSH and LH , but also on normal Prolactin level. Hyperprolactinemia is a common finding in patients with reproductive disorders from menstrual irregularities to anovulatin. The aim of this study was to evaluate the serum Prolactin level in patients having Dysfunctional Uterine Bleeding (DUB) in relation to histopathological pattern of the endometrium.

Methods

This work included 50 patients diagnosed as DUB. All were subjected to full and complete history, examination and routine laboratory investigations. Serum Prolactin level were assayed and endometrial biopsy were done under GA , the curettage stent for histopathological examination.

Results

The mean Prolactin level was 32.7 ng/ml , 68%of the patients had hyperprolactinemia. Perimenopausal patients had a significantly higher Prolactin than the younger age. The Acyclic Bleeding recorded in 34 cases , it was cyclic in the other 16 . Patients with endometrial hyperplasia were found to have significant higher Prolactin (43.13 ng/ml) than patients with proliferative endometrium (26.92 ng/ml) and the other types of endometrium (25.80 ng/ml) . Galactorrhea was found only in 22%of the patients.

Conclusions

Hyperprolactinemia may be the primary abnormality in DUB which may results from unopposed estrogen stimulation. There was a link between endometrial hyperplasia and high Prolactin level , while galactorrhea was not an essential feature in DUB. Routine Prolactin assay should be consider in those patients , and trial of medical therapy may be recommended. Further investigations can be performed on a Large sample of patients.

doi: 10.1016/j.cca.2022.04.665

T187

The relationship between iron deficiency and thyroid function in Maldives

Z. Un Naher , S. Ali, S. Moosa, M.A. Mohsin

School of Medicine, Maldives National University, Male', Maldives

Background-aim

Thyroid hormone (TH) regulates metabolic processes essential for normal growth and development as well as regulating metabolism in the adult .Thyroid hormones affect every cell and all the organs of the body, as they are involved in regulating the rate of metabolism . (Mullur, Rashmi., et al.2014). Iron is a component of many enzymes including thyroid peroxidase (TPO) , and is essential for the initial two steps of thyroid hormone synthesis. (Rousset B.,et al.2000). Serum ferritin is an iron storage protein present in almost all cells, the altered level of serum ferritin have been reported in patients with thyroid disease. (Wang, W.,et al 2010)The prevalence of thyroid disorders in Maldives is not available, nevertheless, a goiter rate of 0.6% was observed among children 6months to 5 years and 0.8% in reproductive aged women (Ministry of Health Project Report National Micronutrient Survey Republic of Maldives., 2007).

Research is needed to provide more update information of prevalence of iron deficiency and thyroid disorders and their relationship to guide policy and guidelines on health interventions to comprehensively address thyroid disorders in the country.

Methods

This was a cross sectional study conducted on 400 subjects who were attending IGMH laboratory services 2019-2020. Thyroid Stimulating Hormone (TSH), Free Thyroxin (T4), and serum Ferritin levels were measured in a fully automated analyzer.

Patient with a request for complete blood profile from a clinician with the exception of:

pregnant, pre-diagnosed with iodine deficiency,

autoimmune thyroid disorders (positive for Anti-TPO and Anti-TG),

hepatic disorders and renal diseases excluded from the study.

Non consenting patients

and children under 18 years of age without a legal guardian also be excluded

Instruments and materials:

Blood specimens collected from consenting patients by a trained laboratory technician/phlebotomist. All blood specimens will be obtained through an indwelling intravenous catheter.

Serum ferritin level assessed using immunoassay (Linpisarn S., 1993). Basal serum levels of thyroxine (T4), triiodothyronine (T3), free T4 (fT4), free T3 (fT3), thyroid stimulating hormone (TSH), by using Fluorescence polarization immunoassay (FPIA)(Driggers DA., 1983).

Ethical considerations – We took approval for full ethical clearance from the Ethical Committee of Maldives National University and related departments of the Ministry of Health.

Data collection and management

Relevant information was collected from the patient including demographic details, medical history, laboratory investigations and recorded in a pre designed data sheet.

The results analyzed by SPSS 25 and Unpaired t-test and Pearson's correlation coefficient test will be performed.

Results

As we targeted the goal to find out the relationship between iron status and thyroid hormones in subjects who are attending the IGMH laboratory ,Maldives, 400 subjects were selected. Serum samples were collected and assayed serum ferritin, iron, total iron binding capacity (TIBC), thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), free thyroid hormones (fT4). Hematological indices for iron status confirmed that all subjects were iron-deficient.There was a non-significant correlation between T4 and S Iron ($r = -.035$, $P < 0.05$) and between TSH and S Iron ($r = 0.68$, $P < 0.05$). There was a non-significant correlation between T4 and S TIBC ($r = .121$, $P < 0.05$) and between TSH and TIBC($r = -0.026$, $P < 0.05$).

In the present study, the mean value of TSH (2.18 ± 3.1) was higher among hypothyroid subjects whereas mean value of FT4 (12.77 ± 2.16), and S ferritin (63.12 ± 99.52) and Serum Iron (72.99 ± 35) were found be lower among hypothyroid subjects as compared to euthyroid subjects.

There was a non-significant correlation between T4 and S ferritin ($r = 0.067$, $P = 0.409$) and between TSH and ferritin ($r = 0.086$, $P = 0.287$).

There was a non-significant correlation between T4 and S ferritin ($r = 0.067$, $P = 0.409$) and between TSH and ferritin ($r = 0.086$, $P = 0.287$).

Conclusions

The present study showed that, hypothyroid subjects had low serum ferritin levels. Hence estimation of serum ferritin concentration among hypothyroid subjects could be useful in the evaluation of thyroid hormone status.

Above discussion indicates the reason for perhaps failing to find significance is the sample size and male and female ratio. This study findings that are different from what we expected is making an interesting avenue of research questions.

Though the study failed to show any significant positive correlation between serum ferritin and thyroid hormones, lower level of thyroid status in iron deficient patients suggest that it could be a reflection of disturbed activities of iron dependent enzymes such as thyroid peroxidase that impairs thyroid hormone synthesis. However, a large scale study is recommended to establish the fact.

doi: 10.1016/j.cca.2022.04.666

T188

Association between pre-pregnancy body mass index and triglyceride glycemic index in mid pregnancy in women with gestational diabetes

M. Genova [a], B. Atanasova [a], I. Ivanova [c], K. Todorova – Ananieva [e], K. Tosheva [d], D. Tzvetina [b]

[a] *1Department of Clinical Laboratory, Faculty of Medicine, University Hospital "Alexander", Medical University-Sofia, Bulgaria*
[b] *Clinic of obstetrics and gynecology, Vita Hospital, Sofia, Bulgaria*
[c] *Clinical Laboratory Department, University Hospital "Sv. Ivan Rilski", Medical University – Sofia, Bulgaria*
[d] *Medical Center "Pentagram", Bulgaria*
[e] *University Clinic of Endocrinology, Medical University – Pleven, Bulgaria*

Background-aim

Gestational diabetes mellitus (GDM) is characterized by a relatively diminished insulin secretion coupled with a pregnancy-induced insulin resistance (IR). Increasing pre- pregnancy BMI is a risk factor for the development of GDM. Triglyceride–glucose index (TyG) is a non-insulin based ratio suggested as a useful marker for recognizing IR.

The aim of this study is to evaluate the relationship between pre-pregnancy body-mass index (BMI) and TyG index in GDM and healthy pregnancy.

Methods

Totally 80 pregnant women (age 18-40 years, in the second trimester) are included in the study. They are divided into two groups on the base of 2h 75-g oral glucose tolerance test (OGTT): 1. GDM group (n = 40) and 2. Pregnancy with normal glucose tolerance (NGT) (n = 40). Glucose and triglycerides (TG) are measured in venous blood at fasting state using amperometric method for glucose (analyzer Biosen C-line) and enzyme colorimetric test (GPO-PAP) for triglycerides(Roche-Cobas 6000). Both indices are calculated by formulas: for TyG (ln (fasting triglycerides (TG) (mg/dL) × fasting glucose (mg/dL)/2) and weight (kg)/ height (m^2) for BMI.

Results

Blood glucose, TG, TyG-index and pre-pregnancy BMI are as following: NGT and GDM 78.3 ± 7.07 vs 97.9 ± 14 mg/dl, $P < 0.002$; 177.6 ± 88.6 vs 203,12 ± 70,5 mg/dl, $p = 0.16$; 4,7 ± 0.25 vs 4,91 ± 0.20, $p < 0,0001$; 22,8 ± 4.4 vs 27.8 ± 7.8, $p < 0,0006$.

The correlation between pre-pregnancy BMI and TyG is positive in GDM group $r = 0.270$, $p < 0.0005$ and negative in NGT pregnancy group $r = -0.136$, $p < 0.002$

Conclusions

The existing data about the role of TyG as a potential biomarker in GDM are inconsistent up to date. In any case, the current findings support the clinical utility of TyG index in identification of pathologic IR during GDM. Positive connection is observed between TyG and pre-pregnancy BMI in GDM group. Both markers-pre pregnancy BMI and TyG index could be suitable to evaluate the pathophysiologic characteristic of women with GDM.

doi: 10.1016/j.cca.2022.04.667

T189

Is a morning serum cortisol of 100nmol/L still an appropriate cut-off to predict inadequate short synacthen test response for newer generation of cortisol immunoassay?

J.G. Tan, G.E. De La Pena, P.H.M. Er, M.S. Wong

Lab Medicine, Khoo Teck Puat Hospital, Singapore

Background-aim

Short synacthen tests are commonly performed for patients suspected of adrenal insufficiency. Historically, peak serum cortisol of 500 - 550nmol/L at 30 - 60min after administration of cosyntropin are considered as an adequate adrenal response. However, with the improved analytical specificity of newer generation immunoassays, the peak short synacthen test cut off values are lowered to 400-450nmol/L and are numerically similar to values obtained by LC-MS/MS. Therefore, a common query is whether the traditional morning 100nmol/L serum cortisol as a rule-in test for adrenal insufficiency is still suitable for laboratories using newer generation cortisol immunoassay.

Methods

We searched the laboratory information system for all short synacthen tests requests received by our hospital laboratory between 1st January and 31st December 2021, and studied the relationships between 0, 30 and 60min serum cortisol. Receiver-operating characteristic (ROC) curve analysis was performed to obtain the predictive cut-off value for early morning cortisol. Cortisol was assayed on Roche Cobas e602 using the Elecsys Cortisol II reagent. We used 420nmol/L at 30min as the reference for adequate short synacthen response. 420nmol/L is the 2.5th percentile peak 30min cortisol response for healthy subjects in the Australasian Harmonisation of Endocrine Dynamic Testing (Adult) manual and the cut-offs used at several European laboratories using Elecsys Cortisol II assay.

Results

We retrieved a total of 658 sets of short synacthen tests with cortisol at 0min, 30min and 60min. Of which, 307 sets had the 0min cortisol drawn between 6 and 9 am (early morning cortisol) and were included in the analysis. The median 30min cortisol was 547 nmol/L. Median cortisol at 60min was 10.9% [CI:10.2 - 11.8] higher than the corresponding 30min. An early morning cortisol of 104 nmol/L has 99% specificity [CI:96.9 - 99.9] and 24% sensitivity [CI:14.9 - 35.3] with LR+ 27.8. An early morning cortisol of 358 nmol/L resulted in sensitivity of 99% [CI:92.8 - 100.0] and specificity of 44% [CI:37.5 - 50.6] with LR- 0.03.

Conclusions

Our study has found that a morning cortisol level of more than 358nmol/L may be used to rule-out adrenal insufficiency (99% sensitivity) and a basal cortisol level of less than 104nmol/L may be used to rule-in adrenal insufficiency (99% specificity). The level of 100nmol/L to rule in adrenal insufficiency remains relatively accurate even with Elecsys Cortisol II assay. A morning cortisol of more than 358nmol/L may preclude the need for the laborious short synacthen test.

doi: 10.1016/j.cca.2022.04.668

T190

Paraganglioma and synchronous carcinoid tumor

C. Montero Domínguez [a], L. Martínez Figueras [a], A. Ortiz Temprado [a], V. Ortiz González [a], M.L. Fernandez Ruano [b]

[a] *Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain*
[b] *Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain*

Background-aim

Neuroendocrine tumors are divided into two main types: those of epithelial origin (carcinoid tumors) and those of neural derivation (paragangliomas). Although carcinoid tumors and paragangliomas (PGLs) have overlapping histological features, they have different biologic behaviors.

The clinical presentation of PGLs is heterogeneous, depending on several factors like catecholamine secretion or the compression effect of the mass. The most common head and neck PGL is carotid body tumor.

Carcinoid tumor produces serotonin which cause symptoms like diarrhea, flushing or wheezing. The majority of carcinoid tumors are found along the gastrointestinal tract and bronchopulmonary system.

The aim of this poster is to present an extremely rare case of a patient affected by this two neuroendocrine tumors.

Methods

Urinary methanephrine (MN), normetanephrine (NMN) and 3-methoxythyramine (3-MT) were measured by HPLC (Agilent® 1200 series, RECIPE®) and urinary vanillylmandelic acid (VMA) and 5-hydroxyindoleacetic acid (5HIAA) were measured by HPLC (Agilent® 1300 series, RECIPE®). Chromogranin A was analized by immunofluorescence (Kryptor®, ThermoFisher Scientific®) and PTH was analized by immunochemiluminescence (Alinity®, Abbott®).

Results

A 67-year-old male, presented with parotid gland tumor in control for several years. Thirteen years ago, he underwent a radical retropubic prostatectomy due to an adenocarcinoma of the prostate. A dimensional increase of parotid gland was detected. Computed Tomography and Octreoscan examination were performed, which showed a mass lesion in the region of the left parotid highly suggestive of PGL. The biochemical analysis revealed an increase in urine NMN: 3938 nmol/24h (<2880), 3-MT: 30170 nmol/24h (<2390) and VMA: 8.3 mg/24h (<6.7) with normal MN and 5HIAA. Laboratory findings were negative for hyperparathyroidism (PTH: 42ng/L (<68 ng/L)) and his serum chromogranin A level was normal (64 ng/ml (<98 ng/mL)). The patient underwent biopsy, whose results revealed a well differentiated PGL. The treatment was radiotherapy. At the same time, in a follow-up PET-CT scan to evaluate his adenocarcinoma of the prostate, a lung nodule in the right lower lobe was detected. The patient underwent pulmonary surgery. The histological study was compatible with lung carcinoid tumor. Genetic testing was requested and it is still pending.

Conclusions

Lung carcinoid tumor account for less than 1% of all lung cancers. Carotid body PGLs are even rarer, and the incidence of both of these tumors is extraordinarily rare. The presence and almost coexistence of two rares entities and an adenocarcinoma of the prostate in this patient raises the hypothesis of a genetic etiology.

The occurrence of PGL is well characterized within Von Hippel-Lindau disease, multiple endocrine neoplasia type 2 (MEN2), and neurofibromatosis type 1. Mutations in the genes encoding subunits of the succinate dehydrogenase (SDHA, SDHB, SDHC and SDHD) have been shown to determine the hereditary PGL syndromes. Lung carcinoid tumors usually occur sporadically, although they can arise in association with Multiple Endocrine Neoplasia type 1 (MEN1). In the literature there is a case of coexistence of PGL with MEN1. However, in this case, the diagnosis with MEN1 seems very unlikely because the clinical features that characterize this syndrome were absent.

This case highlights the need for ongoing research into the molecular genomics of PGLs and lung carcinoid tumors.

doi: 10.1016/j.cca.2022.04.669

T191

Association between the ratio of estimated average glucose to fasting plasma glucose levels and pancreatic beta-cell function in prediabetes and type 2 diabetes mellitus

N. Eun-Hee

Health Promotion Research Institute, Korea Association of Health Promotion, Seoul, Republic of Korea

Background-aim

Type 2 diabetes (T2DM) is a disease characterized by progressive failures in pancreatic beta-cell function (BCF) and insulin resistance (IR). Identifying and managing the associated decrease in glucose tolerance should be initiated early to prevent or delay beta-cell failure and reverse IR. The homeostasis model assessment (HOMA) is one of the methods for assessing BCF. This study aimed to determine the correlation between the glycated hemoglobin (HbA1c)-derived estimated average glucose (eAG)/fasting plasma glucose (FPG) ratio and HOMA in prediabetes and T2DM.

Methods

This study selected subjects who underwent a health checkup at 16 health-promotion centers in 13 Korean cities between 2019 and 2021. The subjects comprised 3,003 patients with normoglycemia, 3,414 with impaired fasting glucose and 4,178 with diabetes. HbA1c-derived eAG was calculated using Nathan's regression equation. BCF and IR were assessed by HOMA-® and HOMA-IR, respectively. Multivariable regression analyses were used to determine the correlation between the eAG/FPG ratio and HOMA.

Results

The FPG level was negatively correlated with HOMA-® ($r = -0.353$, $P < 0.001$) and positively correlated with HOMA-IR ($r = 0.332$, $P < 0.001$). The eAG/FPG ratios were significantly lower in groups with higher FPG ($P < 0.001$). The eAG/FPG ratio was positively correlated with HOMA-® ($r2 = 0.015$, $P < 0.001$) and negatively correlated with HOMA-IR ($r2 = 0.010$, $P < 0.001$) in prediabetes and T2DM.

Conclusions

The eAG/FPG ratio was associated with both HOMA-® and HOMA-IR, which suggests that this ratio reflects BCF and IR in prediabetes and T2DM. Measurement of the eAG/FPG ratio could be useful for monitoring BCF and IR in adults with prediabetes and T2DM.

doi: 10.1016/j.cca.2022.04.670

T192

Effect of pH on the correlation between urine density and urine osmolality

M. Boudaya, N. Aissaoui, F. Gargouri, S. Fendri, F. Ayedi, R. Marrekchi, K. Jamoussi

Laboratory of biochemistry Sfax university hospital, Tunisia

Background-aim

Urine osmolality represents the test of choice for the evaluation of the kidneys concentration ability. In practice, urine density (UD) is used to estimate urine osmolality.

Our objective was to study the effect of pH on the correlation between measured osmolality and refractometer-determined DU (UDRef).

Methods

Urine osmolality was determined on urine samples using the Fiske® Microosmometer Model 210 osmometer. UD was determined by the ATAGO® refractometer. pH was determined by MISSION® test strips. Samples were classified into 4 groups according to pH: pH H 5; pH H 6; pH H 7 and pH ε 8. Statistical analysis was performed by SPSS version 20 and XLSTAT software.

Results

One hundred twenty urine samples were analyzed, including 34 with pH H 5; 51 with pH H 6; 20 with pH H 7; and 15 with pH ε 8. UDRef and osmolality were well correlated for pH 5, 6, and 7 with correlation coefficients 0.92 ($p<10\text{-}4$), 0.78 ($p<10\text{-}4$), and 0.97 ($p<10\text{-}3$), respectively. No correlation was observed at pH ε 8 (R^2Ref = 0.15, p = 0.6).

Conclusions

Urine density determination by refractometer is correlated to urine osmolality when pH is below 8.

doi: 10.1016/j.cca.2022.04.671

T193

Changes in platelet count and platelet indices in relation to disease duration and patient age in long-term type 1 diabetes mellitus

G. Chausheva [b], Y. Bocheva [b], S. Shefket [b], K. Tsochev [d], T. Chalukova [f], M. Boyadzhieva [e], G. Valchev [a], R. Pancheva [c], V. Iotova [d]

[a] *Clinic of Diagnostic Imaging, UMHAT "Sv. Marina", MU-Varna, Bulgaria*
[b] *Dept. of Clinical Laboratory, MU-Varna, Bulgaria*
[c] *Dept. of Hygiene and Epidemiology, MU-Varna, Bulgaria*
[d] *Dept. of Pediatrics, MU-Varna, Bulgaria*
[e] *IInd Dept. of Internal Diseases, MU-Varna, Bulgaria*
[f] *Ist Dept. of Internal Diseases, MU-Varna, Bulgaria*

Background-aim

Platelets (Plt) are cellular fragments with active role in the pathogenesis of thrombosis and atherogenesis. We analyzed Plt parameters in long-term type 1 diabetic (T1D) patients. Our aim was to investigate whether Plt' morphology in patients with long-term type 1 diabetes mellitus is altered, compared to healthy controls.

Methods

A total of 183 participants were studied: 124 (53.2% men) with T1D, aged 42.7 ± 10.4 years, diabetes duration 25.3 ± 8.2 years and 59 sex matched healthy control subjects (54.1% men), aged 45.1 ± 9.1 years (p = 0.11), studied in 2018-2020. Plt parameters (PLT, MPV, PLC-R, PCT, PDW) were derived from the results of complete blood count (Sysmex XN1000). Plt morphological evaluation was performed by microscopic observation of blood smears, stained with a Romanowsky stain. Descriptive, t-test and Pearson's correlation statistical analyses were performed.

Results

We found higher results of Plt parameters (PLT, MPV, PCT, P-LCR, PDW) in T1D patients compared to controls: PLT - 280.60 ± 76.016x10 ˆ 9/ L vs 253.08 ± 47.098 x10 ˆ 9/L, MD- 27.520 x 10 ˆ 9 / L, t = 2.999, p = 0.003; PCT - 0.299 ± 0.070%. vs 0.2647 ± 0.048%, MD = 0.0345%, t = 3.069, p = 0.003; MPV -10.562 ± 1.172fL vs 10.222 ± 1.16fL, MD = 0.340fL, t = 1.840, p = 0.067; PDW - 12,933 ± 2,189fL vs 12,248 ± 1,867fL, MD = 0.685 fL, t = 1.872, p = 0.063; P-LCR - 31.527 ± 8.123% vs 29.557 ± 7.354%, MD = 1.969, t = 1.428, p = 0.155. For 69.4% of all diabetic patients, the evidence for Plt anisocytosis was positive. These patients had MPV > 10fl (72.7% men). Duration of diabetes correlated significantly with: PLT (r = - 0.208; p = 0.021); MPV (r = 0.167, p = 0.063), P-LCR (r = 0.180, p = 0.061). Significant weak correlations were found between age and: MPV r = 0.200, p = 0.026; P-LCR r = 0.277, p = 0.004; PLT r = -0.254, p = 0.004; PCT, r = -0.213, p = 0.026.

Conclusions

Plt parameters were higher in patients with long-term T1D, compared to healthy controls. We found a significant positive correlation between MPV, PDW, P-LCR and duration of T1D and patient age, while the correlation with PLT and PCT was negative. These suggest that with advancement in the age and diabetes duration, the proportion of reactive platelets increases with a corresponding predisposition to atherosclerosis.The study was funded by a research grant DN 13/3, 14 Dec 2017, from the Scientific Research Fund at the Ministry of Education and Science of Bulgaria.

doi: 10.1016/j.cca.2022.04.672

T194

Peripheral blood Smad7 and miR-181b-5p possibly regulate insulin resistance in type 2 diabetes

P. Purohit [a], D. Roy [a], A. Modi [a], R. Shukla [b], P. Sharma [a]

[a] *Department of Biochemistry, AIIMS Jodhpur, India*
[b] *Department of Endocrinology & Metabolism, AIIMS Jodhpur, India*

Background-aim

Type 2 diabetes mellitus (T2DM) is a major global health burden. Various microRNA (miRNA) contribute to its pathogenesis, among which hsa-miR-181-5p plays a major role. Smad7 is an inhibitory Smad protein that regulates insulin resistance in mice models. The current study aimed to investigate the expression of hsa-miR-181b-5p and Smad7 in peripheral venous blood of T2DM patients.

Methods

We recruited 30 T2DM individuals and 25 healthy controls, aged 18-60 years, after informed consent.

Fasting venous blood samples were collected and analyzed for blood glucose, HbA1c, lipid profile, insulin, and insulin resistance indices (calculated from biochemical parameters). Total RNA was extracted, reverse transcribed and amplified by real time PCR. GAPDH and RNU6 were used as internal control genes for Smad7 and miR-181b-5p, respectively. All statistical analyses were performed on Microsoft Excel.

Results

The two groups significantly differed in body mass index ($p < 0.001$), waist-hip ratio ($p < 0.001$), HbA1c ($p < 0.001$), fasting blood glucose ($p < 0.001$), total cholesterol ($p = 0.005$), triglycerides ($p = 0.019$), fasting insulin ($p < 0.001$), and HOMA-IR ($p < 0.001$). The miR-181b-5p expression was significantly and positively correlated to fasting blood glucose ($> = 0.262$, $p = 0.027$), HbA1c ($> = 0.275$, $p = 0.021$), and HOMA-IR ($> = 0.275$, $p = 0.021$). The fold change expression was 5.11 times upregulation for miR-181b-5p and 1.86 times downregulation for Smad7 in T2DM subjects compared to healthy individuals.

Conclusions

The findings suggest that miR-181b-5p and Smad7 may be crucial players in the development of insulin resistance in T2DM, and represent potential therapeutic markers.

doi: 10.1016/j.cca.2022.04.673

T195

Plasma creatinine measurement in diabetic patients. Should we advocate the enzymatic method?

M. Boudaya, F. Ayadi, S. Fendri, N. Aissaoui, F. Gargouri, R. Marrekchi, K. Jamoussi

Laboratory of biochemistry, Sfax University Hospital, Tunisia

Background-aim

The prevalence of chronic renal failure is constantly increasing. Diabetes seems to be the main cause. For a long time, creatinine (CRE) has been considered the simplest biochemical marker for the assessment of renal function. Several methods are used for the determination of creatinine including classical colorimetric assays (Jaffe's kinetic method, enzymatic method)

Our objective was to compare the two methods of creatinine measurement: enzymatic method and compensated kinetic Jaffe method in a diabetic population.

Methods

This study was performed in the biochemistry laboratory of Hedi Chaker hospital of Sfax during the period of April 2019 until June 2019. We analysed 150 samples from type 1 and 2 diabetic patients. The determination of CRE values by the kinetic method of Jaffe and by the enzymatic method were performed on DxC600 of Beckman Coulter.

The statistical comparison of the results obtained with the two methods of analysis was performed using XLSTAT software version 2019.

Results

CRE levels measured by the enzymatic method ranged from 31 ⌈mol/L to 597 ⌈mol/L (Mean 73.300 ⌈mol/L). These levels were 27 ⌈mol/L to 626 ⌈mol/L (Mean 77.460 ⌈mol/L) by the kinetic Jaffe method.

When comparing the means of CRE assayed by Jaffe method and those by enzymatic method, we observed a significant difference ($p < 0.0001$). However, this difference was clinically insignificant since the differences in means ($= 5.67\%$) did not surpass the Total Variability Limitfor both assay methods ($< 12\%$).

There is a good correlation between the two methods (Pearson = 0.581, $p > 0.0001$)

The regression line equation (Passing and Bablock) is: CRES = 1.089 * (CREE) + (-3.229)

Concordance analysis of CRE results using the Bland-Altman plot revealed the presence of a bias equal to 4.16 ⌈mol/L (mean of differences).

We observed an overestimation of the creatinine values determined by kinetic method of compensated Jaffe compared to those by enzymatic method.

The mean blood glucose level was 11.708 m mo/L.

A study of the correlation between the difference in creatinine levels determined by the two methods (CRES-CREE) and blood glucose levels showed a good correlation (Pearson coefficient = 0.348; $p < 0.001$) .

Conclusions

The determination of serum creatinine by enzymatic method in the diabetic population appears to be more reliable than that determined by compensated jaffé.

doi: 10.1016/j.cca.2022.04.674

T196

Importance of plasma separation for a deferred dosage of thyroid hormon FT4 and TSH

M. Boudaya, F. Gargouri, N. Aissaoui, F. Ayedi, S. Fendri, R. Marrekchi, F. Ayadi

Laboratory of biochemistry, Sfax University Hospital, Tunisia

Background-aim

Thyroid stimulating hormone (TSH) and free thyroxine (FT4) are the two main parameters of thyroid assessment. Sometimes samples are stored after centrifugation for delayed determination. Our objective was to study the importance of separation before plasma storage at +4°.

Methods

This work involved 22 blood samples collected on lithium heparinate. Each tube was centrifuged and assayed immediately, then plasma was aliquoted into an eppendorf and the remainder was left in its primary tube. Redosing after storage for 1 week at +4° was performed for both separated and non-separated plasma. The assays were performed on Architect Ci 4100 (Abott) by immunological methods. Statistical study was done by SPSS20.

Results

Comparison of TSH means assayed immediately and those assayed on separated plasma after storage at +4°C for one week showed no significant difference while a significant increase was noted on non-separated plasma.

For FT4, comparison of the means of FT4 measured immediately and those measured on separated plasma after storage at +4°C for one week showed no significant difference, where as a significant increase was found for FT4 measured on non-separated plasma.

Among the 22 patients studied, the diagnosis was changed for 2 patients for the non-separated samples.

Conclusions

Storage for one week at +4°C showed that thyroid parameters are stable on separated plasma where as they are unstable on unseparated plasma and this instability could lead to misdiagnosis.

doi: 10.1016/j.cca.2022.04.675

Clinica Chimica Acta 530 (2022) S150–S152

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Evidence Based Medicine, Guidelines

M076

Cost implications of dual and duplicate amylase and lipase requesting in the work up of acute pancreatitis at Tygerberg Tertiary Hospital, Cape Town, South Africa

T. Jalavu [c], A. Cook [a], A. Zemlin [b]

[a] *Berkshire and Surrey Pathology Services Clinical Laboratory, Royal Surrey County Hospital, Guildford, Surrey, United Kingdom*
[b] *Division of Chemical Pathology, Faculty of Medicine and Health Sciences Stellenbosch University and National Health Laboratory Services (NHLS), Tygerberg Hospital, Cape Town, South Africa*
[c] *Stellenbosch University and National Health Laboratory Service, South Africa*

Background-aim

Acute pancreatitis (AP) is a sudden inflammatory process of the pancreas with variable involvement of adjacent organs. It is reportedly the most common gastrointestinal cause of hospital admissions in the USA with an annual incidence of 4 to 30 per 100 000. Diagnosis of acute pancreatitis (AP) requires two of the three features to be present: characteristic abdominal pain, raised serum amylase and/or lipase enzymes >3 times the upper limit of normal (ULN) or consistent imaging results according to the revised Atlanta consensus guidelines 2012. Lipase is the preferred test to screen for AP, but many clinicians still perform amylase or both in the same patient.

The aim of the study was to investigate the extent of dual and duplicate amylase and lipase testing in suspected AP cases and the cost implications thereof.

Methods

A retrospective audit of all amylase and lipase requests was conducted over a one-month period. Only inpatient requests were included because clinical notes were available on the hospital electronic document management system. Amylase and lipase results for these patients were reviewed together with patient clinical data to determine the sensitivity and specificity of amylase, lipase and dual requesting for the diagnosis of AP using the guideline cut-off criteria of >3ULN.

Results

Lipase showed a much higher diagnostic sensitivity of 90% compared to 50% for amylase. Specificity was shown to be very similar for amylase (98.6%) and lipase (96.9%). Dual requesting of the two enzymes had a sensitivity 83.2% and specificity 97.4%. The audit showed that 25% (n=55) of requests in the one month's period were duplicate for both lipase and amylase. The cost of avoidable/inappropriate testing amounted to ZAR 8000 (557 USD) for the month, extrapolated to ZAR 101 831 (7085 USD) over a 12 month period which is very high for a single test in a resource limited setting.

Conclusions

Lipase has been shown to be more sensitive to screen for AP. Addition of amylase to lipase provides only marginal additional benefit. The extent of dual requesting, duplicate and inappropriate requests is high which has cost implications. Amylase could be phased out of tertiary clinical laboratories to avoid unnecessary expenditure.

doi: 10.1016/j.cca.2022.04.677

M077

Using evidence based medicine to evaluate the strategic evaluation of restrictive and free blood transfusion in different clinical situations

M.H. Chen, M.F. Hsu, K.T. Chen, B.C. Show

Clinical pathology Department, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Background-aim

There are currently two main blood transfusion strategies in clinical transfusion, including restricted blood transfusion strategies and free blood transfusions. The free blood transfusion threshold haemoglobin was 10 g / dL. At the same time, the lower limit of blood transfusion threshold is 8 g / dL for haemoglobin, and the blood transfusion is not performed until the patient has symptoms of anemia. Blood transfusion is a therapeutic measure and a supportive and compensatory therapy. Including surgical blood preparation to prevent excessive blood loss during surgery, severe anemia, and so on. Blood transfusion can be input for different blood components (or "blood products"), including whole blood, packed RBC, washed RBC, and platelet concentrate, etc. Make choices based on patient needs. Recent analyses in many randomized controlled trials (RCTs) report that restrictive blood transfusion methods are as safe as free blood transfusion strategies. In view of the emerging evidence, we conducted a meta-analysis of RCT, using the empirical medical model PICO: (Patient), I (Restrictive transfusion), C (liberal

transfusion), O: (clinical Outcome), to search for keyword usage "Restricted and free blood transfusions." Relevant literature in Cochran, PubEMed and EMBASE.

Methods

A PubMed database search was performed in September 2015. In EMBASE, Medline and Google Scholar were cross-checked to collect patients over 18 years old from 1998 to 2015. Haemoglobin was set to 8.1 ~ 10g / dL for free blood transfusion, and haemoglobin was less than 8g / dL for restricted blood transfusion. . We evaluated each study using the Jadad composite scale and assessed statistical heterogeneity by the Cochrom chi-square test and the I2 test.

The study population, age, male to female ratio, number of cases and participants, blood transfusion threshold, 30-day mortality, incidence of infection, incidence of myocardial infarction, incidence of congestive heart failure, length of hospital stay, and number of patients transfused. The methodological quality of each component study was assessed using the Jadad Comprehensive Scale.

Data analysis was performed using the Cochrane analysis program (RevMan version 5.3.0). Statistical heterogeneity was assessed by the Cochran chi-square test (Q test) and I 2 test. P <.1 and I 2> 50% are considered to imply statistical heterogeneity, and random effects models are used to estimate binary and continuous variables. Otherwise, a fixed-effects model will be used, and inverse variance statistical methods will be used for binary and continuous variables. Binary variables are expressed as hazard ratios (RR) and 95% confidence intervals (95% CI). Continuous variables are expressed as mean and standard deviation (SD) and evaluated using weighted mean difference and corresponding 95% CI

Results

1. Transfusion-related mortality 30 days after surgery

These included restrictive and free blood transfusion thresholds in patients 1763 and 1762, respectively. In the second experimental group (RR = 1.06, 95% CI 0.78-1.45; no difference in postoperative mortality between 30 days P = 0.71), and no statistically significant heterogeneity (|2 = 7.28; P = 0.30; I2 = 18%). According to the Begg test, publication bias was not significant in all included studies (P = 1.00) or the Egger test (P = 0.52; 95% CI was -1.24–2.14)

2. Incidence of pneumonia and wound infections after blood transfusion

There was no significant difference in the incidence of pneumonia between the restricted and free-infusion threshold groups (RR = 0.80, 95% CI 0.61–1.05; P = 0.11). Similarly, there was almost no difference in the incidence of wound infections between the free and restricted infusion threshold groups (RR = 1.28, 95% CI 0.69–2.36; P = 0.43). There was no significant difference in the incidence of pneumonia and wound infection (RR = 0.87, 95% CI was 0.68-1.11; P = 0.27. According to the Begg test (P = 0.23) or the Egger test (P = 0.27; 95% CI was-1.19–0.37).

3. Incidence of myocardial infarction

A total of 1313 and 1308 patients were included in the report for the number of restricted and free transfusions. There was no restrictive and free transfusion threshold for myocardial infarction (RR = 1.55, 95% CI 0.96-2.50; significant difference between P = 0.07), and no statistical heterogeneity was found (|2 = 2.20; P = 0.70 ; I2 = 0%). According to Begg test (P = 0.08) or Egger test (P = 0.51; 95% CI is -1.93-1.19).

4. Incidence of congestive heart failure

There were a total of 1333 and 1323 patients with restricted and free blood transfusion thresholds for congestive heart failure (RR = 1.32, 95% CI 0.83-2.11; no significant difference between P = 0.25) and no statistics Significant heterogeneity (|2 = 1.52; P = 0.82; I2 = 0%). Not obvious according to Begg test (P = 0.81) or Egger test (P = 0.89; 95% CI is -1.40–1.56).

5. Whether to extend hospital stay

There were 3157 patients admitted to the hospital, including the restrictive and free blood transfusion thresholds of the patients and 1576 1581, respectively. There were significant differences between the two groups (mean difference = 0.14, 95% CI was -0.13-0.42, P = 0.31), And found no statistically significant heterogeneity (∴2 = 5.45, DF = 5, P = 0.36, I2 = 8%). According to Begg test (P = 1.00) or Egger test (P = 0.07; 95% CI is -2.53-0.16).

Conclusions

Only RCT was evaluated in this report analysis because it optimizes follow-up and data quality while minimizing selection bias and other confounding factors. All 10 RCTs included in the analysis have adequate methodologies and sensitivity analyses were conducted to explore the impact of each trial on the combined effect estimates for each result. Let us find that the omission of any study has an overall effect on the overall results. No significant impact. In addition, no significant bias was observed according to the Begg and Egger tests.

Analysis of the RCT showed no significant difference in 30-day mortality between the restricted and free transfusion strategy groups. Surgical patients reported no significant differences in in-hospital mortality between the two groups, with free and restricted infusion threshold groups recording 2.0% and 1.4% mortality, respectively. A retrospective study of individuals undergoing surgery found that RBC transfusions were not associated with changes in mortality. Restrictive transfusion strategies are therefore not associated with any statistically significant differences in 90-, 120-, or 365-day mortality.

When comparing the restricted infusion strategy with the free infusion strategy, no significant difference was found in the rates of pneumonia or wound infection. A recent analysis of reports including eight RCTs indicates that restrictive blood transfusion strategies can reduce the risk of infection in orthopedic patients by 35%. It has also been shown that blood transfusions are associated with increased susceptibility to infections and transfusion-related lung injury, suggesting that free blood transfusions increase the risk of these adverse consequences. To validate this report, we added 2 other RCTs to our analysis. However, in the restricted transfusion threshold group, we did not find a significant reduction in the incidence of pneumonia or wound infection.

However, 39% of patients in the restricted transfusion threshold group reduced the number of RBC transfusions during the postoperative period, further reducing transfusion-related complications, and accelerating postoperative recovery and avoiding excessive medical costs. This study showed that there were no significant differences in mortality, pneumonia, wound infections, myocardial infarction, and congestive heart failure rates between the restricted and free transfusion thresholds for RBC transfusion. And reduce the economic burden of using free blood transfusion strategies without increasing the risk of adverse events. Restricted blood transfusion in our hospital accounts for two thirds of the total hospital transfusion, and one third of free blood transfusions are mainly in general wards. The main reason is that after the blood transfusion treatment, patients can be discharged Its hemoglobin has reached a normal value. However, with the normal blood-generating mechanism, patients can avoid re-blood transfusion. This aspect is worth pondering again.

doi: 10.1016/j.cca.2022.04.678

M078

A retrospective epidemiological audit of crab criteria in patients with multiple myeloma at Tygerberg Hospital, South Africa

T. Jalavu [b], K. Ichihara [a], F. Bassa [c], Z. Chapanduka [d], R. Erasmus [b], A. Zemlin [b]

[a] *Department of Clinical Laboratory Sciences, Yamaguchi University, Graduate School of Medicine, Minami-Kogushi, Japan*
[b] *Division of Chemical Pathology, Faculty of Health Sciences, Stellenbosch University and National Health Laboratory Service, Cape Town, South Africa*
[c] *Division of Clinical Haematology, Department of Medicine, Tygerberg Academic Hospital/Stellenbosch University, Cape Town, South Africa*
[d] *Division of Haematopathology, Faculty of Health Sciences, Stellenbosch University, and National Health Laboratory Service, Cape Town, South Africa*

Background-aim

Multiple myeloma (MM) is defined by the International Myeloma Working Group as a cytogenetically heterogeneous clonal plasma cell proliferative disorder usually preceded by an asymptomatic pre-malignant stage called monoclonal gammopathy of undetermined significance (MGUS). Common presenting symptoms and myeloma defining criteria include hypercalcaemia, renal dysfunction, anaemia, and bone lesions (CRAB). MM accounts for about 1% of all cancers and up to 10% of all haematological cancers globally. The global prevalence of MGUS is estimated at 3 to 4% in those above the age of 50 years. The national cancer registry in 2012 reported that MM contributes 0.44% of all cancers in South Africa. This study aimed to investigate the prevalence, common presenting patterns and biochemical findings (in line with CRAB criteria) in MM patients at Tygerberg Hospital.

Methods

Patient files were reviewed for all new and known MM patients seen in the last five years at the haematology clinic. Clinical, demographic and laboratory information was captured for each and a subset is presented here.

Results

A total of 106 cases are presented. The majority were male at 58% (n = 61) and 42% females (n = 45). The mean age was 60 years old with a range of 27 to 87 years. Fifteen percent were 45 years and below (n = 16) and 8% were HIV positive (n = 8) (39 - 60 years old). The mean calcium was 2.5 ± 0.08 mmol/L, total protein was 94 ± 4 g/L and haemoglobin 10.02 ± 1.05 g/dL. The mean creatinine was 187 ± 50 μmol/l and 51% had eGFR below 60 ml/min (n = 54). Fifty-two percent (n = 55) had documented pathological fractures. The mean M-protein size was 27 ± 5 g/L with the largest peak measuring 102 g/L. Only 47% (n = 50) of cases had documented sFLC levels due to limited availability at the time. The main diagnoses were MM with CRAB criteria (78%) followed by MGUS (16%) and Waldenström macroglobulinaemia (3%).

Conclusions

This study describes the common presenting features of MM in a South African tertiary institution. Interestingly, younger patients were affected. All CRAB criteria did not always occur in the same patient therefore clinicians need to have a high index of suspicion for MM when any of the CRAB criteria are present even in younger patients.

doi: 10.1016/j.cca.2022.04.679

Clinica Chimica Acta 530 (2022) S153–S160

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Gastrointestinal Disease

T197

Diagnostic accuracy of forns score for liver cirrhosis in subjects with chronic viral hepatitis

T. Bukhari, L. Jafri, A. Khan, S. Abid, A. Raza, A. Raheem, I. Siddiqui

Aga Khan University Hospital, Karachi, Pakistan

Background-aim

Liver cirrhosis is an irreversible and end stage disease. It results due to chronic liver damage characterized by replacement of normal liver tissue by fibrosis, leading to progressive loss of liver function. Making an early diagnosis of cirrhosis is important for the patients with chronic hepatitis because early antiviral therapy can prevent the progression of cirrhosis and even induce regression. There has been interest to develop surrogate markers for liver Cirrhosis as biopsy is invasive, costly and difficult to standardize. The aim of the study was to assess the diagnostic accuracy of non-invasive index i.e. Forns score for liver cirrhosis in patients of chronic viral hepatitis in terms of sensitivity, specificity and accuracy by doing comparison with Fibroscan.

Methods

It was a cross sectional study conducted at Section of Chemical Pathology, Department of Pathology and Laboratory Medicine in Collaboration with Section of Gastroenterology, Dept. of Medicine, AKU, from Jan to Dec 2018. Total 90 patients (> 18 years of age) with history of chronic viral hepatitis, coming to the Fibroscan clinic were included. Patients with history of autoimmune liver disease and liver carcinoma were excluded. Blood samples withdrawn were analyzed on ADVIA Centaur, Forns score was calculated by taking four parameters: patient's age, total cholesterol, GGT and Platelet count.

Results

Median age of the patients was 38.5 with IQR (21). In study population 59(65.6%) were men & 31(34.4%) women. 26 patients showed reactivity for HBsAg, 63 patients were found chronic with HCV. Proportion of HCV was observed higher as compared with that of HBV. Nineteen patients found with jaundice and only one patient had Ascites.

An area under the Receiver Operating Curve (ROC) curve [AUROC] was generated to determine the diagnostic accuracy of forn's score. It was observed that the form index value > 7.110 has AUROC 0.9928 (95% C.I. 0.9821 to 1.003, p-value < 0.001*) with a sensitivity 100% (95%C.I., 91.19% to 100.0%) and specificity 94 (95%C.I., 83.45% to 98.75%) with higher positive likelihood ratio 16.67.

Conclusions

This study found a correlation between the fibroscan and Forns index in diagnosing liver cirrhosis patients. Forns score is a very sensitive as well as specific noninvasive test that can be used to know the status of fibrosis in chronic hepatitis patients.

doi: 10.1016/j.cca.2022.04.681

T198

Syndrome hemolitic uremic associted to Shiga toxin E-coli

K.V. Falcones Gracia, L. Julia, E. Ricart Alvarez, R. Molina Gasset

Hospital Virgen de los Lirios, Spain

Background-aim

The Shiga toxin E-coli (STEC) infections can manifest within a broad clinical spectrum, such as intestinal asymptomatic infections, watery diarrhea, bloody diarrhea (hemorrhagic colitis) and systemic complications known as Syndrome hemolytic uremic (SUH).

The SHU is a clinical entity, characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure that can progress to chronic renal failure. It is postulated that endothelial damage is a direct consequence of the action of Shiga toxin (Stx) that, released by bacteria, crosses the intestinal barrier and gains access to blood circulation.

Below, we present a case of the SHU chart associated with Stx.

Methods

Man of 16[a] of British origin, on vacation in our province; with progressive abdominal pain diffuse colic-type, this begins 7 days before admission with increasing liquid diarrhea, 48 hours after its onset, have with live red bloody diarrhea going to the emergency service.

On physical examination, he presented regular general conditions. Apyretic, stable constants. AR: no alterations. AC: rhythmic, no blows. ABD: soft, depressible, increased noise. No masses.

In analytics it presents Urea 83 mg / dL, PCR 5.7 mg / dL, BT 3.03 mg / dL, LDH 1249 U / L, Hb 11.8 g / dL and Platelets 20 thousands micro / L.

Haptoglobin 0 mg / dL and direct negative Coombs. Urine: sediment 70 red blood cells / field, Proteinuria 987 mg / day. In peripheral blood smears, 3-4% of schistocytes (3-4 per 100x field) are observed in addition to the presence of red blood cells in the helmet. Determination of ADAMS13, Stx and complement levels (C3, C4, CH50) are requested. Due to the presence of thrombocytopenia, we started treatment with plasmapheresis with fresh plasma is expected pending aetiological results.

Results

At 24 hours after admission and after receiving confirmation of Stx (with normal AMDAS13 of 87%), a diagnosis of SHU by Stx is established, plasmapheresis is suspended and treatment with antibiotics and diuretics is initiated.

The only renal repercussion has been hematuria and proteinuria less than 1 gram/day. During admission, hematologic evolution has improved. A total of 2 pool of platelets have been transfused. He has not required transfusion of red blood cell concentrate.

After five days of treatment, the patient shows clear improvement, his analytical reports Hemoglobin (10.1 g / dl). Platelets 147000. Normal leukocytes. In urine Protein / cr ratio: 128 mg / g. has normal renal function (Cr 0.5 mg / dl), normal ions.

Conclusions

The SHU associated with Stx can occur with acute gastroenteritis, which is an intestinal inflammation caused by an infectious agent or its toxins. It is more frequent and serious in children. The etiology, more common, is bacteria in adults and viruses in children. Concerning the clinic, this will vary if the gastroenteritis is caused by toxins or by enteroinvasive agents.

When a patient requires admission, as in our case, blood culture, coproculture, fresh stool examination, etc. will be performed. About treatment, the diet is essential, and it is proposed to administer antidiarrheal microorganisms to recover normal intestinal flora, as well as hydro electrolytic replacement.

doi: 10.1016/j.cca.2022.04.682

T199

Investigation of serum glutathione peroxidase in patients with liver cirrhosis

D. Terziev [d], D. Terzieva [c], V. Andonov [d], N. Mateva [b], Y. Ronchev [a]

[a] *Clinical Laboratory, University Hospital "Kaspela", Faculty of Medicine, Medical University-Plovdiv, Bulgaria*
[b] *Department of Biostatistics and Medical Informatics, Faculty of Public Health, Medical University, Plovdiv, Bulgaria*
[c] *Department of Clinical Laboratory, Faculty of Pharmacology, Medical University, Plovdiv, Bulgaria*
[d] *Second Department of Internal Medicine, Gastroenterology section, Faculty of Medicine, Medical University, Plovdiv, Bulgaria*

Background-aim

An excessive amount of reactive oxygen species (ROS) is highly toxic to cells. ROS can cause oxidative damage to proteins, lipids, and DNA. Both enzymatic (catalase, superoxide dismutase, glutathione-peroxidases) and non-enzymatic antioxidant system are essential to protect cells from oxidative damage. Glutathione peroxidase (GPx) catalizes the reduction of hydrogen peroxide and is among the protective mechanisms against free-radicals mediated damage in the liver cells. The aim of our study was to investigate serum GPx-1 concentration in patients with diagnosed liver cirrhosis and to compare it with control group consisted of healthy subjects.

Methods

A total of 58 subjects were divided in two groups consisted of 29 patients with liver cirrhosis (45-83 years) and 29 healthy controls (20-60 years). Serum glucose, total bilirubin, total protein and albumin concentrations, transaminase, alkaline phosphatase and gama-glutamil transferase activity (Mindray BS 200e, China) and platelet count (Medonic, Stockholm, Sweeden) were examined in all participants. Serum concentrations of GPx-1 (Human Glutathione Peroxidase 1 ELISA, BioVendor, Czech Republic) were measured with a Sirio S microplate reader, SEAC, Italy. Comparison of quantitative variables between groups was performed by independent samples T-test or Mann-Whitney U test. Data were expressed as mean value (mean) and standard deviation (SD) or standart error (SE). P value < 0.05 was considered significant.

Results

The mean age ± SD of patients with liver cirrhosis was significantly higher than this of control group (64.45 ± 1.98 years vs 35.62 ± 2.42 years, $P < 0.0001$). In the group with liver cirrhosis men were 79.3 % ($n = 23$), and in healthy controls women were 72.4 % ($n = 21$). Mean serum concentration ± SE of GPx was significantly increased in patients with liver cirrhosis compared to healthy controls (3.01 ± 1.24 ng/ml vs 1.38 ± 0.43 ng/ml, $P = 0.008$).

Conclusions

The results of our study show elevated serum concentration of GPx in patients with liver cirrhosis and suggest, that oxidative stress may be an important factor in pathogenesis of liver cirrhosis.

doi: 10.1016/j.cca.2022.04.683

T200

Evaluation of superoxide dismutase in serum in patients with liver cirrhosis

D. Terziev [d], D. Terzieva [c], V. Andonov [d], N. Mateva [b], Y. Ronchev [a]

[a] *Clinical Laboratory, University Hospital "Kaspela", Faculty of Medicine, Medical University-Plovdiv, Bulgaria*
[b] *Department of Biostatistics and Medical Informatics, Faculty of Public Health, Medical University, Plovdiv, Bulgaria*
[c] *Department of Clinical Laboratory, Faculty of Pharmacology, Medical University, Plovdiv, Bulgaria*
[d] *Second Department of Internal Medicine, Gastroenterology section, Faculty of Medicine, Medical University, Plovdiv, Bulgaria*

Background-aim

Oxidative stress is a result of an imbalance between oxidants and antioxidants in the cells that leads to the generation of highly toxic reactive oxygen species (ROS). Antioxidant protection system involves endogenous antioxidants, enzymes, dietary antioxidants, metal binding proteins. One of the antioxidant enzymes of the blood is superoxide dismutase (SOD). Liver is the major organ attaked by ROS. Various risk factors such as drugs, heavy metals, alcohol, radiation, virus, insulin resistance, may induce oxidative stress in liver. The aim of this study

is to analyze the serum concentration of SOD in patients with liver cirrhosis and compare it with this in healthy individuals.

Methods

Our study comprised 29 patients with liver cirrhosis (23 men and 6 women) and 29 healthy subjects (8 men and 21 women). The age of patients with liver cirrhosis ranged between 45 and 83 years, and of controls – between 20 and 61 years. We determined SOD concentrations in serum by the ELISA method (Human Cu/ZnSOD Platinum ELISA, Bender MedSystems, Austria) using the filter photometer Sirio S microplate reader (SEAC, Italy). Statistical analysis was performed using independent samples t-test and the Mann-Whitney U test. $P < 0.05$ was considered to be statistically significant. Data are presented as mean ± standard error and percent.

Results

Patients with liver cirrhosis were significantly older than controls ($P < 0.0001$). Ratios between males and females were in liver cirrhosis patients 79.3%: 20.7% and in controls – 27.6%: 72.4%. In patients with liver cirrhosis mean serum level of SOD was 24.55 ± 1.20 ng/ml. In control group mean SOD concentration was 128.86 ± 22.84 ng/ml. The mean difference of serum SOD between two groups was statistically significant (104.31 ± 22.87 ng/ml, $P < 0.0001$).

Conclusions

The results of this study showed that compared to controls, SOD levels were significantly lower in cirrhosis patients. This results indicate a decreased antioxidant protection in patients with liver cirrhosis.

doi: 10.1016/j.cca.2022.04.684

T201

The liver enzymes in patients with cirrhosis and hepatocellular carcinoma (HCC)

N. Serdarevic [b], M. Frankovic [a]

[a] *Faculty of Health studies, University of Sarajevo, Bosnia and Herzegovina*
[b] *Institute for Clinical Biochemistry and Immunology, Clinics Center University of Sarajevo, Sarajevo, Faculty of Health studies, University of Sarajevo, Bosnia and Herzegovina*

Background-aim

In this study, we investigated alanine aminotransferase (ALT), aspartate aminotransferase (AST), ©-glutamyltranspeptadase (GGT), alkaline phosphatase (AKP), total bilirubin (TBIL) and direct bilirubin (DBIL) in patients with cirrhosis and hepatocellular carcinoma (HCC).

Methods

The study included 120 patients, over 50 years of age, 40 patients with liver cirrhosis, 40 patients with HCC, and 40 patients in the control group. The concentrations of AST, ALT, GGT, AKP were determined on the biochemical analyzer Architect i2000sr, (Abbott) and analyzer Dimension (Siemens).

Results

The study showed that the mean values of the examined parameters were significantly higher in subjects with diagnosed liver cirrhosis and HCC compared to the control group. Examining the difference between ALT, AST, GGT, AKP and bilirubin between the groups, we came to the conclusion that there is a statistically significant difference between the examined parameters ($p < 0.05$) in our group of respondents. The incidence rates were higher in the HCC group for all four enzymes. The most significant finding was for GGT, with the highest incidence rate of 15.7% in the elevated HCC group and cirrhosis 4.1 % compared to the control group ($P < 0.001$). The ALT was not statistically significant for liver cirrhosis, and AST was statistically significant for liver cirrhosis compared control group. The AKP was higher (135.35 (94.9–231.35) U/L in HCC group vs 82.15 (68.1–114.2) U/L compared with liver cirrhosis, $P < 0.001$. It was a certain degree of correlation was established between these five parameters, where all measured parameters were in a statistically significant correlation.

Conclusions

The results confirmed that there is the elevated activity of ALT, AST, GGT, ALP and bilirubin in subjects with liver cirrhosis and HCC compared to the control group of subjects. The serum enzymes evaluation observed to indicate the degree of hepatic damage.

doi: 10.1016/j.cca.2022.04.685

T202

Evaluation and characterization of hospitalized patients with non-alcoholic fatty liver diseases

M.T. Albogami [a], B.Z. Alotaibi [a], M.T. Alotaibi [a], R.T. Alotaibi [a], M.A. Alahmed [a], W. Tamimi [b]

[a] *College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia*
[b] *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical City, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*

Background-aim

Nonalcoholic fatty liver disease (NAFLD), defined as fat accumulation in hepatocytes, may progress to fibrosis or cirrhosis later in life. NAFLD prevalence has increased significantly in direct relation with obesity, life style, liver diseases and metabolic syndrome prevalence. Because NAFLD is underdiagnosed in many hospital populations, therefore, it is important to evaluate and increase the public awareness of this unnoticed condition in order to take more preventive measures and actions. The aim of this study was to evaluate and characterize inpatients who admitted to our hospital during the last 8 years in order to assess the possibility of risk association of developing NAFLD in hospital population.

Methods

A clinical, biochemical, histological and image data for 309 patients with NAFLD were collected from the hospital information system (HIS) Bestcare, ezCaretech company (Seoul, Korea) between year 2010 and 2018. A total of 49 records were initially evaluated. There were lower

number of liver biopsies and therefore, were not included in this initial evaluation. Liver enzymes were performed on general clinical chemistry analyzer Architect C16000 (Abbott, USA). The diagnostic sensitivity and specificity were calculated.

Results

Among the 49 records confirmed with NAFLD, only 39 had performed ultrasound image with 35 positive and 4 negative for fatty liver infiltration with a 90% sensitivity. The obesity, elevated liver enzymes (ELE), diabetes mellitus (DM), hypertension (HTN), heart failure (HF), hypothyroidism (HTY), and dyslipidemia have shown lower sensitivity as 59%, 59%, 47%, 49%, 10%, 23%, and 29% respectively. When obesity, elevated liver enzymes (ELE), diabetes mellitus (DM), hypertension (HTN), heart failure (HF), hypothyroidism (HTY), and dyslipidemia were compared to ultrasound the sensitivity values were found as 66%, 60%, 43%, 49%, 14%, 25%, and 33% respectively. In addition, the specificity were found to be 75% for all with exception of obesity 50% and HF 100%. When obesity and ELE were combined the sensitivity and specificity were improved to 77% and 67% respectively.

Conclusions

The ultrasound has shown to be a good diagnostic tool in NAFLD patients. Obesity and elevated liver enzymes can be combined with ultrasound to improve the diagnostic criteria. Further evaluation is in process to establish a solid conclusion.

doi: 10.1016/j.cca.2022.04.686

T203

Gene expression of lipid metabolizing proteins in antitubercular drug induced liver injury in relation to steatosis

M.K. Chidananada [a], A.N. Aggarwal [d], S.K. Sinha [b], A. Duseja [c], S. Sharma [a]

[a] *Department of Biochemistry, Post Graduate Institute of Medical Education and Research, Chandigarh, India*
[b] *Department of Gastroenterology, Post Graduate Institute of Medical Education and Research, Chandigarh, India*
[c] *Department of Hepatology, Post Graduate Institute of Medical Education and Research, Chandigarh, India*
[d] *Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India*

Background-aim

Hepatocellular liver injury is the most important complication of antitubercular treatment which can be either necrosis or steatosis. Lipid metabolizing proteins namely Perilipin (PLIN2) is involved in lipid droplet formation and triglyceride accumulation, Sterol Regulatory Element Binding Proteins 1c (SREBP1c) is associated with activation of fatty acid synthesis enzymes such as fatty acid synthase, etc. and peroxisome proliferator-activator receptor alpha (PPAR⟨) is associated with activation of fatty acid degradation enzymes such as Carnitine palmitoyl tranferase 1, etc. Gene expression of these proteins may be helpful to study the alterations in lipid metabolism and accumulation which can further lead to steatosis associated with antitubercular therapy (ATT).

Methods

Different biomarkers of lipid metabolism and liver injury along with expression of gene involved in lipid metabolism namely PLIN2, SREBP1c and PPAR⟨ were studied in healthy subjects, naïve untreated tuberculosis patients, tuberculosis patients without ATT hepatotoxicity (non-toxic) and ATT induced liver injury (toxic) patients.

Results

In ATT induced liver injury patients, total cholesterol, HDL, APOA-1 and triglyceride were decreased in 41.6%,41.6% , 37.5% and 8.2% of patients respectively in addition to increase in the levels of conventional liver function parameters like AST, ALT, ALP, total bilirubin, direct bilirubin, GGT and decrease in the levels of haptoglobulin, total proteins, albumin indicating alterations in lipid metabolism in patients on ATT. PLIN2 expression in toxic patients was increased with a fold change of 4.66, 4.09 and 3.21, when compared to healthy, untreated and nontoxic groups. SREBP1c expression in toxic group was increased with a fold change of 2.60, 2.61 and 2.94 as compared to healthy, untreated and non-toxic groups, however; PPAR⟨ expression in toxic group was not altered when compared to healthy, untreated and toxic subjects.

Conclusions

Our observations show alterations in expression of PLIN2 and SREBP1c genes in toxic patients as compared to healthy, untreated tuberculosis and non-toxic groups. Therefore, alterations in the gene expression of these lipid metabolizing proteins can result in lipid accumulation which can further lead to the development of steatosis in antitubercular drug induced liver injury.

doi: 10.1016/j.cca.2022.04.687

T204

Inflammation and mucosal integrity process of esophageal epithelium in recovery after laparoscopic anti-reflux surgery in patients with gastro-esophageal reflux disease

P. Ergun [b], S. Kipcak [c], S. Bor [a], E. Sozmen [b]

[a] *Department of Gastroenterology, Faculty of Medicine, Ege University, Izmir, Turkey*
[b] *Department of Medical Biochemistry, Faculty of Medicine, Ege University, Izmir, Turkey*
[c] *Department of Medical Biology, Faculty of Medicine, Ege University, Izmir, Turkey*

Background-aim

The symptoms of gastroesophageal reflux disease (GERD) recur six months after proton pump inhibitors medication is discontinued in 75-80% of patients with GERD. These patients may require lifelong continue PPI treatment. Some patients may need to laparoscopic anti-reflux surgery (LARS). Symptoms of the disease rapidly disappear following the surgery.

We evaluated the inflammatory process of esophageal mucosa of the cases before and after LARS and compare with healthy controls (HC) at the cellular and tissue level in order to investigate the underlying pathophysiologic mechanism of GERD.

Methods

Esophageal biopsies from 22 patients with GERD pre- and post- LARS (13 men; 43,7 ± 12,6 years) and 23 healthy controls (7 men; 41,9 ± 10,8 years) were taken during upper-GI endoscopy. The post-operative patients (post-op) were included to the study at least 3-4 months after LARS. The biopsies were analysed in Multiplex immunoassay system to scan 40 chemokines and studied in ussing chambers to measure transepithelial resistance (TEER) and tissue permeability. Dilated intercellular spaces (DIS) of cases were examined histopathologically under light microscopy to evaluate epithelial damage.

Results

TEER results of post-op were significantly higher than pre-operative patients (pre-op) ($p = 0.0002$) and healthy controls (HC) ($p = 0.038$). Mucosal permeability of post-op was significantly decreased versus pre-op ($p = 0.007$) and HC ($p = 0.047$). The levels of C-C Motif Chemokine Ligand (CCL-) 1, 3, 4, 7, 8, 17, 21, 24, 26, 27, interleukin (IL-) 1b, 10, C-X-C motif Chemokine Ligand (CXCL)-2,-6,-8 Interferon gamma (IFNG) and macrophage migration inhibitory factor (MIF) of post-op were increased among pre-op at the very least $p < 0.05$ significance. IL-8 and TARC levels of pre-op was significantly higher than HC. DIS score of HC was significantly lower ($p < 0.0001$) to pre- and post-op whereas no significance was found between post-op and pre-op on DIS score.

Conclusions

The TEER and permeability results imply that LARS has made an efficient recovery within esophageal epithelium in patients with GERD at the tissue level. Higher levels of anti-inflammatory chemokines (IFNG, IL-10 and CCL-7) and tissue healing markers (CCL-17, -22, IL-4, -10) may indicate the role of macrophages and neutrophils during recovery phase in post-op.

doi: 10.1016/j.cca.2022.04.688

T205

Clinical study of Aptiva anti-transglutaminase IgA reagent on patients with biopsy results

G. Previtali [a], M.G. Alessio [a], A. Seaman [b], E. Wahl [b], J. Mangat [b], J. Nibecker [b], O. Springfield [b], C. Bentow [b], M. Mahler [b]

[a] *Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy*
[b] *Research and Development, Inova Diagnostics, San Diego, CA, USA*

Background-aim

Antibodies to tissue transglutaminase (tTG) are important factors in diagnosis of celiac disease (CD). Increased anti-tTG IgA titers can be especially important as suggested by the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), where a titer 10 times the upper limit of normal (>10xULN) may consider foregoing invasive intestinal biopsy in diagnosis of CD. Recently, novel assays have been developed which allow for the detection of CD antibodies on a new platform, Aptiva (Inova Diagnostics, San Diego, CA, USA). This study aimed to compare the performance of the novel assays with the results of patients who underwent biopsy.

Methods

130 patients with suspicion of CD that underwent biopsy to confirm diagnosis were tested on Aptiva Celiac Disease IgA containing DGP and tTG which utilizes a novel particle-based multi-analyte technology (PMAT, research use only, Inova Diagnostics, USA) and QUANTA Flash (QF) tTG IgA reagent. Additionally, sixteen pediatric samples that are potential candidates fulfilling criteria of children to avoid biopsy were also tested on the Aptiva Celiac IgA reagent as well as the QF reagent.

Results

59 of the 130 patients with suspicion of CD were confirmed after the biopsy. The Aptiva Celiac Disease tTG IgA showed a sensitivity of 94.9% (IC 95%: 86.1-98.3%) and a specificity of 93.0% (IC 95%: 84.6-97.0%). Comparison with QF tTG IgA showed a negative percent agreement of 100% (IC 95% 94.5-100.0), a positive percent agreement of 92.2% (IC 95% 83.0-96.6) and a total percent agreement of 96.2% (IC 95% 91.3-98.3). Of the 16 pediatric patients, all were positive for tTG IgA with results >10xULN.

Conclusions

Anti-tTG antibodies measured using the novel fully automated PMAT assay showed excellent clinical performance in this CD cohort and high correlation to biopsy results and good correlation to the QF reagents. The tTG IgA results >10xULN in this pediatric study confirms the use of serological testing as part of the criteria for pediatric patients is an alternative to invasive biopsy.

doi: 10.1016/j.cca.2022.04.689

T206

Endorphin and 2-arachidonoylglycerol in the course of IBD in the youth

A. Martyniak, K. Sztefko, P. Tomasik

Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland

Background-aim

Inflammatory bowel disease (IBD) is severe condition, recently affected an increasing number of children and teenagers. Abdominal pain and weight loss associated with diarrhea and diminished appetite are typical in IBD. Opioids are frequently used in the IBD treatment and recently few papers described also positive effect of medical cannabis during IBD recurrence. Their effect is based on antinociceptive and enhancing appetite action. However little is known about secretion of endogenous opioids (endoopioids) and endogenous cannabinoids (endocannabinoids) in the course of IBD. Therefore the aim of this study was to assess endorphin (member of endoopioid family) and 2-Arachidonoylglycerol (2-AG; member of endocannabinoid family) concentration in children suffered from IBD.

Methods

We have studied 38 children with IBD, mean age 12.8 ys ± 2.9. The blood was collected three times – in active phase of the disease (during admission to the hospital, before treatment), 2-4 weeks later

during consolidation of medical treatment and month to 6 months later during remission. As a control group served a group of 34 age-matched healthy children. In all cases fasting samples were taken in the morning. The endorphin and 2AG were measured in the serum using EIA kits (Fine Test; Wuhan, China and Abclonal Technology; Wuhan, China).

Results

In the study group before treatment mean concentration of endorphin was 562.3 pg/ml ± 243.4. During treatment it diminish to 453.6 pg/ml ± 217.1, and in remission concentration rose to 477.0 pg/ml ± 263.2. All these values were significantly lower as compare to values observed in control group (745.1 pg/ml ± 285.8) ($p < 0.01$, $p < 0.001$, $p < 0.001$ respectively).

2-AG mean concentration in study group was stable (acute phase 305.9 ng/ml ± 244.5, during treatment 296.1 ng/ml ± 240.9; remission 310.9 ng/ml ± 211.6) and similar to the values observed in control group (321.7 ng/ml ± 233.7).

Conclusions

We concluded that endoopioids system do not act against pain as well diminished appetite in IBD children. The lower concentrations of endorphin in these children than in controls suggest some factors negatively affecting endoopioids concentration in the course of IBD in children. Endocannabinoids are not disturbed in children with IBD.

doi: 10.1016/j.cca.2022.04.690

T207

Anti transglutaminase antibodies could predict the development of celiac disease 5 years before the diagnosis

G. Previtali, M. Seghezzi, V. Moioli, E. Cimpoies, R. Ravasio, G. Guerra, M.G. Alessio

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-aim

Celiac disease (CD) is an autoimmune disease characterized by duodenal damages after gluten exposure in genetically predisposed subjects. Guidelines suggest to perform anti-transglutaminase (tTG) and anti-endomysium (EMA) for CD diagnosis. Many clinical sign and symptoms, not only gastrointestinal, are associated to CD and gluten free diet is the only available therapy. The aim of this study is to evaluate the predictive value for CD development of tTG on a chemiluminescence platform (BioFlash®, Inova Diagnostics).

Methods

10614 patients were tested for tTG between June 2012 and December 2013 at the clinical chemistry laboratory of Papa Giovanni Hospital (Bergamo, Italy). 515 subjects were tTG positive and we follow them up for 5 years.

Results

203 patients were excluded: 190 patients were already CD diagnosed and 13 were lost at follow up. 270/312 (86.5%) patients were diagnosed after the first tTG test or within 5 years of follow up, 42/312 (13.5%) subjects do not receive a confirmed CD diagnosis (CD was excluded for 22 subjects, 20 patients were classified as potential CD). The median tTG values between the two groups were statistically different (342.7 CU vs 35.23 CU; $p < 0.0001$). ROC analysis showed a 95.4% probability to receive a CD diagnosis within 5 years for tTG values over 68.8 CU, it increases to 99.4% for tTG values over 170.5 CU.

Conclusions

A tTG result above 170.5 CU could predict with a very high probability the development of CD within 5 years independently from the pre-test probability.

doi: 10.1016/j.cca.2022.04.691

T208

Thrombin generation in patients with inflammatory bowel disease

L. Lóczi[b], M. Papp[c], N. Sipeki[c], I. Beke Debreceni[a], J. Kappelmayer[a], Z. Bagoly[b]

[a] *Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[b] *Division of Clinical Laboratory Sciences, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[c] *Division of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Background-aim

Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC), both predominantly affecting the gastrointestinal tract. Clinically significant thrombosis is frequent complication in IBD patients. Interestingly, accumulating evidence suggests that microvascular thrombosis and hypercoagulability may also play a significant role in the pathogenesis of the disease. Here we aimed to find out whether thrombin generation is increased in IBD patients as compared to healthy controls, and whether TGA parameters are associated with disease activity in patients.

Methods

In this observational study, 37 patients with IBD (CD/UC: 26/11; male/female: 22/15; age: 34.5 ± 12.8) and 40 age- and sex-matched healthy controls were enrolled. The measurements of whole blood count, fibrinogen and basic clinical chemistry tests including CRP were carried out immediately from blood samples. TGA was performed on stored platelet poor plasma. Lag time, endogen thrombin potential (ETP), peak thrombin, time-to-peak, and velocity index were calculated. Clinical parameters including BMI, thrombosis risk factors, thrombotic history and disease activity (partial Mayo score, Crohn's disease activity index) were registered. In order to correlate results with disease activity, patients were followed and blood samples were taken in both active and quiescent stages of the disease.

Results

As compared to healthy controls, in IBD patients lag time and time-to-peak parameters were significantly prolonged ($p < 0.0001$). ETP was significantly higher in patients vs. controls (1892 ± 64 vs. 1448 ± 42 nMxmin, $p < 0.0001$). Peak thrombin was also significantly increased

in patients as compared to controls (386.7 ± 16.5 vs. 311.1 ± 12.9 nM, $p < 0.0005$). TGT parameters did not differ between CD and UC patients. CRP showed significant positive correlation with all TGT parameters except for time-to-peak, while BMI showed significant positive correlation with peak thrombin in patients and in controls. When patients were followed, ETP and peak thrombin were found to be elevated, while lag time and time-to-peak were shortened in association with the rise in disease activity.

Conclusions

In patients with IBD, the extent of thrombin generation was significantly higher as compared to controls, which might be associated with increased thrombotic risk. Changes in disease activity resulted in parallel changes of thrombin generation, indicating that hypercoagulability might be a feature of the active disease.

Funding: NKFI 128582.

doi: 10.1016/j.cca.2022.04.692

T209

Serum calprotectin as a novel biomarker in inflammatory bowel diseases

A. Gallego, M. Jiménez, J.M. Maesa, C. González, A. León

Clinical Biochemistry, Virgen Macarena Hospital, Sevilla, Spain

Background-aim

Inflammatory Bowel Disease (IBD) is a clinical entity of multifactorial aetiology and not fully understood today. Mostly, it includes two pathologies: Crohn's Disease (CD) and Ulcerative Colitis (UC). Both consist of chronic inflammatory processes at the digestive level, of more or less limited extension and different characteristics according to the pathology.

IBD limits the quality of life of people who suffer from it and its worldwide prevalence has been increasing in recent years.

Likewise, there are markers that could help both an early diagnosis of inflammatory digestive pathologies and an adequate follow-up of these diseases and their treatment.

The aim of this study is to demonstrate the usefulness of various markers of inflammation in the blood for the early detection and proper follow-up of patients with IBD, and thus be able to avoid or delay the use of invasive and expensive tests such as colonoscopy.

Methods

Thirty two sera samples of different patients with suspected inflammatory bowel disease (IBD) were analysed to determine sCAL, C-reactive protein (CRP), Alpha-1 acid glycoprotein (⟨-1AG) and Serum Amyloid A protein (SAA). Also, fecal Calprotectin (fCAL) was simultaneously determined in stool samples of same patients. Spearmen's correlation test (⟩) was used to establish the correlation between the variables and p-values calculated.

Results

Correlation between sCAL and fCAL: ⟩= 0.35 (p-value = 0.086); CRP: ⟩= 0.4 (p-value = 0.02); ⟨-1AG: ⟩= 0.54 (p-value = 0.001); SAA: ⟩= 0.3 (p-value = 0.1). Correlation between fCAL and CRP: ⟩= 0.36 (p-value = 0.06); ⟨-1AG: ⟩= 0.5 (p-value = 0.008); SAA: ⟩= 0.53 (p-value = 0.005).

Conclusions

Since all the analysed variables are acute phase reactants, the association between them are positive -different level correlations. The correlation between sCAL and fCAL is weak (⟩ = 0.35), as well as with CRP and SAA. Only the correlation between sCAL and ⟨-1AG is moderate (⟩ = 0.54, p = 0.001). The fCAL has a moderate correlation with ⟨-1AG and SAA. All the parameters calculated are statistically significant. Considering a moderate significant correlation between serum and fecal calprotectin with serum ⟨-1AG but not with CRP , serum calprotectin could be representative of subacute inflammation intestinal. We need more studies to find out the possible application of serum calprotectin as a blood-based biomarker in IBD.

doi: 10.1016/j.cca.2022.04.693

T210

Drug induced acute pancreatitis

T. Rolić, I. Lukić, S. Mandić, V. Horvat, M. Lukić, V. Šerić

Institute of Clinical Laboratory Diagnostics, Osijek University Hospital, Josipa Huttlera 4, HR-31000 Osijek, Croatia

Background-aim

Acute pancreatitis is a common reason for hospitalization and the incidence continues to rise. Establishing the etiology in order to prevent recurrence is important. There are over 100 drugs reported to cause acute pancreatitis in the literature.

Methods

Hereby is presented a case of 71-year-old women with drug induced acute pancreatitis. Her diagnosis was confirmed 2 years ago as PV with confirmed JAK2 mutation. As a stem cell disorder with elevated absolute red blood cell count recommended therapy is phlebotomy to remove excess cellular elements by lowering blood viscosity. Limited mobility of the patient lead hematologists to prescribe an hydroxycarbamidum (Litalir).

After two weeks of taking the drug she was admitted to the Emergency Department due to abdominal pain and vomiting. The onset of symptoms has been related with the time shorty after starting the therapy. Laboratory findings showed elevated count of erythrocytes ($5.71x10^{12}$/L), hemoglobin (163 g/L) and hematocrit (0.508 L/L) according clinical finding of PV, and highly elevated alfa amylase (895 U/L) and lipase (2748 U/L). Enzymes were measured by Beckman Coulter AU 480 analyzer (Beckman Coulter, Inc., Brea, USA), by spectrophotometric methods and the red blood count is measured by Sysmex XN 2000 (Sysmex, Inc., Lincolnshire, Illinois, USA) analyzer.

Results

These findings raised suspicion of acute pancreatitis caused by drug therapy. After therapy discontinuation there were no symptoms of acute pancreatitis present.

Although only a minority of cases associated with acute pancreatitis are linked to drugs in recent years many medications have been linked to drug-induced pancreatitis and there are several scientific papers

mainly describing pancreatitis through case reports. Reviews claim that drug induced acute pancreatitis is accountable for 3-5% of all cases of acute pancreatitis. The exact cause of pancreas injury is not well understood, and future investigation will help establish earlier diagnosis.

Conclusions

It is important to follow up cases and to report side effects since acute pancreatitis is a very common, but yet not described side effect of hydroxycarbamide.

doi: 10.1016/j.cca.2022.04.694

T211

Characterization of rotavirus among children under five years with gastroenteritis at Kenyatta National Hospital, Kenya

J.O. Zablon [b], N. Shavia [c], R. Lihana [a], B. Mwinyi [b]

[a] *Kenya Medical Research Institute, Kenya*
[b] *Kenyatta National Hospital, Kenya*
[c] *Masinde Muliro University of Science and Technology, Kenya*

Background-aim

Severe diarrhea is a common occurrence among children below the age of five years worldwide, and the major cause remains infection from rotavirus. The mortality from rotaviruses stands at an estimated 215,000 annually worldwide; 200,000 of these being in Africa alone. The main objective of this study was to characterize the strains of rotavirus among children with gastroenteritis below five years at Kenyatta National Hospital Nairobi County. The research design was cross-sectional, and the sample size was 355 participants. The study participants were identified during clinical examination by clinicians in outpatient and inpatient departments

Methods

. Stool samples were collected and tested for rotavirus using Enzyme Linked Immuno-assay, one step multiplex qRT-PCR genotyping assay and whole genome sequencing using next-generation sequencing. The prevalence of rotavirus was 16.34% (58/355). Rotavirus infection among males was 10.42% (37/355) as compared to those of females 5.91% (21/355). The distribution of Rotavirus between outpatient and inpatient was 9.30% (33/355) outpatient while 7.04% (25/355) inpatient.

Results

The distribution of Rotavirus between outpatient and inpatient was 9.30% (33/355) outpatient while 7.04% (25/355) inpatient. There was no statistical significance of rotavirus infection between gender, inpatient, and outpatients. The most prevalent G-type being G1 48.3% (28/58), followed by G2 22.41% (13/58), G3 15.51%% (9/58), and G9 5.17%% (3/58) with mixed infections which included G1, 2, 5.17% (3/58) and G2, 3, 1.72% (1/58). The P-type, P [8] 46.55% (27/58) was most prevalent followed by P4 24.13% (14/58) and P [6] 20.68% (12/58). There were mixed infection which includes P [4, 8] 5.17% (3/58), and P [4, 6] 1.72% (1/58). The G-P combination showed that G1 P [8] 41.37% (24/58) was more prevalent followed by G2 P [4] 22.41%, (13/55) G3 P [6] 15.51% (9/58) and G9 [P8] 5.17% (3/58). The mixed infections included G1, 2 P [4, 8] 5.17% (3/58) and G3, 2 P [4, 6] 1.72% (1/58). The study revealed the prevalence of rotavirus has decreases to 16.34% and the most prevalent genotype was G1P[8].

Conclusions

There was no statistical difference in rotavirus infection in regard with gender, inpatient and outpatient. Rotavirus infection affected more males than females. The recommendation of the study is to increase vaccination of rotavirus among children to reduce gastroenteritis caused by rotavirus and frequent surveillance to monitor emerging genotypes.

doi: 10.1016/j.cca.2022.04.695

Clinica Chimica Acta 530 (2022) S161–S162

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Geriatric Medicine

M266

Establishment of biological variation and age-related reference interval model of 22 common biochemical analytes in elderly through real world big data mining

M. Ma, L. Xia, X. Chen, Y. Yin, J. Wu, L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China

Background-aim

Many countries, including China, have entered the aging society. However, reference intervals (RIs) for elderly are rarely established due to difficulties of selecting reference individuals. Therefore, this study aims to analyze the factors affecting the biochemical analytes and establish the RI and age-related RI models for biochemical analytes through mining real world big data.

Methods

The data of 97220 individuals download from electronic health records. Three derived databases were established for the purpose of the study. The first derived databases including 97220 individuals is to build age-related RI models after identifying outliers by Tukey method. The second derived database consists of the elderly, used to establish biological variation models and RI for biochemical analytes of the elderly. The difference between the elderly and non-elderly were compared using the third derived databases.

Results

The gender was the main source of variation of biochemical analytes for the elderly in biological variation models. The distribution of creatinine and uric acid was significantly different in the RI of biochemical analytes for the elderly established by gender. Age-related RI models for biochemical analytes which affected by age most were built and visualized. Biochemical analytes RI showed various pattern of changes from the non-elderly to the elderly.

Conclusions

The study analyzed the factors affecting biochemical analytes in the elderly. Moreover, RI and age-related RI models of biochemical analytes for the elderly were established, to provide the vital sight into biological process and to assist clinical use of various biochemical analytes to monitor the status of various diseases for the elderly.

doi: 10.1016/j.cca.2022.04.697

M267

Establishment of biological variation and age-related reference interval for thyroid hormones in the elderly using real-world big data mining

C. Ma[a], X. Chen[a], L. Xia[a], L. Xiaoqi[a], X. Guo[a], X. Guo[a], S. Lin[b], L. Qiu[a]

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China*
[b] *Department of Health Care, Peking Union Medical College Hospital, China*

Background-aim

Thyroid hormone levels are affected by age, season, gender and other factors. To explore the factors that affect thyroid hormone level can provide a theoretical basis for the establishment of personalized clinical decision. The purpose of this study was to establish a biological variation model of thyroid hormones for the elderly based on multi-factor method, analyze the factors affecting thyroid, and explore the pattern of thyroid hormone changes with age by using polynomial composite model.

Methods

According to the purpose of the study, two derivation set was established. The first derivation set including 53191 individuals is to establish the age-related reference intervals (RIs) models for thyroid hormones. Furthermore, the second derivation set of the elderly is to build up the biological variation model and establish RIs of thyroid hormones for the elderly.

Results

Biological variation indicated that FT3 significantly affected by gender (standardized regression coefficient: 0.205, $P < 0.01$) and age (standardized regression coefficient: -0.258, $P < 0.01$), and total triiodothyronine (TT3) decreased with age. (standardized regression coefficient: -0.171, $P < 0.01$) The age-related RIs models showed a various change patterns with age for thyroid hormones.

doi:10.1016/j.cca.2022.04.697

Conclusions

The RIs of thyroid hormones for the elderly were established, which were different with RIs provided by the manufacturer. The biological variation and age-related reference interval models beneficial for physicians to monitor the status of various thyroid diseases for the elderly.

doi: 10.1016/j.cca.2022.04.698

Clinica Chimica Acta 530 (2022) S163–S188

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Hematology, Hemostasis

T212

Shifts of transfusion demand in cardiac surgery after implementation of rotational thromboelastometry (ROTEM)

I. Rodriguez Martin, C. Sanchez Mora, V. Sanchez Margalet

Clinical Biochemistry, Hospital Virgen Macarena, Spain

doi: 10.1016/j.cca.2022.04.700

Background-aim

Viscoelastic tests (rotational thromboelastometry, ROTEM®), together with the implementation of a specific algorithm for coagulation management in cardiac surgery, enable perioperative coagulopathy to be better controlled.

Methods

Retrospective cohort study including 675 patients who underwent cardiac surgery with cardiopulmonary bypass. The incidence of allogeneic blood transfusions and clinical postoperative complications were analyzed before and after ROTEM® implementation.

Results

Following viscoelastic testing and the implementation of a specific algorithm for coagulation management, the incidence of any allogeneic blood transfusion decreased (41.4% vs 31.9%, P = 0.026) during the perioperative period. In the group monitored with ROTEM®, decreased incidence of transfusion was observed for packed red blood cells (31.3% vs 19.8%, P = 0.002), fresh frozen plasma (9.8% vs 3.8%, P = 0.008), prothrombin complex concentrate administration (0.9% vs 0.3%, p = 0.599) and activated recombinant factor VII (0.3% vs 0.0%, p = 0.603). Increased incidence was observed for platelet transfusion (4.8% vs 6.8%, p = 0,530) and fibrinogen concentrate (0.9% vs 3.5%, p = 0.066), tranexamic acid (0.0% vs 0.6%, p = 0.370) and protamine administration (0.6% vs 0.9%, p = 0.908). Similar results were observed in the postoperative period, but with a decreased incidence of platelet transfusion (4.8% vs 3.8%, p = 0.813). In addition, statistically significant reductions were detected in the incidence of postoperative bleeding (9.5% vs 5.3%, P = 0.037), surgical reexploration (6.0% vs 2.9%, P = 0.035), and length of Intensive Care Unit (ICU) stay (6.0 days vs 5.3 days, P = 0.026).

Conclusions

The monitoring of hemostasis by ROTEM® in cardiac surgery, was associated with decreased incidence of allogeneic blood transfusion, clinical hematologic postoperative complications and lengths of ICU stay.

T213

Comparison of APTT values measured on ACL top and STA compact analyzers

M. Andjelkovic [a], M. Petrovic [b], I. Nikolic [a], M. Mitrovic [a], M. Stanojevic Pirkovic [a]

[a] *Faculty of Medical Sciences, Department of Biochemistry, University of Kragujevac, Serbia*
[b] *Clinical Center Kragujevac, Department of Biochemistry, Kragujevac, Serbia*

Background-aim

Activated Partial Tromboplastin Time (APTT) is used as a procedure for evaluation of the intrinsic coagulation pathway and also for monitoring of patients receiving anticoagulant therapy. Our purpose was to compare the values of APPT obtained by ACL Top (Instrumentation Laboratory, USA) with the values obtained using STA compact (Diagnostica Stago SAS, France).

Methods

A total of 200 samples from patients were analyzed. Samples were processed by ACL Top, and immediately thereafter by STA compact. The principle of APTT test in both analyzers consisted of measuring the clotting time of the patient's recalcificated plasma in the presence of contact activator and factor XII activator. All statistical analyses were performed using MedCalc Statistical Software version 19.0.7. The significance of differences for the paired means was calculated using the Wilcoxon Rank Sum test. The correlation study was performed using Spearman's correlation test. Comparison of assays was performed using Passing-Bablok regression analysis. The difference of measurements was assessed by calculating the bias using Bland-Altmann plots.

Results

According to Wilcoxon Rank Sum test there is statistically significant difference between APTT values obtained by ACL TOP and STA compact ($p < 0.0001$). The examined ranges were 14.2-60.6s as measured by ACL TOP and 23.2-74.2s as measured by STA compact. The results were highly correlated ($r = 0.815$). In Passing Bablok regression analysis $y = -0.876 + 1.121x$ (intersection confidence interval (-4.7965-2.6000), slope confidence interval (1.000-1.2566)) equation was found. Bland-Altman comparison of ACL TOP and STA compact analyzer for APTT

measurement results revealed that the limits of agreement were between -15.7 to 8.7 and the mean difference was 3.5s.

Conclusions

There was a significant correlation among examined parameters, but values of APTT measured by STA compact were statistically different from the same parameter measured by ACL TOP. Our findings suggests that in patient's APTT monitoring the same analyzer should be used in order to provide comparability of the results.

doi: 10.1016/j.cca.2022.04.701

T214

Assessment of hemocromatosis in politransfunded patients

E. Lepe Balsalobre, R. Rubio Sánchez, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville, Spain

Background-aim

Patients with high transfusion requirements (> 4 red blood cell concentrates / month) are at risk of developing posttransfusional iron overload. The result is an iron accumulation that slowly leads to tissue dysfunction of target organs. The alteration of serum ferritin is considered mild if it is <1000 ng/mL, moderate if it is between 1000 and 2500 ng/mL and severe if it is >2500 ng/mL.

There are several alert methods, such as the CH20 alert, which can inform the physician early when the 20 units of transfused red blood cell (CH) concentrates are exceeded, thus initiating treatment to avoid further future consequences.

The objective of this study was to assess the increase in serum ferritin in polytransfused patients, as well as the notification to the physicians responsible for inclusion in chelating therapy.

Methods

Retrospective and observational analysis of the CH20 alert registry of our center in 2018.

Results

78 patients were included that were divided into 3 groups:

Group with iron overload (n = 31, 40%): had average serum ferritin levels of 2,705 ng / mL. 83% of the physicians responsible were informed, but 65% of them did not perform chelating treatment. The average of transfused units was 56CH; the majority of the diagnoses were myelodysplastic syndromes and acute leukemias.

Group without iron overload (n = 25, 32%): average serum ferritin levels of 602 ng/mL. 68% of the physicians responsible were informed. The average of transfused units was 21CH; the majority were hematological patients.

No risk group (n = 22, 28%): average serum ferritin levels of 93 ng/mL. In no case were the physicians responsible informed. The average of transfused units was 24CH.

Conclusions

Because hematologic and oncological patients receive periodic transfusions and have prolonged life expectancy, if serum ferritin is greater than 1000 ng/mL and clinical conditions allow, they should be included in iron chelation programs.

Measures such as the CH20 alert make it possible to detect patients who need chelation onset, being important to carry out therapeutic interventions or to schedule more continuous follow-up visits.

doi: 10.1016/j.cca.2022.04.702

T215

About a case: Crioaglutinine disease

K. Falcones Gracia, L. Julia, A.M. Santo Quiles, R. Molina Gasset, E. Ricart Alvarez

Hospital Virgen De Los Lirios, Spain

Background-aim

The Cryogglutinins are cold IgM antibodies that react against red blood cell membrane antigens. Their presence in blood samples can cause spontaneous agglutination of erythrocytes at room temperature. This phenomenon is detected by a surprising decrease in red blood cell count and hematocrit value, which does not correlate with the hemoglobin concentration, causing a marked increase in the mean corpuscular hemoglobin concentration (CHCM). Its binding effect is reversed after incubating the sample for 30 minutes at 37 ° C. On the other hand, the Sysmex XN-1000 analyzer allows to obtain by optical method parameters such as RBC-O that can quickly correct the effects caused by the presence of these antibodies.

Methods

A 65-year-old British male patient with a history of colon cancer who visited his primary care physician in our country in May 2018 to monitor his pathology. He is asked for complete analytics and is summoned at his health center for extraction. The peripheral blood sample for hematimetry study is extracted by venipuncture in a 3.0 mL BD Vacutainer® tube with EDTA-K3 anticoagulant. After the reception in the laboratory, it is analyzed in the Sysmex XN-2000 analyzer. The red blood cell count (RBC 0.53x106 / uL) and the hematocrit value (5.0% HCT) were surprisingly low and did not correlate with the hemoglobin concentration (Hb 15.0 g / dL).

Results

Consequently, hematological indices, especially HCM and CHCM, showed a spurious increase, while the platelet and leukocyte count had apparently valid results. After performing visual inspection to rule out the presence of aggregates, it is decided not to report these results and after talking with your doctor a new sample is requested, obtaining the results Hb 13.4 g / dL, RBC 0.85x106u / L and HCT 8, 0% In the smear a massive agglutination of the red series is observed, morphologically normal and without schistocytes. The study was completed by quantifying the immunoglobulins IgA, IgG, IgM, complement proteins C3 and C4, and coombs test, increasing IgM concentration and direct positive Coombs test. The presence of hemolytic anemia was ruled out. It was decided to process in manual mode by selecting the RET channel obtaining HB 13.9 g / dL, RBC 4.05x106u / L and 44.48% HCT.). Subsequently, the sample was incubated at 37 ° C for 30 minutes and after reprocessing it by the analyzer in normal impedance mode, Hb 14.1 g / dL, RBC 3.5x106 u / L and 32.4% HCT were observed.

Conclusions

The presence of cold antibodies causes agglutination of erythrocytes at temperatures below 37 ° C, which can cause serious problems when interpreting the blood count. The experience of laboratory professionals can detect this phenomenon and correct it by incubating the sample, or by processing by an optical method with a preheating of the sample at 41 ° C, which significantly reduces the delivery time of results.

doi: 10.1016/j.cca.2022.04.703

T216

Early detection of coagulation disorders in sepsis by using delta neutrophil index and DIC score

P. Peneva [b], Y. Bocheva [b], K. Yankov [b], S. Nikolova [a]

[a] *Department of Social Medicine and Healthcare Organization, Medical University – Varna, Bulgaria*
[b] *UMHAT "St. Marina", Medical University – Varna, Bulgaria*

Background-aim

Sepsis is a serious life-threatening clinical syndrome caused by a severe infection in the body.

Disseminated intravascular coagulation (DIC) is a frequent complication in sepsis. The present study evaluated how changes in DIC score (ISTH (The International Society on Thrombosis and Haemostasis) criteria for DIC) and Delta Neutrophil Index (DNI) values indicate clinical progression from infection to sepsis with initial coagulation disorders.

Methods

A prospective clinical follow-up was conducted. In total we enrolled 82 patients. About 37 of them were infected patients without any criteria of sepsis, whereas 45 patients covered the sepsis criteria according to SEPSIS-3 (2016). DNI was calculated as the difference between the leukocyte differential in the MPO channel and the leukocyte differential in the nuclear lobularity channel using Advia 2120i hematology system (Siemens). D-dimer, fibrinogen and prothrombin time were assessed by SYSMEX CA-1500(Siemens). Pierson Correlations tested the associations between DNI and DIC score as significant factors for sepsis and septic shock development. Receiver operating characteristic (ROC) and logistic regression models assessed whether DNI and DIC score could indicate the probability of developing sepsis.

Results

Sepsis development and DNI were positively correlated ($r = 0.363$, $p = 0.001$) whereas sepsis progression was strongly associated with DIC score ($r = 0.608$, $p = 0.0001$). DNI was a significant predictive factor for developing sepsis (Exp(B) = 1,329, p = 0,007) and septic shock (Exp (B) = 1,430, p = 0,001). Results from the ROC curve analysis indicated that a cut-off value of 1.5 of DNI indicates development of sepsis [AUC = 0.764, $p = 0.0001$] with 73% sensitivity and 87% specificity, whereas for DIC score the cut-off value is 2.5 points [AUC = 0.858; $p = 0.0001$] with 84% sensitivity and 76% specificity.

Conclusions

Our results show that the initiation of sepsis is associated with coagulation disorders despite the absence of overt DIC. Parallel calculation of DNI and DIC score might be valuable tools for early detection of sepsis with underlying coagulation disorders. It is crucial to identify the initial sepsis-induced coagulopathy for appropriate management in the prodromal phase of DIC.

doi: 10.1016/j.cca.2022.04.704

T217

Burkitt's lymphoma/Leukemia in a 4-year-old boy

H. Kabbaj [a], S. Nader [a], Y. Tabache [c], M.H. Besaad [a], S. El Hayani [a], S. Errahmani [c], Z. Alhamany [b], N. Erreimi [c], M. Nachef [c], A. Belmekki [a]

[a] *Medical Biology Laboratory, Cheikh Zaid International Hospital/International University of Health Sciences, Rabat, Morocco*
[b] *Pathology laboratory of Cheikh Zaid Hospital, Morocco*
[c] *Pediatrics Department of Cheikh Zaid Hospital, Morocco*

Background-aim

Burkitt's lymphoma is an unusual and aggressive mature B-cell tumor characterized by high proliferation. Most cases of Burkitt's lymphoma have tumor masses with involvement of the bone marrow in advanced forms. However, rare cases may involve only the bone marrow without clinical or radiographic evidence of a tumor mass, called pure Burkitt's leukemia. In the past, these patients were considered to have B lymphoblastic leukemia "L3" with a mature phenotype.

This work aims to draw the attention of both clinicians and biologists to the diagnostic difficulty that this pathology can present and to the need for interdisciplinary dialogue in its management.

Methods

We report the unusual case of a young child diagnosed with Burkitt's lymphoma with spinal cord invasion.

Results

The child initially complained about arthralgia and the radiological imaging found hepatic, splenic, renal and pancreatic nodules.

A kidney nodule biopsy revealed Burkitt's lymphoma. At the same time, a myelogram was performed and showed an acute lymphoblastic leukemia.

Conclusions

Through this observation we emphasize the importance of interdisciplinary dialogue in the management of this pathology.

doi: 10.1016/j.cca.2022.04.705

T218

Reticulocyte hemoglobin (CHR) by Mindray BC 6800 plus for the assessment of iron deficient erythropoiesis in rheumatologic disorders

E. Urrechaga [a], I. Perez [c], A. Martinez [b], M. Merino [c], G. Muguerza [c]

[a] *Biocruces Bizkaia Research Institute. Baracaldo, Spain*
[b] *Facultad de Ciencia y Tecnología, Universidad de País Vasco. Leioa, Spain*
[c] *Laboratorio. Hospital Galdakao – Usansolo. Galdakao, Spain*

Background-aim

The main cause of anemia in rheumatologic disorders is anemia of chronic disease (ACD). The evaluation of iron deficiency, absolute or functional, and the requirements for erythropoiesis is important to select the appropriate treatment, since iron supplements are beneficial in iron deficiency anemia (IDA) but may be deleterious for pure ACD patients. We study the value of reticulocyte hemoglobin content (CHr), measured on BC 6800 Plus analyzer (Mindray Diagnostics, China) for the detection of iron deficient erythropoiesis in those patients.

Methods

Eighty anemic female outpatients with rheumatic diseases were enrolled and divided depending on the iron status and inflammatory markers:

IDA: s-ferritin < 10 μg/L, C reactive protein CRP < 5 mg/L.

ACD with iron deficiency: s-ferritin 10-150 μg/L, CRP > 5g/L.

ACD: s-ferritin > 150 μg/L, CRP > 5 mg/L.

Blood samples were processed in reticulocyte mode of the BC 6800 Plus. S-iron, transferrin and s-ferritin were assayed in a Cobasc711 analyzer with Roche Diagnostic reagents. Differences among groups were examined using ANOVA and post-hoc comparisons. $P < 0.05$ was considered significant. Receiver operating characteristic curve analysis to establish the performance of CHr for detecting iron-restricted erythropoiesis, gold standard transferrin saturation (Tsat) < 20 %.

Results

The number of patients with IDA, ACD with iron deficiency and ACD were 20, 35 and 25 respectively. The results in IDA group reflected iron restricted erythropoiesis, mean CHr 26.5 pg. Similar results were obtained in the ACD and iron deficiency group, CHr 27.3 pg. These values were not statistically different $P = 0.632$. Patients were divided according to their erythropoietic status, iron restricted or adequate iron supply based on Tsat (58 and 23 patients, respectively). Mean CHr in these groups were statistically different (26.9 vs 30.1 pg $P < 0.0001$). ROC analysis results area under curve 0.888 (95% CI 0.845-0.914) at cut off 30.0 pg, sensitivity 85.7%, specificity 82.1%.

Conclusions

The evaluation of erythropoiesis status and iron requirements in patients with rheumatic diseases is difficult, because of the presence of a chronic inflammatory process. CHr is a reliable parameter for detecting iron deficiency, absolute or functional, thus improving the management of anemic patients by recognizing those who will benefit from therapy.

doi: 10.1016/j.cca.2022.04.706

T219

Different interpretations of huge bicuspid peak in capillary electrophoresis: lesson from three case series

J.J. Lee, J. Ryu, S.Y. Cho

School of Medicine, Kyung Hee University, Seoul, South Korea

Background-aim

Monoclonal gammopathy results from the proliferation of a single clone of plasma cells that produce a homogeneous monoclonal protein. In contrast, polyclonal gammopathy is associated with a heterogeneous group of benign conditions, including reactive or inflammatory process. They should be exactly differentiated using electrophoresis tests, for detection of monoclonal gammopathy and diagnosis of multiple myeloma. Capillary electrophoresis (CE) has different characteristics with conventional gel electrophoresis (GE) in mechanism and interpretation. Therefore, the differentiation between monoclonal gammopathy and polyclonal gammopathy can be more difficult or confusing than in GE. Here, we reported the three cases showing a huge bicuspid peak from which we can take lessons for the exact differentiate monoclonal, oligoclonal and polyclonal gammopathies in CE.

Methods

In this study, protein electrophoresis in serum and urine were detected in CE via capillary 2 (Sebia, France). They were reconfirmed by agarose GE and immunofixation electrophoresis (IFE) in a Hydrasys analyzer (Sebia, France) using Hydragel 15 HR gels (Sebia, France).

Results

Case 1. In a 63-year-old male patient, serum CE showed a huge bicuspid huge peak which is immunosubtracted in IgM, and kappa fractions. GE was also performed for reconfirmation and corresponding with previous CE results that presented monoclonal gammopathy of IgM and kappa types accompanying with polyclonal background.

Case 2. In a 85-year-old male patient, serum CE showed a huge bicuspid huge peak which cannot be exactly differentiated only with the immunosubtracion types. Therefore, AGE was performed for confirmation and presented polyclonal increased of IgG with an oligoclonal profile of IgG and IgM.

Case 3. In a 62-year-old female patient, serum CE presented a huge bicuspid peak in gamma region, which is immunosubtracted in IgG, IgM, and lambda fractions, respectively. AGE was performed for reconfirmation and showed compatible results with CE, which presented biclonal gammopathy accompanying with polyclonal background. However, one month later, follow up tests of GE revealed that the previous biclonal pattern was converted into polyclonal gammopathy with an oligoclonal pattern of IgG and IgM.

Conclusions

In our cases to show a huge bicuspid peak in gamma region of CE, two cases were diagnosed to monoclonal gammopathy accompanying with thick polyclonal backgrounds, and the other one was revealed to oligoclonal gammopathy accompanying with thick polyclonal backgrounds. Monoclonal gammopathy and oligoclonal gammopathy cannot be easily differentiated when they are accompanied by thick and huge polyclonal backgrounds, especially in CE. Therefore, we suggest that GE and its follow-up tests can be a very helpful and essential tool for differentiation between monoclonal and oligoclonal gammopathies which are partly buried in a big polyclonal peak.

doi: 10.1016/j.cca.2022.04.707

T220

Sigma metrics for evaluating the performance of complete blood counts

E. Urrechaga [a], A. Martinez [b], M. Merino [c], G. Muguerza [c], I. Perez [c]

[a] *Biocruces Bizkaia Research Institute. Baracaldo, Spain*
[b] *Facultad de Ciencia y Tecnología, Universidad de País Vasco. Leioa, Spain*
[c] *Laboratorio. Hospital Galdakao – Usansolo, Galdakao. Spain*

Background-aim

Clinical decisions for diagnosis and patient management are based on the information provided by the Laboratory. Clinical laboratories fol-

low quality control programs to ensure the reliability and accuracy of the results and the overall quality of the report submitted to clinician. We aim to calculate sigma metrics for Complete Blood Counts (CBC), using total allowable error (TEA) requirements, in order to identify areas for improvement in patient care.

Methods

CBC were performed in a Mindray BC-6800Plus analyzer. Red cell count (RBC), hemoglobin (Hb), hematocrit (HTC), mean cell volume (MCV), mean cell Hb, (MCH), mean cell Hb concentration (MCHC), platelets counts (PLT), mean platelet volumen (MPV) leucocyte counts (WBC), neutrophil absolute count (NEUT), lymphocytes (LYMPH) , monocytes (MONO), eosinophils (EO), basophils (BAS).

Data from quality controls Mindray BC-6D were used for the sigma calculation of each parameter during the 60 days period in use.

The total analytical error (TE) for each measurand is compared to the total allowed error (TEa). http://www.westgard.com/biodatabase

TE = bias + 1.96*CV and sigma calculated Sigma (TEa -bias)/CV

Results

Except MCHC and BAS all parameters got TE less than their corresponding TEa. Mindray BC- 6800 Plus analyzer has excellent quality (sigma > 6) for NEUT, WBC, Hb, RDW, sigma > 3 for platelets, MPV and LYMPH. Red cell derived indices are not independent parameters; MCH, MCHC and HTC are calculated from the primary parameters RBC, Hb, MCV (sigma 3.0, 8.4, 2,9, respectively) which is the reason for the lower sigma values, acceptable in clinical practice, sigma > 2.5 for MCH and HTC.

Conclusions

The application of Sigma metrics to CBC demonstrates the need to maintain strict limits in the quality control of the primary parameters of the analyzer, in order to ensure the quality required in the derived parameters. Sigma metrics is a procedure that can be applied in Hematology laboratories and a good method to ensure and compare the quality of analyzers.

doi: 10.1016/j.cca.2022.04.708

T221

Plasma cell Leukemia: Two case reports

S. Nader, A. Bentahila, S. El Hayani, H. Kabbaj, M. Tber, M. Ztout, A. Lamanaouer

Hematology, Medical biology laboratory, Cheikh Zaid International Hospital/ International University of Health Sciences, Rabat, Morocco

Background-aim

Plasma cell leukemia is a rare malignant hemopathy defined by the presence of more than 20% of plasma cells of the leukocyte formula or a rate of circulating plasma cells greater than 2 × 109 / L (2G / L). It can be primary, in 60% of cases, and manifest itself immediately on a leukemic mode, or it can be secondary, in 40% of cases, complicating an already known multiple myeloma.

Methods

Two case reports of multiple myeloma transformed into plasma cell leukemia. This was diagnosed in the medical biology laboratory at Cheikh Zaid International Hospital in Rabat and was found in a five year period (from 2015 to 2019 included).

Results

The diagnosis of plasma cell leukemia was established on the cytological study of the blood smear for both patients which revealed an infiltration of the white line by plasmoblasts of 28 and 30% respectively.

Conclusions

Through the two observations we emphasize the importance of blood smear in detecting plasma cell leukemia in patients with multiple myeloma, a rare pathology with very poor prognosis.

doi: 10.1016/j.cca.2022.04.709

T222

Screening for Covid-19 with cellular morphometric parameters on the routine hematology analyzer DXH 800

M. Vasse [b], M. Ballester [d], D. Ayaka [c], D. Sukhachev [e], F. Delcominette [c], E. Sukhacheva [a], F. Habarou [c], E. Jolly [c], T. Pascreau [b], E. Farfour [c]

[a] *Beckman Coulter Eurocenter, Nyon, Switzerland*
[b] *Biology Department, Foch Hospital & UMR-S 1176, Suresnes and Kremlin-Bicêtre, France*
[c] *Biology Department, Foch Hospital, Suresnes, France*
[d] *Emergency Unit, Foch Hospital, Suresnes, France*
[e] *LabTech Ltd, Saint-Petersburg, Russian Federation*

Background-aim

Coronavirus disease (COVID-19) caused by SARS-CoV-2 is characterized by high contagiousness requiring isolation measures. Currently, diagnosis is based on the RT-PCR and/or chest computed tomography (CT) scan, but these methodologies are time-consuming and may delay the diagnosis. CBC-Diff analysis is the first step in patient assessment and may contribute to the diagnosis of COVID-19. Morphological changes of the immune cells can be identified by electro-optical analysis on the hematology analyzer DxH 800 (Beckman Coulter, Inc., Brea, CA). We studied whether the analysis of cellular population data (CPD), provided as part of CBC-Diff analysis by the DxH 800 analyzers can help to identify SARS-CoV-2 infection.

Methods

The study included 322 consecutive patients from the emergency unit with positive RT-PCR (Allplex 2019 nCoV Assay, Eurobio, Les Ulis, France) and 285 consecutive patients with clinical suspicion for COVID-19, but who had negative RT-PCR and CT-scan not suggestive of SARS-CoV-2 infection. We also included 137 subjects with a normal CBC-Diff, referred to our institution without evidence of infection and when prevalence of SARS-CoV-2 was very low in France. Blood was collected in EDTA-K3 tubes and analyzed within 6 h after collection.

Results

The majority of CPD was different between the 3 groups; CPD of patients (with or without COVID-19) were significantly different of CPD of controls. Four, six and nine CPD for NE, LY, and MO, respectively, were significantly different between COVID-19+ and COVID-19- patients. Using ROC analysis, we identified parameters, which were able to discriminate COVID-19+ patients from COVID-19- patients. The best parameter was SD-V-Mo (Standard Deviation of Monocyte Volume), with AUC 0.819, sensitivity of 91.59% and specificity of 63.03% at the cut-off > 21.71. MN-V-Mo (Mean Monocyte Volume) demonstrated AUC 0.742 with sensitivity of 76.64%, specificity of 65.85% at cut-off > 180. SD-AL2-MO (Standard Deviation of Axial Light Loss for Monocytes) provided AUC 0.722 with sensitivity of 85.67%, specificity of 52.11% at cut-off > 17.51. Currently CPD are research use only; their clinical utility has not been established.

Conclusions

Consideration of CPD could constitute a first step and potentially aid in the early diagnosis of COVID-19.

doi: 10.1016/j.cca.2022.04.710

T223

Validation and clinical application of a novel platelet function analyzer (ANYSIS 200) in cardiology patients

B. Lee [b], Y.H. Chung [b], K.A. Lee [a], S. Shin [c]

[a] *Department of Laboratory Medicine, College of Medicine, Yonsei University, Seoul, South Korea*
[b] *Division of Cardiology, Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, South Korea*
[c] *Korea University, Guro Hospital, Seoul, South Korea*

BACKGROUND-AIM

Evaluation of antiplatelet therapeutic responsiveness is crucial in the management of patients with cardiovascular disease. This study aimed to assess a new platelet function analyzer (Anysis-200) and to compare it with VerifyNow in cardiology patients.

Methods

Anysis-200 measured platelet function with blood migration distance (MD) until clogging of flow passage, which is comparable to aspirin resistance units obtained using VerifyNow. Platelet assays were simultaneously conducted with Anysis-200 and VerifyNow and compared. A total of 125 citrated blood samples were collected from 85 cardiology patients who were referred for platelet function testing.

Results

In the Anysis-200 assay, the intraclass correlation coefficient (ICC) was 0.960 (95% confidence interval [CI], 0.948–0.970). The MDs before and after taking aspirin were 174 ± 51 and 247 ± 27 mm, respectively ($p < 0.0001$). Compared with VerifyNow (reference), the sensitivity and specificity of Anysis-200 was 91.5% and 75.5%, respectively (area under the curve, 0.829). The agreement rate between two devices was 0.682 (95% CI, 0.551–0.812; Cohen's kappa coefficient), which is the second highest level among the agreement comparisons.

Conclusions

Conclusively, Anysis-200 system has equivalent accuracy and precision, and moderate agreement to VerifyNow. This new platelet function test can significantly improve prognosis and survival by identifying patients who are not responding to aspirin in order to prevent thrombosis and allow replacing aspirin with other effective treatments.

doi: 10.1016/j.cca.2022.04.711

T224

Hematological changes among Ethiopian petroleum station workers

S. Getu [a], M. Melku [b], E. Shiferaw [b]

[a] *Department of Medical Laboratory Science, Debre Tabor University, Ethiopia*
[b] *Department of Medical Laboratory Science, University of Gondar, Ethiopia*

Background-aim

Petrol is the non-specific term for petroleum which is used for inside combustion of engines. Petrol filling workers are highly vulnerable to occupational exposure to these harmful substances which leads to hemato-toxicity and blood disorders such as leukemia, aplastic anemia and dysplastic bone marrow. Thus, the current study aimed to assess hematological parameters of petrol filling workers in Gondar town, North West Ethiopia.

Methods

A comparative cross-sectional study was conducted from January to March 2019 in Gondar town, North West Ethiopia. A total of 110 study participants comprising 55 study groups and 55 controls group were recruited by convenient sampling technique. Socio-demographic data was collected using structured questionnaire and 3 ml of venous blood was collected for the determination of hematological parameters. The data was entered using Epi info 7.2.0.1 and analyzed by SPSS version of 20. Mean, standard deviation, median and interquartile ranges were used to present the data. Independent t-test and Mann-Whitney U- test were used to compare the mean difference between parametric and non-parametric hematological parameters, respectively. Moreover, Pearson and Spearman's rank-order correlations analysis were used to describe the association between hematological parameters and duration of work. P value of < 0.05 was statistically significant.

Results

The current study figure out mean red blood cell count and hemoglobin level as well as the median hematocrit, mean cell hemoglobin concentration, platelet count, absolute lymphocytes count and red cell distribution width values of petrol filling workers showed a significant increment compared to control group. On the other hand mean cell hemoglobin value of petrol filling workers showed a significant decrement compared to healthy controls. Moreover, duration of petrol exposures showed a significant strong positive correlation with red blood cell

count and mean cell hemoglobin concentration. However, a significant strong negative correlation was observed with mean cell volume.

Conclusions

Findings of the current study showed that majority of hematological parameters indicated an increment in petrol filling workers compared to healthy controls which might be associated with exposure to petrol chemicals. However, further longitudinal study with larger sample size should be conducted.

doi: 10.1016/j.cca.2022.04.712

T225

The development of the laboratory algorithm for screening and diagnosis of hemoglobinopathies

I. Zhilenkova [b], M. Zenina [b], N. Chernysh [a], A.V. Kozlov [b]

[a] *Almazov National Medical Research Centre, Saint-Petersburg, Russia*
[b] *The North-Western State Medical University named after I.I. Mechnikov, St.-Petersburg, Russia*

Background-aim

Disorders of globin chain synthesis are among the most common genetic disorders worldwide. Russia is a low prevalence area of this pathology. However, we are also involved in this problems as a result of migration of ethnic groups with a high frequency of haemoglobinopathy. Now there is a need to create an algorithm for screening and diagnosis of hemoglobin disorders, including available laboratory methods and technologies.

Methods

The study was carried out in the North-Western region of Russia. The algorithm was created on the basis of an examination of 150 patients (1-58 years) with hemoglobin disorders. The comparison group consisted of 200 patients (1-54 years) with microcytic anemia due to other cases.

The diagnosis of haemoglobinopathies involves measuring RBC parameters and calculated indices (incorporate at least 2 of the parameters provided by the automated hematologic analysers); quantification of hemoglobin fractions by the capillary electrophoresis (Sebia, France); assay for the identification of globin gene mutations based on PCR and reverse-hybridization.

Results

It was found that the most common form of hemoglobin disoders in our area is beta thalassemia (92%).

The laboratory algorithm for screening of hemoglobin disorders for low prevalence areas involves:

Step 1 - screening. It is based on RBC parameters and combined use of the index Menzer = MCV/RBC and Sirdah = MCV - RBC - 3×Hb with cutoff for detecting thalassemia <11.5 and <25, respectively. It is an available method for identifying patients with a high probability of thalassemia in all laboratories with an automatic hematology analyser.

In the case of rare form hemoglobin disoders screening is based on assessing the individual risk by determining the family origin of the patient by the questionnaire.

Step 2 - the study of hemoglobin fractions for all patients with a high probability of hemoglobin disoders. In the study the capillary electrophoresis allowed to confirm the diagnosis in 97% of cases.

Step 3 - molecular diagnostic. It is required only for patients with suspected ⟨-thalassemia

Conclusions

The algorithm has a sensitivity and specificity of 98%. It is effective and accessible for areas of low prevalence and allows to directly search for carriers of hemoglobin disorders without additional costs.

doi: 10.1016/j.cca.2022.04.713

T226

Neopterin in serum and cerebrospinal fluid of children with acute Leukemias – Case report

J. Berska [a], J. Bugajska [a], K. Pawińska-Wąsikowska [b], W. Balwierz [b], K. Sztefko [a]

[a] *Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland*
[b] *Department of Pediatric Oncology and Hematology, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland*

Background-aim

Neopterin (Neo) is a nonspecific marker of cellular immune response. Elevated level of Neo has been reported in the malignant diseases including leukemia. However, there is a lack of information of Neo concentration in serum and cerebrospinal fluid (CSF) in children with acute leukemias. Therefore the aim of the study was to assess the changes of neopterin levels in serum and CSF at the time of diagnosis and during treatment

Methods

Four clinical cases of children with newly diagnosed acute leukemias are presented. Based on cytomorphology and immunophenotyping assessment of bone marrow, two children were diagnosed with acute myeloid leukemia (AML-M5)(11-year-old boy and 8.5-year-old girl). They were treated according to AML-BFM 2012 and 2019 protocol, respectively. Two other children had acute lymphoblastic leukemia (ALL)(3-year-old boys). Both patients were treated according to AIEOP-BMF 2017 protocol. One of reported children had an initial central nervous involvement.

Neopterin concentration was determined (ELISA, IBL International) in serum and CSF samples before the 1st, 2nd and 3rd cycle of chemotherapy (AML-patients) and at first, 15. and 33. day of treatment (ALL-patients). In addition, at the same time points of therapy blood morphology (Sysmex XN1000) and CRP serum concentration (Vitros 4600, Ortho Clinical Diagnostics Inc.) have been routinely determined.

Results

In AML-patients the concentration of Neo, both in serum and CSF, decreased with progress of treatment. Dramatic decrease in serum level of Neo ($>85\%$) was observed during therapy. The observed decrease in Neo level was associated with an increase of leukocytosis.

In ALL-patients, simultaneous increase in concentration of Neo in serum and CSF was observed during therapy. At 33 day of treatment

Neo level in serum was 5 to 10 times higher whereas Neo level in CSF was 10 to 100 times higher as compared to the values before chemotherapy. In these patients decreasing white blood cell counts was observed.

Regardless of the type of leukemia, CRP concentration decreased during treatment in all study patients.

Conclusions

Neopterin level in serum and CSF strongly depends on type of leukemia, as well as therapy protocol and time point of measurement during oncologic treatment.

doi: 10.1016/j.cca.2022.04.714

T227

Differential role of phospholipase d isoforms in hemostasis and thrombus formation

L. Huang [a], T. Chen [a], K. Lin [b]

[a] *Department of Pharmacology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[b] *Institute of Biomedical Sciences, Mackay Medical College, New Taipei City, Taiwan*

Background-aim

Phospholipase D (PLD) 1 and PLD2 are involved in many biological processes and diseases including cancer, immunity, and Alzheimer's disease. PLD also plays a crucial role in platelet activity; however, our previous study has revealed that the regulatory role of PLD in humans and mice seems different. Thus, this study further confirmed whether PLD regulate platelet activation via a different mechanism between humans and mice.

Methods

In this study, platelet function analyzer-100 (PFA-100) was used to determine the effect of PLD isoforms on normal hemostasis. Thrombosis and stroke model in mice were used to observe the effect of PLD isoforms on thrombosis and stroke. Moreover, the animal behavior post-stroke was also determined to evaluate the neuroprotective effect of PLD isoforms.

Results

In PFA-100 study, Our data revealed that either PLD1 inhibition or PLD2 inhibition did not significantly affect closure time, but concurrent inhibition of PLD1 and PLD2 could significantly prolong closure time in human whole bloods, suggesting Both PLD1 and PLD2 inhibition can affect normal hemostasis. To clarify the discrepancy of the role of PLD in humans and mice, two animal models of ADP-induced pulmonary thrombosis and middle cerebral artery occlusion/reperfusion-induced brain injury were performed in mice. For the model of ADP-induced pulmonary thrombosis, the data revealed that PLD1 inhibition significantly increased survival rate and decreased thrombosis, whereas PLD2 inhibition did not exert protective effects. For middle cerebral artery occlusion/reperfusion-induced brain injury, the data revealed that PLD1 inhibition, but not PLD2 inhibition, exerted a significant reduction of infarct size and edema. The results obtained from these two animal models indicate that PLD1 may play more crucial role in thrombosis and stroke than PLD2.

Conclusions

This study further demonstrated that pharmacological inhibition of PLD1 showed similar protective effect to genetic deletion of PLD1 in thrombosis and stroke, and confirmed that PLD1 plays more crucial role in thrombosis and stroke in mice. Collectively, these findings obtained from our present and previous findings revealed PLD plays differential role between humans and mice.

doi: 10.1016/j.cca.2022.04.715

T228

Evaluation of coagulation profile in Bosnian children with type-1 diabetes mellitus and effects of glycaemic control on coagulation indicies

S. Tihić-Kapidžić [a], A. Čaušević [b], J. Fočo-Solak [a], M. Malenica [b], S. Hasanbegović [d], N. Babić [c], E. Begović [a]

[a] *Department for Clinical Biochemistry and Immunology, Clinical Center University of Sarajevo, Bosnia and Herzegovina*
[b] *Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina*
[c] *Department of Human Physiology, Medical Faculty, University of Sarajevo, Bosnia and Herzegovina*
[d] *Pediatric Clinic, Clinical Center University of Sarajevo, Bosnia and Herzegovina*

Background-aim

Among several diabetes-related complications, Type-1 Diabetes mellitus also affects the coagulation factors, and it is implicated as prothrombotic condition characterized by dysregulation of the haemostatic mechanisms. Hyperglycemia, hyperinsulinemia, and insulin resistance in diabetics alter hemostasis leading to increased risk of thrombotic events in diabetic individuals. We aimed to evaluate the coagulation profile among children in Bosnia and Herzegovina with Type 1 Diabetes Mellitus (T1DM) and compare it to their healthy peers; as well to analyze the effects of glycemia control on coagulation indicies.

Methods

The study included 100 healthy children and 100 children with T1DM treated with insulin in the Department of Paediatrics, University Clinical Center Sarajevo. The control group was age- and sex-matched to the study group. We analyzed hemoglobin A1c (HbA1c), platelet count (PLT), International Normalized Ratio (INR), activated Partial Thromboplastin Time (aPTT), fibrinogen, antithrombin III (AT-III) and protein C.

Results

There was no significant difference in PLT count, INR and fibrinogen values between the groups ($p = 0.170$, $p = 0.075$ and 0.246; respectively) while aPTT, AT-III and protein C values showed significant differences ($p = 0.004$, $p < 0.001$ and $p = 0.032$; respectively). aPTT and AT-III were significantly lower in children with T1DM in comparison to healthy children (aPTT 32.54 + /-4.93 sec vs 33.94 + /-3.81sec; AT-III 94.04 + /-16.25 % vs 111.92 + /-12.10%). Protein C was significantly higher in children with T1DM in comparison to healthy children (protein C 99.32 + /-21.53% vs 94.33 + /-14.90%). Significant negative correlation was observed between HbA1c and aPTT (Spearman's rho = -0.261, $p < 0.001$), as well as HbA1c and AT-III (rho = -0.461,

p < 0.001). A positive correlation was observed between HbA1c and protein C (rho = 0.280, p < 0.001).

Conclusions

Coagulation profile in children with T1DM differs from healthy children. Poorly-managed T1DM and higher HbA1c values are associated with reduced aPTT and AT-III levels, and increased levels of protein C. Assesment of coagulation profile is essential in the prevention and treatment of complications in children with T1DM.

doi: 10.1016/j.cca.2022.04.716

T229

Plasma levels of XA inhibitors in patients with venous thromboembolism and hereditary thrombophilia

I. Paskaleva [a], E. Doncheva [a], D. Dineva [a], V. Tampas [a], V. Ganev [b]

[a] *Laboratory Department, National Heart Hospital, Sofia, United States*
[b] *Medical Genetics Laboratory, University Hospital Lozenetz, Sofia, United States*

Background-aim

In the last decade, direct oral anticoagulants (DOACs) has been approved and commonly used in acute and long-term treatment of venous thromboembolism (VTE). Although DOACs have predictable pharmacokinetics and pharmacodynamics, recent studies have documented inter-individual variability in plasma levels of DOACs. There is a lack of sufficient data on the levels of DOAC in patients with VTE and underlying thrombophilia (THRPH). The aim is to determine plasma levels of DOAC in outpatients with venous thrombosis receiving Xa inhibitors (apixaban or rivaroxaban) for secondary prophylaxis.

Methods

The study included 20 patients, of whom 12 received apixaban 2 x 5 mg and 8 received rivaroxaban 20 mg daily. All patients were tested for Factor V Leiden G1691A and Prothrombin gene mutation G20210A; activity assay for Protein C, Free Protein S and antithrombin; antibody tests ACL IgG/IgM, beta-2 glycoprotein 1 IgG/IgM were studied. Most of them are heterozygous carriers of Factor V Leiden G1691A (n = 12), three are homozygous FVL, one is double heterozygotes, two are with protein C deficiency and two are with free protein S deficiency. VTE (venous thromboembolism) includes the common manifestation of deep venous thrombosis (DVT) of the lower or upper extremities, and less frequent pulmonary embolism (PE n = 9). The patients were below 50 years, 11 male and 9 female.

Results

We used calibrated chromogenic anti-Xa assay DiXaI (Biophen, Hyphen, France) on CS 2000i (Sysmex). Plasma levels of apixaban (n = 12) in patients with THRPH were 73 ng/ml (48.5 – 100 ng/ml) vs 72 (45-106) ng/ml in patients with VTE without THRPH (n = 30). The coefficient of inter-individual variation was 25%. Plasma levels of rivaroxabane (n = 8) in patients with THRPH were 262 ng/ml (200 - 370) vs 247 ng/ml (140 – 410) in patients with VTE without THRPH (n = 20) with inter-individual variation of 36%. Results are presented as median (5-th – 95-th percentiles).

Conclusions

We found no differences in plasma anti-Xa levels of apixaban and rivaroxaban in patients with and without hereditary thrombophilia. No recurrence of VTE was reported at three-year follow-up. DOACs appear to have effective levels of anticoagulation in patients with THRPH, treated for secondary prevention of VTE.

doi: 10.1016/j.cca.2022.04.717

T230

Genetic characteristics of AML with different risk category according to NCCN guidelines

j. Zou [a], y. Nie [a], l. Chen [a], k. Ru [b,c]

[a] *SINO-US-Diagnostics, Tianjin, China*
[b] *SINO-US-Diagnostics, Tianjin, China*
[c] *Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences, China*

Background-aim

Acute myeloid leukemia (AML) is characterized by considerable clinical and biological heterogeneity. NCCN defines AML as favorable-, intermediate-, adverse-risk. The stratification criteria are improving, but with the development of sequencing technology, genetic abnormalities of AML that can be inferred as a risk factors continue to be uncovered. Similarly, although some AMLs are classified by NCCN as the same risk category, clinical experience suggests that there are differences in clinical. The purpose of this study was to analyze genetic abnormalities between different groups.

Methods

Karyotyping and next-generation sequencing (NGS) were performed in 293 AML patients. Genomic DNA was selected to detect 114 known genes closely related to blood tumors by NGS. Risk classification was carried out according to NCCN guidelines version 2.2020.

Results

The 293 AML patients were divided into three groups, of which 64 were in the favorable-risk, 114 in the intermediate-risk, and 85 in the adverse-risk. Correlation analysis showed that as a distinct risk factor, FLT3-ITD was positively correlated with DNA methylation genes (DNMT3A, IDH2). It was also be found that the mutation rate of DNMT3A in the poor-risk (24.71%) was more frequent than in favorable-risk (14.06%). As a continually genetic alteration in AML-M4eEo, KIT mutations were often accompanied with sex chromosome deletions and ASXL2 mutations. Interestingly, KIT and ASXL2 might had higher mutation rates in both favorable-risk and adverse-risk than intermediate-risk, meaning these mutations carry more risk in patients with no-M4Eo. Importantly, splice-related genes (U2AF1, ZRSR2, SRSF2) were be found that its had rarely incidence in favorable-risk AML. In further risk analysis, their OR values showed a higher risk than the favorable-risk AML. Using an online public database, the clinical survival data of 662 AML patients were demonstrated that splice-related mutations, especially U2AF1, reduced overall survival (OS) in AML patients.

Conclusions

DNMT3A mutations, as events related to FLT3-ITD mutations, may play an important role in the clonal evolution of AML patients. In favorable-risk AML patients, KIT and ASXL2 mutations were more likely to be observed in MA4Eo patients. The latest NCCN risk stratification do not discuss KIT mutations favorable-risk AML patients, its specific role remains to be studied. Splice-related genes have a certain adverse effect on the prognosis of AML patients, which can be further confirmed by studies.

doi: 10.1016/j.cca.2022.04.718

T231

The effects of conivaptan and boric acid on peripheral blood cells after renal ischemia in unilateral nephrectomized rats

B. Can [b], F. Kar [b], E. Kar [b], M. Özkoç [b], H. Şentürk [a], G. Kanbak [b], İ.Ö. Alataş [b]

[a] *Department of Biology, Eskisehir Osmangazi University, Eskisehir, Turkey*
[b] *Department of Medical Biochemistry, Eskisehir Osmangazi University, Eskisehir, Turkey*

Background-aim

The antidiuretic hormone (ADH) has an important role on body fluid homeostasis through its specific G protein-coupled receptors. However, the hypersecretion of ADH has previously reported to be associated with pathogenesis of acute/chronic renal failure and ischemia-related diseases. Therefore, ADH receptors may be preferred as an important therapeutic target. This study designed for the purpose of investigating the effects of conivaptan as an antidiuretic hormone antagonist and boric acid as an antioxidant agent on peripheral blood cells after renal ischemia in unilateral nephrectomized rats.

Methods

Fourty Sprague-Dawley male rats were randomly divided into 5 groups: Control (Sham-operated), I/R, I/R + dimethyl sulfoxide (DMSO), I/R + conivaptan, and I/R + conivaptan + boric acid. The right kidney nephrectomy was performed in all groups and animals allowed to recover for 15 days. After recovery, renal ischemia was performed for 45 minutes by occlusion of the left renal artery. At the end of the ischemia, kidney was reperfused; 5% DMSO (i.v.), 10 mg/mL conivaptan (i.v.; in 5% DMSO) and 50 mg/kg boric acid (i.p.) performed to the related groups at the onset of the reperfusion. Blood samples were taken at 6th hours of the reperfusion (at the same time in the control). In order to determine the abnormalities of blood count, red blood cell (RBC), hemoglobin (HBG), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), white blood cell (WBC), mean platelet volume (MPV), neutrophil (NEUT), monocyte (MONO) and lymphocyte (LYMPH) measurements were performed in whole blood samples using commercial kits. NEUT/LYMP, PLT/LYPMH and NEUT/PLT ratios were also calculated.

Results

According to the statistical analyzes, ischemia-reperfusion injury alone caused several changes in the CBC parameters but there were no remarkable differences compared to the control. In particular, conivaptan was significantly reduced the MCH ($p = 0{,}0002$), MCHC ($p = 0{,}0003$), and MONO levels ($p = 0{,}008$) compared to the I/R group. Although DMSO significantly decreased PLT levels ($773{,}2 \pm 49{,}05 \times 10^3$/L) compared to the I/R group ($888 \pm 55{,}85 \times 10^3$/L) and to the control group ($861{,}6 \pm 67{,}89 \times 10^3$/L), conivaptan treatment either alone or combined with boric acid prevented the decrease of PLT levels ($853{,}6 \pm 41{,}35$ and $889 \pm 44{,}2 \times 10^3$/L, respectively). There were no significant differences in terms of RBC, HGB, HCT, WBC, LYMPH parameters, and also NEUT/LYMP, PLT/LYPMH and NEUT/PLT ratios among the groups.

Conclusions

Ischemia-reperfusion injury in unilateral nephrectomized rats caused no considerable changes in CBC parameters at the early injury period. Conivaptan alone significantly reduced monocyte levels, which play a major role in cytokine synthesis, this effect was not observed in combined therapy. We also observed that DMSO was significantly reduced platelet levels, which may be important in animal models used DMSO as a solvent for drugs.

doi: 10.1016/j.cca.2022.04.719

T232

Value of routine preoperative hemostasis testing before elective surgery, results from regional center for transfusion medicine stip

V. Dejanova

Institut of Transfusion Medicine, United States

Background-aim

Our study aimed to analyze the results of routine preoperative haemostasis testing in patients undergoing elective surgery in order to prevent perioperative bleeding.

Methods

This is a retrospective review of patients with scheduled elective surgeries(tonsillectomy,orthopedic surgery,thoracic surgery,gynecology,urological surgery,endoscopy and other procedures),with the exception of neurosurgical patiens.The data is collected from our monthly repost(from My Term) from 2015 to 2018 Regional Centre for Transfusion Medicine Stip.

Results

Of the total 7168 made haemostasis,4863 cases(67.8%) were for preoperative screening,56% were men and 44% were women,with a median age of 44 years.Of these,96.1% had normal cagulation tests,while 191 patiens(3.9%) showed abnormal results.However,more than half of the anormalities found in asymptomatic patients were not confirmed after a second sample analysis.In 73 patients there was only prolongation of PT (mean value od PT was 13sec.) in 52 patients there was a prolongation of PT and aPTT(mean value of aPTT 33.8sec.),and TT was prolonged only in 9 patients(mean value of TT 23.9sec.)The prolongation of the time s explained by the patient's medical history(administration of antiplatelet drug,antibiotics,antirheumatics and other drugs, prostate,cancer,alcohol and drug abuse)Among them,7 patients had liver diseases,two patients with lupus anticoagulant antibodies(LA) and one,11year old child with von-Willebrand disease,confirmed in

ITM Skopje.Other patients the next control had normal findings.None had a lack of coagulation factor in the later detailed examination.

Conclusions

Our analysis shows that only 3.9% of patients had a haemostasis disorder,which could be detected by taking anamnesis,family history or filling in a questionnaire for bleeding risk.Can preoperative hemostasis test predict the risk of perioperativ bleeding?Many studies have shown that unless personal,family history and clinical examination predict an increased risk of bleeding no additional hemostasis test are needed. Excessive preoperative testing causes distress,delay in treatment and unnecessary,costly trials.In the published UK guidelines fo 2003,preoprative coagulation screening is considered inappropriate unless indicated by clinical and family risk.These recommendations were confirmed in 2008 by the British Committe on Standards in Hematology.Official guidelines from various associations of anesthesiologists-NICE guidelines 2016,to streamline preoperative screening no longer recommended routine coagulation testing.but emphasize the need to asses bleeding risk based on personal,family bleeding history and use of bleeding risk questionnaire.Methods such as thromboelastometry ROTEM provide rapid intraoperative diagnosis of the cause of bleeding,but are not practical for use in risk assessment befor operation.

doi: 10.1016/j.cca.2022.04.720

T233

The region of HBB mutation may influence the screening ability of calculated RBC indices in ®-thalassemia minor

J. Park, S. Shin

Department of Laboratory Medicine, College Of Medicine, The Catholic University Of Korea, Seoul, Republic of Korea

Background-aim

®-thalassemia is an interited disorder of blood caused by mutations in the HBB gene that is responsible for ®-globin production. Individuals with the ®-thalassemia trait (®-TT) are usually asymptomatic and may be unaware of their carrier status unless diagnosed by testing. A definitive differential diagnosis between ®-TT and iron deficiency anemia (IDA) is based on the result of HbA2 electrophoresis, serum iron levels, and ferritin calculation. In this study, we compared the ability of eight different calculated RBC indices to distinguish ®-thalassemia minor (®-Tm) from IDA, and estimated the genotype-phenotype correlation between the HBB mutation site location and its influence on the screening ability of the calculated RBC indices.

Methods

Relevant literature including genetically confirmed Korean ®-Tm cases with well-documented clinical summaries and relevant information was reviewed to investigate the mutational gene- or domain-specific laboratory and clinical association. Eight calculated RBC indices were selected and estimated to distinguish Korean ®-Tm confirmed by molecular analysis to differ from IDA.

Results

Twenty one ®-Tm cases carried one heterozygous mutation of HBB. Missense mutations such as p.Met1Arg, p.Leu115Pro, and p.Gln128Arg accounted for half of all the reported mutations (10/21). Deleterious mutations including frameshift (c.270_271del), nonsense (p.Lys18* and p.Glu122*), and splice site (c.93-1G > C and c.315 + 1G > A) mutations were identified in 52% (11/21). Common mutations such as p.Met1Arg and p.Lys18* were identified in exon 1 of HBB, whereas private familial mutations including c.270_271del, p.Leu115Pro, and p.Gln128Arg were dispersed between exon 2 and exon 3. Interestingly, cases with HBB mutations in exon 1 were screened by all eight calculated RBC indices, whereas HBB mutations located on between intron 1 and exon 3 were detected only by a part of these calculated RBC indices ($P < 0.05$).

Conclusions

The region of HBB mutation may influence the screening ability of calculated RBC indices, including RBC counts and RBC indices such as mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width.

doi: 10.1016/j.cca.2022.04.721

T234

A case report of Sysmex XN series Malaria flag

S. Lee [a], S. Namgoong [a], M. Choi [a], C. Park [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*

[b] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

Methods for malaria diagnosis include peripheral blood smear through microscopic examination, the rapid and specific detection of malaria antigens, and polymerase chain reaction (PCR). The major symptoms of malaria infection are headache, fatigue, abdominal distension and muscle pain etc. If fever, anemia and thrombocytopenia ($< 100 \times 10^3$/L) are observed, malaria should be suspected first. A quick diagnostic kit using immunochromatography can be used for screening, but the standard method for malaria diagnosis is microscopic examination.

Methods

Syemex XN series (Sysmex Corporation, Kobe, Japan) has been commercialized since 2019 by applying a function that automatically detects infected RBCs (Red blood cell) with malaria protozoa within one minute. It uses flow cytometry to discriminate blood cells combined with CBC (Complete blood count) and differential count without additional reagents. If malaria is suspected, “pRBC?” (parasitized RBC) flag is displayed to inform the testing personnel.

All CBC tests performed by Asan medical center are being screened for malaria. We report a case that had malaria flag and confirmed by peripheral blood smear examination since malaria screening system applied at our laboratory at October 2018.

Results

A 21-year-old male with cytopenia and splenomegaly was referred. The CBC results were WBC (White blood cell) 4.01 x 10^3/L, RBC 3.12 x 10^6/L, Hemoglobin 10.0 g/dL, Platelet 114 x 10^3/L, and Reticulocyte

10.24% and malaria particles were found in the WNR main (SFL-FSC) scattergram. Infected RBCs with Plasmodium vivax were observed on the thin and thick blood smears stained with Wright-Giemsa. The patient was not suspected of malaria clinically and could be treated quickly with hydroxy chloroquine.

Conclusions

In this case, it suggests that malaria flag is a useful indicator of rapid diagnosis. From October 2018 to present, only one malaria positive was found through malaria screening system and it is necessary to improve sensitivity and specificity. However, since delay in early diagnosis and treatment may result in serious death, it may be helpful to diagnose malaria with an automated hematology analyzer in areas where there is no clinically suspected malaria or no malaria epidemic.

doi: 10.1016/j.cca.2022.04.722

T235

Clinical significance of cell population data (CPD) on Sysmex XN in the differential diagnosis of the lymphocytosis

M. Seghezzi, G. Previtali, V. Moioli, P. Dominoni, G. Guerra, S. Buoro

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-aim

The development of a new generation of hematological analyzers shows a high degree efficiency to identify cellular abnormalities. The cell population data (CPD) can be used for the screening of several hematological and non-hematological disorders. The Sysmex XN-9000 (XN-module) besides the conventional hematologic parameters, generate 22 CPD parameter of neutrophils, lymphocytes (LY) and monocytes. Their assessment may be useful in the diagnosis of myelodysplastic syndromes and sepsis, but there are few studies about the application of LY CPD parameters in differential diagnosis of lymphocytosis (i.e., viral diseases from lymphoproliferative disorder). The aim of this study was to evaluate the usefulness of the LY CPD parameters to differentiate among acute lymphoblastic leukemia (ALL), other lymphoproliferative disorders (abn-LY), non-neoplastic lymphocytosis (react-LY), acute myeloid leukemia (AML) and other hematological disease (oth-HD).

Methods

The study population included 742 patients (16 with ALL, 150 with abn-LY, 34 with AML, 59 with reac-LY and 488 with oth-HD). We evaluated the following LY CPD parameters obtained by XN-module: LY-X, LY-Y, LY-Z, LY-WX, LY-WY and LY-WZ. The diagnostic performance was evaluated by ROC curve analysis.

Results

LY-WX, LY-WY and LY-WZ showed an area under the ROC curve (AUC) of 0.86, 0.85 and 0.75 respectively in the discrimination between react-LY and other groups; LY-X and LY-Z showed an AUC of 0.60 and 0.69 respectively in the discrimination between abn-LY and other groups; LY-X showed an AUC of 0.82 in the discrimination between ALL and other groups. The combination of LY-WX, WY and WZ parameters in react-LY-factor showed an AUC of 0.87, differentiating react-LY from other diagnostic groups.

Conclusions

The lymphocytes positional parameters provided by XN-module are useful to differentiate react-LY (react-LY-factor) and ALL (LY-X) from other diagnostic groups. These parameters are very useful for detecting changes in the lymphocyte population; in fact, the identification of these alterations can induce the need to carry out a blood smear review in samples without morphological flagging.

doi: 10.1016/j.cca.2022.04.723

T236

Alterations of the complete blood count in natalizumab-treated patients with multiple sclerosis

V. Moioli, M. Seghezzi, G. Previtali, P. Dominoni, G. Guerra, S. Buoro

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-aim

Natalizumab is a drug for the treatment of patient with relapsing remitting multiple sclerosis (RRMS). Its action prevents the binding between VLA-4 with VCAM-1 implaying a reduction of the leukocytes migration through the endothelium, reducing the inflammatory infiltrate. However, the use of this monoclonal antibody appears to cause an increase in circulating stem cells in patients subjected to this therapy. The aim of the study is to investigate the alteration of hematological parameters, including cell population data, in patients with natalizumab therapy.

Methods

443 peripheral blood samples were analyzed with the automated analyzer sysmex XN-9000 and the microscopy revision were done. The outcomes of interest have been analyzed with a stratified statistical analysis for natalizumab use or not. The quantitative variables were evaluated as mean, standard deviation, median, interquartile range and the p-values were considered significant only below 0.05.

Results

From the analysis of the results obtained for the leukocyte parameters it is emerging as the values are significantly higher in patients treated with natalizumab compared to the control groups. Erythroblasts ($0.02*10^9$/L), monocytes ($0.68*10^9$/L), lymphocytes ($4.17*10^9$/L), immature granulocytes ($0.04*10^9$/L), eosinophil ($0.26*10^9$/L), basophils ($0.06*10^9$/L) and the LY-WY parameter (993.30ch) are increased in treated patients compared to naives. The counting of monocytes and immature granulocytes is significantly increased in natalizumab patients compared to patients treated with other drugs (p-value 0.001 and 0.0001 respectively). It has been observed that the treatment with natalizumab is the only that induce an increase of eosinophils, which decreases in patients with RRMS ($0.26*10^9$/L vs $0.14*10^9$/L) and basophils, that decrease into patients under others therapy ($0.06*10^9$/L vs $0.03*10^9$/L). The increase in leukocytes count in that sense finds confirmation in literature data and appears attributable to the inibition of VLA-4, which sequests the cells in the peripheral circle, preventing the correct homing.

Conclusions

The long-term consequences on hematopoiesis are not been defined yet so other studies are required in order to evaluate any other alterations.

doi: 10.1016/j.cca.2022.04.724

T237

Comparison study between a lateral flow immunochromatographic assay and haemoglobin H staining for the screening of alpha thalassaemia

J.Q. Tham, G.L. Wee, H.J. Yeo, C.H. Yeoh, P.Y. Heng, M.S. Wong

Department of Laboratory Medicine, Khoo Teck Puat Hospital, Singapore

Background-aim

In Khoo Teck Puat Hospital (KTPH) Department of Laboratory Medicine, alpha-thalassaemia screening is done by microscopic examination for precipitated Haemoglobin H (HbH) in red blood cells stained with brilliant cresyl blue. Screening for HbH inclusions can be time-consuming in individuals with alpha-thalassaemia minor. In order to increase screening efficiency, we evaluated a lateral flow immunochromatographic assay iLAB ⟨THAL IC Strip Test (IC Strip, i + MED Laboratories, Thailand) to replace microscopy.

Methods

Routine samples for thalassaemia screening (n = 97) were concurrently set up using both assay methods. The results from IC Strip were compared against that of HbH microscopy performed by experienced technologists. Samples with discordant results were sent for alpha-thalassaemia genotyping for resolution. The findings were tabulated using a binary matrix and positive and negative agreement between assays calculated.

Results

The positive and negative agreement between IC Strip and HbH microscopic examination was 88% and 81% respectively. There was concurrence between 26 negative and 57 positive alpha-thalassaemia samples. Analysis of 14 discordant samples showed that IC Strip had 86% concordance with alpha-thalassaemia genotyping, implying that the IC Strip was more sensitive than microscopy. The average analysis times for microscopy is 30 minutes for positive samples and between 60 to 120 minutes for negative samples. Analysis by IC Strip requires only 20 minutes.

Conclusions

Our evaluation demonstrated that alpha-thalassaemia can be expeditiously and accurately performed using IC Strip assay. Due to its ease of use, relative low cost, short analysis time and clear interpretation, it can replace microscopy in alpha-thalassaemia screening work-up in a routine laboratory.

doi: 10.1016/j.cca.2022.04.725

T238

Evaluation of activated lymphocytes (RE-LYMP, AS-LYMP) on hematological analyser sysmex XN-1000 with manual method

D. Kajinić, K. Grdiša

Department of Hematological Biochemical Diagnostics,General County Hospital Požega, Požega, Croatia

Background-aim

Novel hematological parameters for rapid monitoring of the immune system response as reactive lymphocytes (RE-LYMP) and T cell-independent plasma cells (AS-LYMP) obtained from a routine blood count can improve and speed up diagnostic procedure. Reference interval of total reactive lymphocytes are 0-5%, as a % of all white blood cells (WBC). The aim of this study is to evaluate agreement between manual and automated findings of activated lymphocytes.

Methods

Venous blood (K2E, BD vacutainer) from patients (n = 112) whose differential blood count showed total reactive lymphocytes % more than 1 % measured by Sysmex XN1000 were taken for manual differentiation. A medical laboratory scientist counted and described 200 WBC on a stained microscope slide (May-Grunwald-Giemsa staining). Method comparison were assessed in MedCalc statistical program.

Results

Bland and Altman(B&A) plot of RE-LYMP (measured by Sysmex) and atypical lymphocytes-suspect reactive, suspect neoplastic and plasma cells (counted in blood smear) revealed the BIAS of -0,3 units (95% CI -1,0846 to 0,5614, upper limit 8,3537 and lower limit -8,8769) not a constant error but statistically a significant difference. B&A analysis of AS-LYMP (measured by Sysmex) and plasma cells (counted in blood smear) revealed the BIAS of 2,4 units (95% CI 1,6488 to 3,1603, upper limit 7,2767 and lower limit -2,4676) existence of constant error and statistically a significant difference. Westgard desirable specifications for lymphocytes count is 10,2 for within-subject biologic variation and Croatian Centre for Quality Assessment in Laboratory Medicine (CROQALM) defined table of allowable deviation for lymphocytes as 20%.

Conclusions

The B&A plot method only defines the intervals of agreements. Acceptable limits must be based on clinical necessity, biological considerations and lead to improvement of patient management and help start or modify treatment faster. A microscope will always be gold standard for cell differentiation, but new parameter RE-LYMP in this study showed low BIAS and limits that fit in Westgard and CROQALM defined criteria so we can conclude growing contribution on patient management. AS-LYMP parameter showed significant difference, poor agreement and the need for improvement.

doi: 10.1016/j.cca.2022.04.726

T239

Von Willebrand factor levels in patients with systemic lupus erythematosus and antiphospholipid syndrome

N.K. Tóth [c], T. Tarr [a], P. Soltész [a], F. Sarkady [c], J. Kappelmayer [b], L. Muszbek [c], Z. Bagoly [c]

[a] *Department of Clinical Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[b] *Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[c] *Division of Clinical Laboratory Sciences, Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Background-aim

Patients with systemic lupus erythemasosus (SLE) are subject to significant morbidity and mortality due to atherosclerotic diseases, which cannot be fully explained by traditional risk factors. Antiphospholipid syndrome (APS) is characterized by recurrent thrombosis and pregnancy morbidity in the presence of antiphospholipid antibodies (aPL). APS can be primary or secondary, often associated with SLE. To find out whether levels of von Willebrand factor (VWF) and factor VIII (FVIII) might differ in patients with SLE and/or APS as compared to healthy individuals and if levels are associated with thrombotic episodes.

Methods

In this observational study, 137 patients with SLE and/or APS and 140 age and sex-matched healthy controls were enrolled. Patients were grouped: SLE without APS (n = 56), SLE with APS (n = 33), primary APS (n = 22), SLE with aPL without clinical symptoms of APS (n = 20), aPL without clinical symptoms of APS (n = 6).VWF antigen levels and factor VIII (FVIII) activity using chromogenic assay were measured from platelet poor plasma samples. Clinical parameters including age, sex, BMI, smoking habit, traditional risk factors, thrombotic history, and disease activity were registered.

Results

VWF antigen levels and FVIII activity were significantly elevated in SLE and APS patients (primary or secondary) as compared to controls. In asymptomatic subjects with circulating aPL antibodies, VWF antigen levels and FVIII activity were within reference range. No association was found between SLE/APS autoantibody titers, complement levels, and FVIII, VWF, fibrinogen levels. Among SLE patients, VWF and FVIII levels were signicantly elevated in those with a history of atherothrombosis. A VWF antigen level or a FVIII level in the upper tertile conferred a significant risk for arterial but not venous thrombotic events or pregnancy morbidity in SLE patients (VWF: OR: 29.6; 95% CI:5.12-170.7, $p < 0.001$; FVIII: OR: 15.07; 95% CI:3.08-73.6, $p = 0.001$).

Conclusions

Elevated VWF and FVIII levels are associated with increased atherothrombotic risk in SLE patients.

Funding: NKFI 128582.

doi: 10.1016/j.cca.2022.04.727

T240

Evaluation of platelet reactivity in patient with recurrent ischemic stroke and clopidogrel resistance

D. Dineva, I. Paskaleva

Laboratory Department, National Heart Hospital, Sofia, Bulgaria

Background-aim

High on-treatment platelet reactivity (HPR) is associated with an increased risk for ischaemic complications. Assessment of platelet response is useful for individualisation of antiplatelet therapy and determining the appropriate dose. The purpose of the assays was to investigate the suppression of platelet reactivity.

Methods

We are reporting a clinical case of 51-year-old woman with recurrent ischemic stroke: first ischemic stroke (1 years ago) during aspirin therapy (100 mg/day); second ischemic stroke during clopidogrel therapy (75 mg/day). The patient was referred at our laboratory for thrombophilia examinations. We performed assessment of antiplatelet therapy with Multiplate impedance aggregometry (MEA) by ADP tests.

Results

Coagulation tests for thrombophilia screening in this patient were in reference ranges: Protein C - 138 % (70-140%); Free PS - 113% (60-113%); AT III - 115% (80-120%). Genetic tests for R506Q mutation and G/A 20210 mutation have shown normal genotype. The patient's platelet count were also normal – PLT 163 G/l (140 – 400 G/l). We found very high residual ADP-induced platelet aggregation 133 U (optimal inhibition at 15-45 U) on clopidogrel therapy after the second ischemic stroke. We switched to more potent and effective P2Y12 inhibitor – prasugrel 10 mg/d. After 20 days we found again insufficient response with high ADP-aggregation of 89 U. Additional platelet inhibition was observed after 1 month when the prasugrel dose was increased to 15 mg/d: ADP-test was 58 U.

Conclusions

We studied platelet reactivity using MEA to determine the more aggressive and appropriate P2Y12 receptors blocker – 15 mg/d prasugrel in order to prevent subsequent thrombotic event.

doi: 10.1016/j.cca.2022.04.728

T241

Prevalence of gene mutation in congenital thrombophilia for Serbian population

N. Stupar, M. Ilic

Aqualab Plus Laboratories, Belgrade, Serbia

Background-aim

In Aqualab plus laboratories, genetic analyses of congenital thrombophiliae were carried out in both genders by the Polymerase Chain Reaction PCR method. The investigations were performed in the period

from 01st January 2017 to 31st August 2019 in 695 patients from which 653 were women and 42 men.

Thrombophilia is a disorder of haemostatic system which increases inclination to blood clotting and arise of arterial and venous thromboses. Thrombophilia may be congenital (hereditary) and acquired. The congenital ones are the consequence of genetic mutations, and the presence of two or more mutations is possible. If the result is positive, mutations bearers may be heterozygotes (when they have only one out of two genes that underwent mutation), which means that the mutation is less manifested and homozygotes (when both genes underwent mutation), when mutation is more strongly manifested. Congenital tromophiliae are inherited autosomal dominantly, which means that one of the parents surely has the same disorder. In addition to the existence of congenital thrombophilia, at the same time both congenital and acquired thrombophilia may exist.

In this study the examination of the presence of the following mutations are presented:

- R506Q (G1691A, rs6025) in the gene for coagolation factor V (Leiden V, FV)
- G20210A (rs1799963) in the gene for coagulation factor II (Prothrombin II, PII)
- C677T (c.665CT, A222V, rs1801133) in the gene for methylentetrahydrofolate reductase (MTHFR)
- 4G/5G polymorphism in the gene for plasminogen activator Inhibitor -1 (PAI-1)

Methods

Polymerase Chain Reaction PCR method from EDTA blood

Results

On the basis data obtained in the mentioned period it is obvious that out of the 695 total number of respondents, most of them are women, 653, whereby more of them are newly discovered women (100 homozygotes and 268 heterozygotes) for MTHFR and PAI-1(217 homozygotes and 294 heterozygotes) in respect to FV and FII, which makes them significantly more frequent.

Conclusions

Given that this type of examination is carried out once in life time, the number of newly discovered is significant in respect to the total number of respondents.

doi: 10.1016/j.cca.2022.04.729

T242

Associations between delta neutrophil index and protein C in septic patients

P. Peneva [b], Y. Bocheva [b], K. Yankov [b], S. Nikolova [a]

[a] *Department of Social Medicine and Healthcare Organization, Medical University – Varna, Bulgaria*
[b] *UMHAT "St. Marina", Medical University – Varna, Bulgaria*

Background-aim

Sepsis is a serious life-threatening clinical syndrome caused by a severe infection in the body. Accurate and timely diagnosis are extremely important for the life of patients though, the management of sepsis and septic shock are difficult. Delta Neutrophil Index [DNI] is calculated as the difference between the leukocyte differential in the MPO channel and the leukocyte differential in the nuclear lobularity channel using an automatic hematology analyzer. The present study evaluates the relationship and the accuracy of DNI and Protein C as predictive factors for sepsis.

Methods

A prospective clinical follow-up study was conducted from January 2017 to May 2018 in a Bulgarian ICU. We enrolled 82 patients. About 37 of them were infected patients without any criteria of sepsis and 45 patients covered the sepsis criteria, according to SEPSIS-3 (2016). DNI and Protein C were measured in all patients. DNI was determined by using Advia 2120i hematology system (Siemens) and Protein C was assessed by SYSMEX CA-1500(Siemens). Correlations and logistic regressions were performed to test for associations and whether DNI and Protein C were prognostic factors for sepsis and septic shock. Independent t-test was used to assess the differences in the means of both indicators in patients with and without sepsis.

Results

Our results showed that both Protein C and DNI had lower mean values in sepsis patients, however significant differences were observed in Protein C values ($t = -28{,}47$, $p = 0.0001$).

Protein C and DNI were negatively associated in sepsis patients ($r = -0.302$, $p = 0.007$). Significant associations were additionally found between Protein C and sepsis development ($r = 0.563$, $p = 0.0001$) and DNI and sepsis development ($r = 0.363$, $p = 0.001$). DNI was a significant predictive factor for developing sepsis ($Exp(B) = 1{,}329$, $p = 0{,}007$) and septic shock ($Exp(B) = 1{,}430$, $p = 0{,}001$).

Conclusions

Early detection and treatment of sepsis are essential to improve patient's outcome. There are correlations between routine markers for detection and tracking of inflammation, and some other indicators, which, if specifically researched and targeted, would significantly improve early diagnosis of sepsis. DNI and Protein C may be valuable tools in assessing the early diagnosis of sepsis.

doi: 10.1016/j.cca.2022.04.730

T243

Thrombocytopenic thrombotic Purpura associated with Goujerot Sjögren syndrome

A. Bentahila [b], A. Belmekki [c], S. Nader [a], M. Ztout [a], H. Kabbaj [a], S. El Hayani [a], M. Tber [a]

[a] *Cheikh Zaid Hospital, Rabat, Morocco*
[b] *Hospital Cheikh Zaid, Rabat, Morocco*
[c] *Military Hospital, Rabat, Morocco*

Background-aim

Association between thrombocytopenic thrombotic purpura and Goujerot Sjögren syndrome

Methods

Clinical case:

We report the case of a 34-year-old patient who displayed a Gougerot-Sjögren syndrome with neurological symptoms under corticosteroid therapy and photosensitivity in her case history.

The clinical and biological picture of non cholestatic jaundice and hemorrhagic syndrome (ecchymotic purpura of the upper and lower limbs) evoked a PTT. The ADAMS 13 activity was collapsed (<1%), and the search for anti-ADAMS 13 autoantibodies was positive (>15 U / ml).

The assessment in search of an autoimmune disease including connectivitis found positive ANA at 160 with a speckled fluorescence, and positive anti SSA at 137.79.

Results

Discussion and conclusion:

The association of Gougerot-Sjögren syndrome with PTT is very rare in literature, with an incidence of 5.4%. The SGS then appears not very symptomatic and is frequently associated with antiSSA antibodies, as in our patient's case. The association with SGS does not seem to worsen the prognosis of PTT.

The occurrence of PTT could be linked to endothelial activation by direct action of anti-cardiolipin antibodies or by other mechanisms (deficiency of von Willebrand factor protease activity). The evolution is pejorative without immunosuppressants, and the discovery of a PTT must therefore encourage to seek a SGS, which could lead to the modification of the therapeutic strategy in the direction of an early corticotherapy, as in our patient's case.

Conclusions

Discussion and conclusion:

The association of Gougerot-Sjögren syndrome with PTT is very rare in literature, with an incidence of 5.4%. The SGS then appears not very symptomatic and is frequently associated with antiSSA antibodies, as in our patient's case. The association with SGS does not seem to worsen the prognosis of PTT.

The occurrence of PTT could be linked to endothelial activation by direct action of anti-cardiolipin antibodies or by other mechanisms (deficiency of von Willebrand factor protease activity). The evolution is pejorative without immunosuppressants, and the discovery of a PTT must therefore encourage to seek a SGS, which could lead to the modification of the therapeutic strategy in the direction of an early corticotherapy, as in our patient's case.

doi: 10.1016/j.cca.2022.04.731

T244

To slice or skim? A case of clinical confusion due to erroneous M-protein quantification

A. Babalhavaeji [b], P.C. Chan [a]

[a] *Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre and LMP, University of Toronto, Canada*
[b] *Laboratory Medicine and Pathobiology, University of Toronto, Canada*

Background-aim

Quantification of the M-protein by serum protein electrophoresis (SPE) is an important tool in the management of monoclonal gammopathies. It is commonly performed by integrating the M-protein peak on an electropherogram based on a perpendicular drop (PD) algorithm that includes both M- and background-proteins above baseline. Alternative approach using a tangential skim (TS) algorithm became available in recent years but its adoption remained scanty. We report a case of how using TS could have helped reducing clinical confusion about relapse in a multiple myeloma patient in remission.

Methods

We followed the course of treatment on a multiple myeloma patient over 16 months using both PD and TS approaches on the Sebia Capillarys II Electrophoretic System. Correlation with other standard-of-care parameters such as serum free light chains (BindingSite), albumin, creatinine and CBC were also made.

Results

At diagnosis the patient had an IgG kappa M-spike of 44 g/L (PD) migrating in gamma region. Following chemotherapy and autologous stem cell transplantation (SCT), the M-spike decreased to 6 g/L with a normal free light chain ratio of 1.64 (0.26-1.65). Two months post-transplant, the M-protein increased from 6 to 10 g/L (PD) although the non-involved gamma fraction increased from 3 to 21 g/L. Together with decreases in red cell, platelet and white cell counts, a relapse was suspected. A bone marrow biopsy was performed but did not reveal any significant plasmacytosis or clonal restriction. Prior to SCT, quantification of M-spike using TS and PD differed, on average, by 1.2 ±0.3 (SD) g/L or 12± 5%. Re-analysis of the M-spike by TS gave a result of 1 g/L as compared to 10 g/L by PD, indicating the inclusion of mostly polyclonal gammaglobulins in the latter. Serum albumin and creatinine did not change significantly and the patient had not worsened clinically.

Conclusions

Contrary to the PD method, the TS method produced results in line with the patient's clinical and laboratory findings. Our case demonstrates how the choice of integration method in the quantification of the M-spike could create confusing results for the clinicians and undue stress to the patient.

doi: 10.1016/j.cca.2022.04.732

T245

Evaluation and management of Anemia among the women of Bangladesh

J.H.B. Masud

Public Health Informatics Foundation, Bangladesh

Background-aim

Anemia is recognized as a risk factor for a number of adverse outcomes in the women and that including hospitalization, morbidity, and mortality. Anemia is primarily caused by iron deficiency but also by other micronutrient deficiencies such as vitamins A, B2, folate, B12, and minerals like selenium, and copper. Bangladeshi women are mostly affected by anemia. In Southeast Asia, it has been estimated that iron deficiency anemia is the cause of 26% of all anemia in reproductive women. The inclusion of micronutrient-rich foods in the daily diet, such as red meat, green leafy vegetables and some nuts or seeds, is often not

affordable for populations living under conditions of poverty in countries like Bangladesh. In this context, food fortification could also be a cost-effective contribution to food-based approaches for preventing anemia by increasing the intake of iron and other micronutrients, a lack of which can cause anemia. The complementary and alternative medicine has a role for the treatment of anemia. There is a scarcity of evidence on the etiology of anemia and their relative proportion to the causes of anemia. So, we need to explore the appropriate management for a female patient with anemia. The aim of this study to assess how the complementary and alternative medicine play a role for the management of anemia.

Methods

An observational study was conducted in September to November 2019 in a district hospital in Bangladesh. We selected 30 patients for assessment of anemia management. Tablet. Arubin (complementary and alternative medicine) was given for the treatment of anemia. Tablet. Arubin was given twice daily for one month each patient. After one month, the patients were assessed by clinical examination as well as hemoglobin test.

Results

More than 70% patient's hemoglobin raised from 7g/dl to 11g/dl. Among the respondents 10% had no change. The symptom of the anemia disappeared after 2 weeks of treatment.

Conclusions

Anemia reduced more significantly when the women provided tablet. Arubin. So, the complementary and alternative medicine may reduce the anemia. However, we need a large-scale study for generalization our results.

doi: 10.1016/j.cca.2022.04.733

T246

HFE genotype and allele frequencies in the Balearic islands for the diagnosis of hemochromatosis

P. Argente Del Castillo Rodríguez [a], B. López Andrade [b], M. Mesquida [a], C. Gómez Cobo [a], M.A. Ballesteros Vizoso [a], B. García García [a], J.A. Delgado Rodríguez [a], J.M. Bauça [a]

[a] *Department of Laboratory Medicine, Hospital Universitari Son Espases, Spain*
[b] *Haematology Department, Hospital Universitari Son Espases, Spain*

Background-aim

Hereditary hemochromatosis (HH) is an inherited iron overload disorder. General population screening for HH, before organ damage is present, has not been established. Real world experience tells us that awareness of both physicians and the general population is a cornerstone in the detection of HH, since current molecular studies in our population are performed mostly as a confirmatory test after physician suspicion due to high transferrin saturation or in the presence of an already diagnosed family member.

In most cases, HH is caused by homozygous p.Cys282Tyr (C282Y) or p. His63Asp (H63D) mutations in the HFE gene. Our aim was to evaluate the genotypes seen in our institution and examine allele frequencies of both mutations.

Methods

All hemochromatosis mutation requests received in our laboratory during January 2014 and December 2019 were included in the study. Requests were based on high ferritin levels, high transferrin saturation indices or due to family history of iron overload or HH.

HH most frequent polymorphysms (C282Y and H63D) were analyzed by Polymerase Chain Reaction and melting curve analysis with FRET probes on the LightCycler 2.0 thermocycler (Roche). Genotype frequencies were evaluated, and allele frequencies were calculated.

Results

A total of 1338 patients were included and classified in six groups depending on their genotype: normal C282Y normal H63D (N = 588); heterozygous C282Y normal H63D (N = 107); normal C282Y heterozygous H63D (N = 417); homozygous C282Y normal H63D (N = 37); normal C282Y homozygous H63D (N = 111), double heterozygous (N = 78).

Given these results, genotype frequencies were 54.7% normal, 36.9% heterozygous and 8.3% homozygous for H63D and 83.4% normal, 13.8% heterozygous and 2.7% homozygous for C282Y.

The mutated H63D allele was present in 26.8% of our attended population, and mutated C282Y allele was found in 9.7%.

Conclusions

H63D polymorphism is more prevalent in our population than C282Y, which is in accordance with previous studies in our country, whereas in other regions the most frequently mutated allele for HH is C282Y.

doi: 10.1016/j.cca.2022.04.734

T247

Mass-fix detects M-protein class-switch: A rare finding in myeloma with clinical implications

J. Murray, D. Murray

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, United States

Background-aim

Our laboratory has recently transitioned from traditional gel electrophoresis to a mass spectrometer based method for the detection and monitoring of plasma cell disorders. This process has afforded us an opportunity to observe new phenomenon in the treatment responses of patients over time. Recently, A class switch of a monoclonal protein was observed in the context of a treated multiple myeloma (MM) patient.

Methods

Isotype class switching has seldom been reported in cases of MM and further investigation was warranted. M-protein subclass typing was subsequently performed as well as immunoprecipitations coupled to liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results

This highly unusual incident occurred three years post-diagnosis and two years after an autologous stem cell transplant. The patient in question is a 62 year old woman with a history of IgA MM, but recent laboratory testing clearly demonstrates the rising of an IgG monoclonal protein. The emergence of new M-proteins in the setting of bone marrow reconstitution is not a rare phenomenon, and these emergent clones are typically thought of as unrelated to a patient's clonal plasma cell population. In this instance, however, MALDI-TOF (Matrix Assisted Laser Desorption Ionization – Time of Flight) mass spectrometer testing demonstrated that this IgG monoclonal had a light chain mass identical to the mass of the MM's clonotypic IgA. These matching light chain masses were highly suggestive of a uniform amino acid structure and, therefore, a singular B-cell progenitor. These findings were confirmed by LC-MS/MS.

Conclusions

This case demonstrates the clinical value of molecular mass identification for monoclonal protein isotyping by MALDI-TOF mass spectrometry. It also provides an avenue for considering immunoglobulin isotype class switching, memory B-cells, and the current understanding of MM pathogenesis.

doi: 10.1016/j.cca.2022.04.735

T248

Reference intervals for 10 platelet parameters on Mindray BC-6800 plus hematology analyzer

H. Kim, M. Hur, S. Kim, H. Moon, Y. Yun

Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, South Korea

Background-aim

Recently introduced Mindray BC-6800Plus hematology analyzer (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) has adopted new technologies to measure platelets (PLTs) and PLT parameters. We established new reference intervals (RIs) for 10 PLT parameters on BC-6800Plus, including percentage immature PLT fraction (%-IPF), absolute IPF (A-IPF), high fluorescent IPF (H-IPF), mean PLT concentration (MPC), mean PLT matter content (MPM), mean PLT volume (MPV), plateletcrit (PCT), PLT distribution width (PDW), PLT large cell counts (P-LCC), and PLT large cell ratio (P-LCR).

Methods

Blood samples from 1,238 healthy individuals (704 men and 534 women) were analyzed using BC-6800Plus. RIs for PLT parameters were defined using non-parametric percentile methods, according to the Clinical and Laboratory Standard Institute guidelines (EP28-A3C).

Results

PLT parameters all showed non-parametric distributions. The RIs for PLT parameters were as follows: %-IPF, 0.6 – 5.6%; A-IPF, 1 – 13 x 10^9/L; H-IPF, 0.1 – 1.0%; MPC, 21.8 – 26.6 g/L; MPM, 2.2 – 2.8 pg; MPV, 8.4 – 12.2 fL; PCT, 0.2 – 0.4%; PDW, 15.5 – 16.7 fL; P-LCC, 39 – 101 x 10^9/L; and P-LCR, 14.5 – 40.9%.

Conclusions

The new RIs for PLT parameters on BC-6800Plus would provide fundamental data for clinical practice and future researches.

doi: 10.1016/j.cca.2022.04.736

T249

Necessity of manual reticulocyte counts for accurate and precise result

A.R. Cho, H.S. Lim, K.R. Lee

Department of Laboratory Medicine, Seoul Clinical Laboratories (SCL), Yong-in, South Korea

Background-aim

The reticulocyte count, reported as percentage of total RBCs reflects erythropoietic activity of bone marrow. The objective of this study was to compare of manual test and automated analyzer in high level reticulocyte count.

Methods

During the study period (from September to November 2019), 9,901 samples of reticulocyte count were ordered with complete blood counts (CBCs). The test of CBCs and reticulocyte count were analyzed by Sysmex XN-2000 (Sysmex, Kobe, Japan) and high level samples of reticulocyte count (above 5.0%) were retested by manual method.

Results

Of the 9,901 samples, 136 samples were showed result above 5.0 %. The comparison between the results obtained by sysmex XN-2000 and the manual method had a good correlation except 20 samples. 20 (14.7%) samples were showed discrepancy result with manual test and sysmex XN-2000.

Conclusions

Manual reticulocyte stain have been replaced by automated hematology analyzers, which is easily and rapidly performed and have much greater precision. But automated test result should be confirmed by manual method at High level of reticulocyte count.

doi: 10.1016/j.cca.2022.04.737

T250

Relationship of economic status with the occurrence of anemia in the third trimester of pregnant women at Caile Health Center, Bulukumba regency, South Sulawesi

R. Fajar, N.I. Kurniastuti, D. Mustikasari

Department of Computational Biology, Yogyakarta State University, Indonesia

Background-aim

Anemia in pregnancy is a condition of the mother with hemoglobin (Hb) levels < 11 gr% in the first and third trimesters while in the second

trimester hemoglobin levels < 10.5 gr%. Anemia in pregnancy is called "potentional danger to mother and child", therefore anemia requires serious attention from all parties involved in health care. Anemia in pregnancy is an indicator of poor health. The main cause of anemia is inadequate food intake based on family income. This study aims to look at the relationship of economic status with the incidence of anemia in third trimester of pregnant women.

Methods

This research uses analytic design with cross sectional approach. The target population in this study were all of the pregnant women in the Caile Region, Bulukumba Regency. The sample in this study was the third trimester of pregnant women who came to the Caile Health Center, Bulukumba Regency who had fulfilled the inclusion and exclusion criteria with a total of 87 people. In this study using univariate analysis in the form of frequency distribution and bivariate analysis with chi-squared test.

Results

The results of the analysis of the relationship between economic status and the incidence of anemia in the third trimester of pregnant women showed that there were 25 out of 39 people (61.0%) of mothers whose income was < PMW (Provincial Minimum Wage) had anemia, while among pregnant women earning ε PMW there were 16 of 47 people (39.0%) had anemia. Chi-squared statistical test results showed that the value of p = 0.005 (p δ 0.05), RP 3.460 (95% CI = 1.421 - 8.425), it can be concluded that mothers with income < PMW increase the incidence of anemia by 3.4 times compared with the mothers with income > PMW. The third trimester of pregnant women with low economic status are more at risk of anemia. This relates to people's purchasing power. Counseling provided is to increase compliance with the Fe tablet and to choose foods that contain high protein but with a low price.

Conclusions

Based on the results of the research, theoretical study and elaboration, the researcher concluded that economic status plays an important role in the incidence of anemia in the third trimester of pregnant women at Caile Health Center, Bulukumba Regency, South Sulawesi.

doi: 10.1016/j.cca.2022.04.738

T251

Performance evaluation of BC6800 hematology analyzer

E. Avci, S. Demir, R. Nar, H. Aybek, K. Akpinar

Medical Biochemistry Department, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Background-aim

In medical laboratories, performance requirements and method verification studies should be done, before starting with the new analyzer. The verification of performance specifications confirms that the instrument and/or test method performs as the manufacturer intended when utilized in a laboratory environment. Complete Blood Count (CBC) is a simple and cheap analysis that is frequently done in medical laboratories. Complete blood count devices often perform analysis based on electrical impedance and optical density methods. In our study, it was aimed to perform Mindray BC 6800 automatic hematology analyzer performance evaluation newly established in our medical laboratory.

Methods

We analyzed 44 patient samples, on different platforms, Siemens ADVIA® 120, Sysmex XN-350 and Mindray BC 6800. The averages of all three devices for each parameter, binary device comparison and compatibility between the three devices were evaluated by the Fleiss kappa coefficient. The data were evaluated with the SPSS package program.

Results

When we compared three auto analyzers, there was no significant difference was in all CBC parameters except eosinophil, MCHC, and platelet. The lowest accordance was found in the monocyte (Mindray & Siemens r = 0.727), and the highest was in the hemoglobin (Mindray & Sysmex r = 0,998).

Conclusions

The manufacturer cannot perform the actual testing for a clinical laboratory but may assist by providing samples to test, by providing procedural instructions, and/or by performing mathematical calculations and analysis. A comparison of the newly set up device with the previous one is an important issue. In our study, we studied 44 samples to compare three devices and reveal performance. Our findings showed the new auto analyzer could use.

doi: 10.1016/j.cca.2022.04.739

T252

[LDQUO] Peripheral blood involvement by AITL: A case report and review of the literature [RDQUO]

G. Jain, C. Kumar, A. Chopra, S. Bakhshi

All India Institute of Medical Sciences, India

Background-aim

Angio-immunoblastic T cell lymphoma (AITL) is an aggressive variant of peripheral T cell lymphoma occurring in elderly adults without any gender predisposition. It accounts for 1–2 % of all non-Hodgkin lymphoma. Although characterized by some peculiar histological features, diagnosis of AITL can sometimes be challenging and a definite diagnosis requires a complete immunophenotypic and molecular workup

Methods

Peripheral Blood (PB) involvement has not been studied in detail and there is a paucity of published data about the leukemic presentation of AITL. We present a case of a 38-year-old female diagnosed as AITL with PB involvement.

Results

Flow cytometry (FCM) examination of PB showed 40% abnormal lymphoid cells which were CD45+, CD4+, CD2+, cCD3+, CD5+, CD10+, CD16+ and TCR gamma/delta restricted.

Conclusions

PB involvement by AITL appears to be more common and under-reported than reported by previous studies. Nevertheless, detection of these tumoral T lymphocytes needs to be assessed in large case studies for assessing the true incidence of PB involvement. FCM analysis is an effective and reliable approach in the identification of leukemic phase of AITL and can lead to timely and effective intervention.

doi: 10.1016/j.cca.2022.04.740

T253

Extra-nasal NK-T cell lymphoma: A rare case with a rarer presentation

C. Kumar, I. Haider, P. Tanwar, S. Bakhshi

All India Institute of Medical Sciences, India

Background-aim

Extra-nodal (NK/T) cell lymphoma of nasal type (ENKL) is an aggressive type of lymphoma which is localized primarily to nasal and upper airway region. ENKL without apparent nasal involvement is called extra-nasal ENKL and can involve skin, testis, intestine, muscle, or present as a disseminated disease in few rare cases where it is known as extra-nasal ENKL.

Methods

A 65-year old male presented with fever, bilateral orbital swelling, right parotid swelling, mediastinal nodes, pleural effusion and hepatomegaly. Contrast enhanced computed tomography (CECT) of whole body revealed an ill-defined soft tissue mass in the orbital region, mediastinal nodes with bilateral pleural effusion

Results

Cytology of pleural fluid showed monomorphic lymphoid cells which on flow-cytometric immunophenotyping (FCMI) were positive for CD45, CD2 and CD56. Histopathological examination of the right eye swelling showed many atypical lymphoid cells which were positive for CD56 and negative for other B and T cell markers. Morphological and immunophenotypic features confirmed a diagnosis of extra-nasal ENKL.

Conclusions

Being a rare variant, diagnosis of extra-nasal ENKL is challenging and often difficult. FCMI can act as a valuable tool in early diagnosis of such cases having unusual presentation.

doi: 10.1016/j.cca.2022.04.741

T254

Diagnosis of hemoglobinopathy S homozygote in a patient without symptomatology

I. Portell-Rigo [b], S. Guardia-Alés [b], M. Molina-Arrebola [c], J. Ruiz-Cara [b], M. Benayas-Bellido [b], C. Avivar-Oyonarte [a]

[a] *Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

[b] *Clinical Analysis Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

[c] *Haematology and Haemotherapy Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

Background-aim

Structural hemoglobinopathies are caused by mutations at the level of the amino acid sequence in the hemoglobin globin chain (Hb). Hb S is the most prevalent type and is generated by a mutation in 6th position of the Hb ®-globin gene, where Glutamic Acid is replaced by Valine (®6 Glu- > Val). This mutation can be inherited from both parents and be homozygous S/S. Hb S polymerizes, increasing the rigidity and viscosity of the erythrocyte membrane. It promotes dehydration and a change in morphology to the so-called ''sickle'' shape, causing episodes of vessel occlusion and hemolytic anemia in these patients. Most of them present anemia with a hematrocit (HTC) of between 15-30% as well as reticulocytosis.

Sickle cell syndromes present great clinical heterogeneity and may be asymptomatic until adulthood or present serious crises from childhood. High performance liquid chromatography (HPLC) is considered the reference method for diagnosis.

Methods

Describe the unexpected finding and diagnosis of hemoglobinopathy S homozygote in an asymptomatic patient.

Results

A 28-year-old man from Senegal underwent a control test and the following findings were revealed:

Blood Count: Red blood cells 2.82 x10^6/µl (4.3-5.75 x 10^6/µl), Hb 8.6 g/dl (13.5-16.5 g/dl), HTC 24.8 % (39.5-50 %), mean corpuscular volume 88 fl (80-101 fl), mean corpuscular Hb 30.7 pg (27-34 pg), mean corpuscular Hb concentration 34.8 g/dl (31.5-36 g/dl), reticulocytes 9.63 % (0.5-2.8 %).

Biochemistry: Glucose 100 mg/dl (74-106 mg/dl), Total Bilirubin 1.74 mg/dl (0.3-1.2 mg/dl), Indirect Bilirubin 1.37 mg/dl (0-0.75 mg/dl), Lactate dehydrogenase 319 U/L (208-378 U/L).

Glycosylated Hb (HbA1c): HPLC (Horiba®) determination was performed and no band was observed for Hemoglobin A (HbA1c: 0%). In addition, the chromatogram indicated the presence of an anomalous band for Hb. A consultation was conducted with Hematology to assess the possibility of a haemoglobinopathy. The Hb fractions were determined with the equipment in ®-thalassemia mode, obtaining the following results: HbA0 0%, HbA2 2.6% (0-3.8 %), Hb Fetal (F) 12.6 % (0-2 %), Hb S 84.8 % (0-0.5 %). Positive sickle cell test.

The sample was sent away to confirm the haemoglobinopathy diagnosis by another methodology (capillary electrophoresis) and the results were confirmed: absence of HbA0, Hb A2 2.6 %, Hb F 13.1% and Hb S 84.3%.

The outcomes are compatible with homozygous haemoglobinopathy S (HbSS, sickle cell or sickle cell anemia) with elevated Hb F that may correspond to associated hereditary persistence.

Conclusions

In HbA1c analysis by HPLC, abnormal Hb can be detected and serve as a ''diagnostic screening'' for patients with hemoglobinopathy (as in this case). With the data from the Hb analysis using the two methodologies and the positive sickle cell test, the diagnosis of homozygous SS haemoglobinopathy is confirmed.

The laboratory plays an important role in detecting these pathologies, and the protocol established between Clinical Analysis and Hematology allows for the early diagnosis of these patients and more rapid implementation of the necessary clinical actions.

doi: 10.1016/j.cca.2022.04.742

T255

Acquired Hemophilia A during pregnancy: A case report

S. Guardia-Alés [a], J. Ruiz-Cara [a], M.d.R. Pérez-Moyano [b], M.A. Molina-Arrebola [b], I.M. Portell-Rigo [a], C. Avivar-Oyonarte [a]

[a] *Clinical Analysis Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*
[b] *Haematology and Haemotherapy Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*

Background-aim

Acquired hemophilia A (AHA) is a rare disease, mediated by an immune process, in which autoantibodies are developed in the coagulation cascade against factor VIII. This causes alterations in the hemostatic functions, which can trigger serious bleeding that compromise the patient´s life.

The incidence of this condition is approximately 1-1.5 cases per million people, although this increases in pregnant women and in the postpartum period as well as in both sexes at 68-70 years of age.

Methods

Describing a clinical case of AHA during pregnancy.

Results

A 34 year old pregnant woman attendet the hospital for a gynecological check-up. When the lab performed a basic coagualtion, which had previously presented normals values, the revised values were: prothrombin time (PT) of 9.9 seconds (s), prothrombin time 100% (>60%), INR 0.87 (0.8-1.2), and activated partial prothrombin time (APPT) of 40.1 s (<34s). The platelet values were normal.

On detecting this value, it was decided to extend the coagulation study, the elongated APPT (39.8s) was confirmed and the mixture test with normal plasma was also performed, obtaining a baseline APTT of 28.3s, 28.6s at one hour, 29.3s at two hours and 30.4s at three hours. Because of abnormal values, the following parameters were studied: Factor VIII 10.7% (50-150), Factor IX 96.2% (65-150), Factor XI 57.2% (70-150), Factor XII 119.1% (50-150), lupus anticoagulant 21.3s and a ratio of 0.62 (0.8-1.2), von Willebrand factor 118.7% (50-150), the specific factor VIII inhibitor 1.42 BU/mL (<0.6). The occlusion time with collagen was normal, the IgG and IgM anticardiolipin were negatives as well as the IgG and IgM anti-beta-2-glicoprotein.

The results confirmed the AHA with a factor VIII deficiency and the presence of an inhibitor. It was decided that the patient should be admited to hospital to avoid the potentially serious consequences of AHA. Factor VIII was administered.

Conclusions

This pathology is characterized by the appearance of bleeding in patients with no prior history of it, and who have characteristic laboratory profile, namely a prolonged APTT, normal thrombin and prothrombin time, a normal platelet count, low levels of Factor VIII and the presence of antibodies against this factor.

The treatment aims to either increase the factor VIII, in which case recombinant factor VIII and/or desmopressin is used; or to erradicate the inhibitor, using immunosuppressants.

doi: 10.1016/j.cca.2022.04.743

T256

Evaluation of coagulation function by rotation thromboelastometry in critically ill patients with severe Covid-19 Pneumonia

I. RodrÍguez MartÍn, J. Montenegro Martinez, M. Santana Morales, J.M. Guerrero Montávez

Hospital Universitario Virgen Del Rocio, Spain

Background-aim

Critically ill patients with COVID-19 pneumonia suffered both high thrombotic and bleeding risk. The effect of SARS-CoV-2 on coagulation and fibrinolysis is not well known.

Methods

Retrospective cohort study including 84 patients, during 16 months, divided into two groups: patients with severe SARS-Cov-2 pneumonia (group 1, N = 42) and patients with severe non-COVID-19 pneumonia (group 2, N = 42). We evaluated coagulation standard parameters (hemoglobin, platelet count and conventional laboratory coagulation tests) in group 1 vs group 2 and coagulation standard parameters on day of admission (T0) and 10 (T10) days after admission to ICU and coagulation function using rotational thromboelastometry (ROTEM) in patients with severe SARS-Cov-2 pneumonia .

Results

84 patients were enrolled into the study. Similar results in conventional laboratory coagulation tests were detected in group 1 and group 2: prothrombin time (15.14s vs 14.76s, p = 0.212), international normalized ratio (1.21 vs 1.19, p = 0.112), activated partial thromboplastin time (32.17s vs 25.52s, p = 0.06) , fibrinogen level (6.15 mg/dl vs 3.39 mg/dl, p = 0.208), hemoglobin (11.81 g/dl vs 11.20 g/dl, p = 0.139) and platelet count (208.98x103/ul vs 288.74 x103/ul, p = 0.123). However, a statistically significant difference was observed in the D-dimer count (2442.11 ng/ml vs 370 ng/ml, p = 0.03). In addition, statistically significant increase in D-dimer count during Intensive Care Unit (ICU) stay (T0 = 2442.11 ng/ml vs T10 = 8564.39 ng/ml, p = 0.000) in group 1 were detected. Finally, blood thromboelastometry profiles were consistent with hypercoagulability characterized by higher clot strength (MCF or maximum clot firmness close to upper limit in FIBTEM test, MCF median value = 25.9 mm). Clotting time presented normal results in INTEM (163.41 s) and EXTEM (68.74 s). No sign of secondary hyperfibrinolysis were found during the study period. In six patients a deep vein thrombosis and in six patients a thromboembolic event. Eighteen patients (43%) died during hospitalization due to coagulopathy produced by SARS-Cov-2 pneumonia.

Conclusions

The results observed in our study support hypercoagulability in a severe inflammatory state, rather than a Consumption Coagulopathy

(DIC) state. More studies are needed to better understanding of coagulopathy produced in patients with severe COVID-19 pneumonia.

doi: 10.1016/j.cca.2022.04.744

T257

Blood coagulation testing and hematological features of thalassemia carriers in pregnant

S. Zhu [a], J. Yin [b]

[a] *Cancer Research Institute Ghent(CRIG), Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium*
[b] *Clinical Laboratory, The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, China*

Background-aim

The incidence rate of thalassemia in Guangxi is very high, and the carriers of the thalassemia gene are also very numerous. In this study, we focused on the coagulation function and hematological parameters of pregnant women with thalassemia gene.

Methods

In this study, the prothrombin time(PT), international normalized ratio(INR), activated partial thromboplastin time(APTT), thrombin times(TT), fibrinogen(FIB), D-dimer(DD), red blood cells count(RBC), hemoglobin(Hb), haematocrit(Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration(MCHC) and red cell distribution width (RDW) parameters of 460 pregnant women were collected and analyzed, including 49 cases in group -⟨/⟨⟨, 49 cases in group ⟨T/⟨⟨, 114 cases in group –/⟨⟨, 5 cases in group –/⟨⟨T, 7 cases in group –/-⟨, 28 cases in group ®+/N, 105 cases in group ®0/N and 103 cases in normal group.

Results

APTT was significantly prolonged in the ®0/N group($p < 0.01$). RBC of each group of ⟨- thalassemia was significantly higher than that of the normal group($p < 0.01$), MCV and MCH were significantly lower ($p < 0.01$). Compared with the normal group, RBC, Hb, Hct, MCV, MCH, MCHC and RDW parameters in the ®+/N and ®0/N group were statistically significant($p < 0.01$). MCV, MCH can be used as a promising indicator to distinguish group –/⟨⟨, ®+/N and ®0/N, and sensitivity and specificity are more than 0.9.

Conclusions

In ®0/N group, the APTT prolonged significantly, and the hematological parameters changed significantly. Abnormal red blood cells can increase the risk of thrombosis, so pregnant women with thalassemia gene should take precautions.

doi: 10.1016/j.cca.2022.04.745

T258

Investigation of genetic biomarkers associated with low platelet count in normal karyotype acute myeloid leukemia

C. Park [a], J.W. Yun [b]

[a] *Department of Laboratory Medicine & Genetics, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, South Korea*
[b] *Veterans Medical Research Institute, Veterans Health Service Medical Center, Seoul, South Korea*

Background-aim

Acute myeloid leukemia (AML) is associated with the risk of bleeding due to systemic coagulopathy and the disease-related lack of platelets. Leukemic blasts could alter platelet activation in vitro in previous report. The aim of the study is comprehensive investigation of genetic biomarkers contributing to low platelet count in normal karyotype AML (NK-AML).

Methods

Among 200 AML cases from The Cancer Genome Atlas, 37 NK-AML with no known driver mutations were enrolled in this study. Among them, AMLs with platelet count $< 100 \times 109/L$ ($N = 24$) were classified as platelet-decreased AML (PD-AML) and the others ($N = 13$) as control. Using the RNA-seq expression data, differentially expressed gene (DEG) analysis and pathway analysis was done. Using the result of DEG, biomarker performance test for predicting PD-AML was done through ROC curve analysis.

Results

In DEG analysis, 175 genes were differentially expressed in normal karyotyped PD-AML. Among them, most differentially expressed genes were CHIH3 ($p = 0.0003$), CSF1R ($p = 0.0007$), HTR3E ($p = 0.0015$), CILP2 ($p = 0.0023$), and LOC64685 ($p = 0.0028$). DEGs were enriched in pathways including GPCR-related signalings, Cytokine-cytokine receptor interaction, cytokines and inflammatory response, JAK STAT molecular variation 1, C-type lectin receptors, and bone remodeling signalings with statistical significance. Based on the biomarkers which were selected through the DEG analysis and functional review, area under curve was up to 0.847 in performance evaluation of biomarkers for predicting PD-AML.

Conclusions

Comprehensive description for cell signaling pathways related with low platelet count in NK-AML was presented in this study. Putative biomarkers which could predict the decreased platelet status and the bleeding tendency in NK-AML were suggested and evaluated. We believe the result of study would contribute to advances in precision medicine and the effective AML treatment.

doi: 10.1016/j.cca.2022.04.746

T259

Evaluation of coagulation function in critically ill patients with severe Covid-19 pneumonia

I. RodrÍguez MartÍn, J. Montenegro Martinez, D. Nuñez Jurado

Hospital Universitario Virgen Del Rocio, Spain

Background-aim

In addition to typical respiratory symptoms, COVID-19 is associated with coagulation abnormalities that lead to thromboembolic complications.

Methods

Retrospective study of critically ill patients admitted to an intensive care unit (ICU) a cause of severe COVID-19 pneumonia (Group 1) and we evaluated coagulation function using coagulation standard parameters on day of admission (T0) and 10 (T10) days after admission to ICU and rotational thromboelastometry (ClotPro). In addition, we compared coagulation standard parameters to patients with severe non–COVID-19 pneumonia (Group 2).

Results

Eighty-four patients participated in our study. Traditional coagulation parameters were similar between group 1 and group 2. Only D-dimer levels (2442.11 ng / ml vs 370 ng / ml, p = 0.03) were significantly higher in COVID-19 pneumonia than in non-COVID-19 pneumonia. In addition, we concluded an increase in D-dimer levels during the hospital stay (T0 = 2442.11 ng / ml vs T10 = 8564.39 ng / ml, p = 0.000). Finally, patients with SARS-CoV-2 pneumonia exhibited hypercoagulant thromboelatometry profiles, characterized by elevated maximum clot firmness (MCF) values.

Conclusions

The results observed in our study support hypercoagulability in a severe inflammatory state, rather than a disseminated intravascular coagulation (DIC). More studies are needed to allow a better understanding of the coagulopathy produced in patients with severe COVID-19 pneumonia.

doi: 10.1016/j.cca.2022.04.747

T260

Anemia, homocysteine and deep vein thrombosis. Is there any link?

M. Boudaya, S. Fendri, R. Ben Salah, K. Jamoussi, Z. Bahloul

CHU Hedi Chaker, laboratory of biochemistry, Sfax, Tunisia

Background-aim

Venous thromboembolic disease is a public health problem whose diagnosis calls for adequate management, this management includes pathologies associated with thrombosis including anemia. In the course of our work, we propose to screen for cases of anemia in patients with deep vein thrombosis.

Methods

This is an observational study involving patients under the age of 60 admitted to the internal medicine department for the management of deep vein thrombosis confirmed by imaging of unknown etiology. A complete blood count was taken for all patients included in the study performed by the sysmex XN1000 machine.

Results

Forty-seven patients were included in our study, mean age 40.8 ± 10.5 years with extremes of 18 and 59 years. The group consisted of 27 men (57.5%) and 20 women (42.5%). The sex ratio was 1.35. Fifteen patients had a body mass index exceeding 25 kg / m2. Venous thrombosis of IM was the most frequent localization observed in 34 patients (72.3%). Twelve patients (25.5%) were anemic. The anemia observed in both sexes was in 50% normochromic normocytic, and in 50% of cases microcytic. Hemoglobin level was significantly lower in folate deficiency (p = 0.05). Four patients (33%) had folate deficiency and hyperhomocysteinemia with normocytic anemia. All these patients received vitamin supplementation.

Conclusions

Adequate management of deep vein thrombosis prompts a full etiological investigation and screening for anemia with possible vitamin deficiency requiring supplementation.

doi: 10.1016/j.cca.2022.04.748

T261

Beyond the in-practice CBC: The research CBC parameters-driven machine learning predictive modeling for early differentiation among leukemias

R.Z. Haider[a], I. Ujjan[b]

[a] *Baqai Institute of Hematology, Baqai Medical University, Karachi, Pakistan*
[b] *Liaquat University of Medical and Health Sciences, Pakistan*

Background-aim

A targeted and timely treatment can be a beneficial tool for patients with hematological emergencies (particularly acute leukemias). The key challenges in the early diagnosis of leukemias and related hematological disorders are their symptom-sharing nature and prolonged turnaround time as well as the expertise needed in reporting confirmatory tests.

Methods

The present study made use of the potential morphological and immature fraction-related parameters (research items or cell population data) generated during complete blood cell count (CBC), through artificial intelligence (AI)/machine learning (ML) predictive modeling for early (at the pre-microscopic level) differentiation of various types of leukemias: acute from chronic as well as myeloid from lymphoid. The routine CBC parameters along with research CBC items from a hematology analyzer in the diagnosis of 1577 study subjects with hematological neoplasms were collected. The statistical and data visualization tools, including heat-map and principal component analysis (PCA,) helped in the evaluation of the predictive capacity of research CBC items. Next, research CBC parameter-driven artificial neural network (ANN) predic-

tive modeling was developed to use the hidden trend (disease's signature) by increasing the auguring accuracy of these potential morphometric parameters in differentiation of leukemias.

Results

The classical statistics for routine and research CBC parameters showed that as a whole, all study items are significantly deviated among various types of leukemias (study groups). The CPD parameter-driven heat-map gave clustering (separation) of myeloid from lymphoid leukemias, followed by the segregation (nodding) of the acute from the chronic class of that particular lineage. Furthermore, acute promyelocytic leukemia (APML) was also well individuated from other types of acute myeloid leukemia (AML). The PCA plot guided by research CBC items at notable variance vindicated the aforementioned findings of the CPD-driven heat-map. Through training of ANN predictive modeling, the CPD parameters successfully differentiate the chronic myeloid leukemia (CML), AML, APML, acute lymphoid leukemia (ALL), chronic lymphoid leukemia (CLL), and other related hematological neoplasms with AUC values of 0.937, 0.905, 0.805, 0.829, 0.870, and 0.789, respectively, at an agreeably significant (10.6%) false prediction rate. Overall practical results of using our ANN model were found quite satisfactory with values of 83.1% and 89.4.7% for training and testing datasets, respectively.

Conclusions

We proposed that research CBC parameters could potentially be used for early differentiation of leukemias in the hematology–oncology unit. The CPD-driven ANN modeling is a novel practice that substantially strengthens the predictive potential of CPD items, allowing the clinicians to be confident about the typical trend of the "disease fingerprint" shown by these automated potential morphometric items.

doi: 10.1016/j.cca.2022.04.749

T262

Testing strategy for laboratory monitoring of Hemophilia A patients treated with Emicizumab

M. Pikta [a], K. Saks [d], M. Ross [c], V. Banys [b]

[a] *Department of Laboratory Medicine, North Estonia Medical Centre, Department of Health Technologies, Tallinn University of Technology,Tallinn, Estonia*
[b] *Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania*
[c] *Hematology Department, North Estonia Medical Centre, Tallinn, Estonia*
[d] *Hematology Department, Tallinn Children's Hospital, Tallinn, Estonia*

Background-aim

Emicizumab is currently approved for prophylaxis in Hemophilia A (HA) for patients with and without inhibitors. Routine monitoring of plasma emicizumab levels is not recommended. However, in selected clinical situations it seems to be useful.

The first aim was to assess the modified one-stage assay (MOSA) for the quantitative measurement of Emicizumab concentration in plasma and bovine chromogenic FVIII assay. Secondly, we evaluated APTT variation in relation to emicuzumab levels in a cohort of severe HA patients treated at 3 Estonian Haemophilia Treatment Centers.

Methods

The emicizumab concentration was analyzed by a modified one stage factor VIII assay (STA ImmunoDef VIII, STA-CK Prest, Diagnostica Stago, France) using emicizumab calibrator and controls (r2 Diagnostics, USA). Residual FVIII activity and FVIII inhibitors were determined by chromogenic FVIII assay containing bovine proteins (Siemens Healthcare, Germany). APTT was assessed by silica clotting time (STA-PTT Automate, Diagnostica Stago, France). All assays were performed on a STA-R Evolution analyzer (Diagnostica Stago, France). Intra- and inter-assay variation was evaluated with 2 levels IQC (Control Plasma N and P: Siemens Healthcare, Germany; Emicizumab Controls Level 1 and 2: r2 Diagnostics, USA).The EQA samples for emicizumb and chromogenic FVIII from INSTAND (Germany) and ECAT (the Netherlands) were used to evaluate assay performance. In total 10 HA patients were included. A history of inhibitors was present in two patients.

Results

Intra- and inter-assay precision of MOSA and chromogenic FVIII met acceptance criteria and were $<10\%$ CV for two levels of controls. The Z-scores of EQA samples were acceptable (in the range ± 2). Pre-treatment emicizumab concentration in plasma ranged 1.5-6.4 μg/mL ($n=4$). Emicizumab plasma concentrations collected at different time points ranged 27.4-79.3 μg/mL. Residual FVIII activity was $<1\%$. On treatment and pre-treatment FVIII inhibitor titers were comparable. On treatment APTT results tended to be normal and ranged between 25.8 and 31.7 seconds.

Conclusions

MOSA and Factor VIII chromogenic assay with bovine components show acceptable precision and accuracy. They provide the possibility to quantify emicizumab concentration gives deeper understanding about HA patient status.

doi: 10.1016/j.cca.2022.04.750

T263

Performance of the alinity HQ body fluid cell count in Thailand

T. Kaosumran, T. Saibungkla

Hematology Department, Fort Suranari Hospital, Nakhon Ratchasima, Thailand

Background-aim

Body fluids are often analysed to investigate health problems. Investigated using a manual technique with microscopic cell counts. A technique requiring highly skilled scientists. While mostly these fluids help organs, joints and the membranes around them to move smoothly, sometimes too much fluid can build up in areas of the body. The fluid is analysed to look for the reason for the buildup.

Methods

Fresh Body Fluid with Heparin anticoagulant collections from 117 patients were analysed using the Alinity hq routine CBC + Diff mode and compared with the WBC & RBC results from the manual counting method and Sysmex XN550 body fluid mode with out any pre-treatment. The evaluation classified by 5 Specimen type, Cerebrospinal Fluid, Peri-

toneal Dialysis Fluid, Pleural Fluid , Ascitic Fluid and Synovial Fluid by using EP evaluator Program.

Results

The comparison results are as follows,

Correlation coefficient of WBC: Alinity hq vs Manual = 0.9796. Alinity hq vs XN550 = 0.9796

Correlation coefficient of RBC: Alinity hq vs Manual = 0.9589. Alinity hq vs XN550 = 0.9172

Correlation coefficient of PMN: Alinity hq vs Manual = 0.9562. Alinity hq vs XN550 = 0.9151

Correlation coefficient of MN: Alinity hq vs Manual = 0.9580. Alinity hq vs XN550 = 0.9122

Conclusions

The Alinity hq provides reliable for total WBC and total RBC counts on Cerebrospinal Fluid, Peritoneal Dialysis Fluid, Pleural Fluid , Ascitic Fluid and Synovial Fluid, even for samples with low cell numbers. Manual counts were still required to accurately differentiate nucleated cells.

doi: 10.1016/j.cca.2022.04.751

T264

Evaluation the diagnostic performance of immature granulocyte cells flag on automated hematology analyzer sysmex XN-series

W. Chieh-Hsi [b], W. Fang-Yu [b], H. Hsiang-Ling [a]

[a] *Department of Biotechnology and Laboratory Science in Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC*
[b] *Division of General Laboratory, Department of Pathology & Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*

Background-aim

Taipei Veterans General Hospital, with an average of 2,000 blood routine tests done daily, is considered to be one of the largest healthcare facilities in Taiwan. Efficiency and accuracy on automated hematology analyzers are important for the clinical laboratories. Immature granulocyte (IG) cells (metamyelocyte, myelocyte and promyelocyte) increase not only in physiological conditions but also in pathological conditions such as bacterial infections or myeloproliferative neoplasm. The Sysmex XN-9100 hematology analyzer includes a white blood cell differentiation channel (WDF) which identifies and counts IG cells. The probability of the presence of IG cells is indicated by the “IG present” flag. This study aimed to evaluate the diagnostic performance of the “IG present” flag, and reduce redundant blood smear reviews.

Methods

A total of 59,886 samples were analyzed on XN-9100 analyzers (Sysmex, Kobe, Japan). The diagnostic performance of the “IG present” flag and IG count (IG%) were studied by comparing the results of the XN-9100 and the reference method (differential using digital cell imaging analyzer, Sysmex DI-60, or optical microscope). Statistical analyses were performed using SPSS.

Results

In 59,886 samples, 4,943 samples had “IG present” flags, and 54,943 samples without “IG present” flags. Immature granulocyte cells were detected by reference method in 3,206 of the samples with “IG present” flag, and in 798 of the samples without “IG present” flag. The sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy rate of “IG present” flag were 80.1%, 96.9%, 98.5%, 64.9% and 95.8%, respectively. In the group that IG was detected by both XN-9100 analyzers and reference method, the mean of IG% reported by XN-9100 analyzers was 7.2%, which observed a positive bias compared to the reference method (mean of IG%: 4.9%).

Conclusions

The “IG present” flag of XN-9100 analyzer showed high diagnostic accuracy for IG cells detection and high NPV to rule out the smears without IG cells, which will help reduce redundant smear review. The “IG present” flag of XN-9100 analyzer is expected to be a potential diagnostic tool to assist clinical laboratories to identify immature granulocyte cells.

doi: 10.1016/j.cca.2022.04.752

T265

SéZARY syndrome: Cytogical Atypia [LDQUO] a difficult diagnosis for a rare disease [RDQUO]

Y. Sbibih, M. Kaabouchi, N. Benhamza, H. Zrouri, A. Saddari, H. Lekfif, A. Moumna, M. Bensalah, R. Seddik

Clinical Hematology Laboratory, University Hospital Mohammed VI, Oujda, Morocco

Background-aim

Sézary syndrome (SS) is defined by the triad of erythroderma, generalized lymphadenopathy, and the presence of clonally related neoplastic T cells with cerebriform nuclei (Sezary cells) in skin, lymph nodes, and peripheral blood.

In addition, one or more of the following criteria are required: an absolute Sezary cell count 21000/L, an expanded CD4+ T-cell population resulting in a CD4:CD8 ratio of 210, and loss of one or more T-cell antigens.

We report a clinical observation of a patient diagnosed SS with atypical lymphocytes on blood smear.

The purpose of this study is to highlight the cytological atypia found in our patient’s blood smear.

Methods

An 84-year-old Moroccan woman was admitted to our university hospital suffering from a generalized pruritus for 2 months, along with a generalized lymphadenopathy.

The patient’s physical examination proved her asthenic and revealed severe generalized erythema with infiltrated skin and scratching lesions.

A full blood count with a blood smear, a myelogram, a lymphocyte immunophenotyping and a skin biopsy were all performed in this case to confirm the diagnosis of Sézary’s syndrome.

Results

The hemogram of this patient showed leukocytosis at 162,440 and lymphocytosis at 129,952.

Examination of the blood smear showed a population of medium-sized lymphocyte cells containing an agranular basophilic cytoplasm with cytoplasmic extensions in the form of blebs , and an irregular nucleus with intermediate chromatin.

Lymphocyte immunophenotyping carried out by flow cytometry (navios beckman coulter) revealed hyperlymphocytosis T expressing the CD3, CD4, CD5 markers with loss of CD7 expressing approximately 99% of the lymphocytes, i.e. 95 G/L.

The myelogram showed an invasion of mature lymphocytes at 45%

The skin lesion biopsies revealed a cellular infiltrate of monotonous atypical lymphocytes with epidermotropism.

The immunohistochemical analysis of the lymphocytes showed a CD3+ , CD4+ , CD2+ , CD5+ , and CD7- , CD8- , CD30-

Conclusions

Finally, after all the investigations, the diagnosis of sézary was retained and the atypical lymphocyte observed was a Sézay cell.

Cytological atypia in Sézary's syndrome has already been reported in the literature, which has been the cause of delayed diagnosis of a rare disease with a poor prognosis.

doi: 10.1016/j.cca.2022.04.753

T266

Aberrant phenotypes in acute leukemias

Y. Sbibih, A. Berhili, N. Benhamza, M. Kaabouchi, A. Saddari, H. Zrouri, H. Lekfif, A. Moumna, M. Bensalah, I. Naji, R. Seddik

Clinical Laboratory of Hematology, University Hospital Mohammed VI, Oujda, Morocco

Background-aim

Aberrant phenotypes in acute leukemias are defined by the expression of one or more antigens on the surface of blasts other than those identifying the leukemic lineage.

This phenomenon can be explained by cytogenetic abnormalities during hematopoiesis affecting the hematopoietic stem cell before its myeloid and lymphoid differentiation.

The identification of phenotypes is done by immunophenotyping, a tool that has become essential for the diagnosis and classification of acute leukemias.

The purpose of our study is to estimate the incidence, characterize the aberrant phenotypes and determine their relevance in the classification and prognosis of the pathology.

Methods

Our retrospective descriptive study carried out at the hematology laboratory of the Mohammed VI University Hospital in Oujda included 61 patients with acute leukemia, ranging in age from 2 months to 76 years, including both sexes,over a period of 8 months.

Samples were processed by flow cytometry using a (beckman Coulter) type cytometer with a panel of monoclonal antibodies.

Results

19 patients among the 61 expressed an aberrant phenotype: 8 children and 11 adults.

In the pediatric population , 62% were suffering from acute myeloid leukemia and 38% from acute lymphoid leukemia.

In AML, the 2 aberrant markers were CD19: B lymphoid marker in 20% of the cases and CD7: Tlymphoid marker in 80% of the cases.

In ALL, CD10: a B lymphoid marker was expressed in 66% of the cases in T-ALL and CD7: a T

lymphoid marker expressed in 33% of the cases in B-ALL.

In the adult population, 80% were suffering from AML expressed either CD19 or CD7, of which only one patient expressed 2 aberrant phenotypes CD 79a: B lymphoid marker and CD7.

And the remaining 20% were suffering from T-ALL, all expressed CD 10.

Aberrant CD 19 expression in AML is of great interest in cytogenetic classification. It is reported in the literature in AML with t (8,21).

Aberrant CD10 expression in T-ALL has been linked with better prognosis according to numerous studies.

Conclusions

The notion of aberrant markers is of great importance in the diagnosis and follow-up of Acute leukemia by flow cytometry. The identification of aberrant phenotypes allows an orientation in cytogenetic classification and is of great interest in the detection of residual disease. Some aberrant phenotypes may also have an influence on the prognosis of the disease.

doi: 10.1016/j.cca.2022.04.754

Clinica Chimica Acta 530 (2022) S189–S192

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Hepatobiliary Disease

T267

Diagnostic performance of MAC-2 binding protein glycosylated isomer (M2BPGI) in predicting liver fibrosis in health checkups

E. Nah [a], S. Cho [a], S. Kim [a], H.s. Kim [b], H. Cho [b]

[a] *Department of Laboratory Medicine and Health Promotion Research Institute, Republic of Korea*
[b] *Korea Association of Health Promotion, MEDIcheck LAB, Seoul, Republic of Korea*

Background-aim

The major histological consequence of chronic liver diseases is progressive liver fibrosis and, eventually cirrhosis. The diagnosis of mild to moderate fibrosis is rarely made because the disease is asymptomatic in early stage. This early diagnosis would allow identification of causal factors responsible for liver infllamation and subsequent application of specific targeted interventions. Liver biopsy has been considered the gold standard for the assessment and quantification of liver fibrosis. Serum Mac-2 Binding Protein Glycosylated isomer (M2BPGi) level has been found to be increased according to the increasing severity of liver fibrosis in patients with chronic hepatitis. The aim of this study is to assess the diagnostic performance of M2BPGi in predicting liver fibrosis diagnosed by magnetic resonance elastography (MRE) as a reference standard and to compare it with APRI and Fibrosis-4 index (FIB-4) in health checkups.

Methods

This was a cross-sectional study. Subjects from health examinees who underwent MRE and M2BPGi test at 8 health promotion centers in Korea were consecutively selected between January 2019 and September 2019. Serum M2BPGi level was measured using the chemiluminescence enzyme immunoassay method (HISCL-5000, Sysmex, Kobe, Japan). The measured levels were indexed using cutoff index (COI). All MRE examinations were performed on MR elastography hardware (MR Touch; GE Healthcare) with a 1.5-T imager by using a two-dimensional MRE protocol. COI values of M2BPGi were compared with MRE results. Receiver operating characteristic (ROC) curve analysis was performed for M2BPGi test as a predicting test for liver fibrosis.

Results

The median (IQR) for COI for fibrosis stages F0 (Normal), F1(Mild fibrosis), F2 (Significant fibrosis) and εF3 (Advanced fibrosis) was 0.49 (0.34–0.61), 0.48 (0.38–0.68), 0.64 (0.43–1.03) and 1.01 (0.75–1.77), respectively ($p < 0.0001$). The COI was significantly higher in subjects with fibrosis stage εF3 compared with fibrosis stage F2 ($P = 0.017$), but not significantly different between subjects with fibrosis stage F1 compared with fibrosis stage F2 ($P = 0.066$). The COI was good for F0F1F2 vs εF3 and fair for F0F1 vs F2F3, but poor for F0 vs F1F2F3. The AUROC of the COI for the screening of fibrosis stage εF1, εF2 and εεF3 was 0.591, 0.698 and 0.853, respectively. The cutoff for COI to exclude advanced fibrosis was 0.75 with 80.0 % sensitivity, 77.9 % specificity, and negative predictive value of 98.9%. The AUROC of M2BPGi for excluding advanced fibrosis was better than those of the FIB-4 and APRI.

Conclusions

Serum M2BPGi was useful for the excluding of significant fibrosis and advanced fibrosis in health checkups.

doi: 10.1016/j.cca.2022.04.512

T268

Serum profile of lactate dehydrogenase and alkaline phosphatase in alcoholic liver disease

S. Lawicki [e], A. Grytczuk [c], E. Gruszewska [a], A. Panasiuk [b], B. Cylwik [d], L. Chrostek [a]

[a] *Department of Biochemical Diagnostics, Medical University of Bialystok, Poland*
[b] *Department of Internal Diseases and Gastroenterology, Provincial Welded Hospital in Bialystok, Poland*
[c] *Department of Laboratory Diagnostics, University Clinical Hospital in Bialystok, Poland*
[d] *Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok, Poland*
[e] *Department of Population Medicine and Civilization Diseases Prevention, Medical University in Bialystok, Poland*

Background-aim

Alcohol-related liver diseases affect the capability of the liver for the synthesis of proteins including enzymes. Among enzymes two expose the liver specific isoenzymes. There are lactate dehydrogenase and alkaline phosphatase. The purpose of this study was to explore the profile of lac-

tate dehydrogenase and alkaline phosphatase isoenzymes in alcoholic liver diseases.

Methods

The serum profile of lactate dehydrogenase and alkaline phosphatase isoenzymes was studied in alcoholic cirrhosis and alcoholic hepatitis by electrophoresis method on agarose gel. The tested group consisted of 80 patients with alcoholic liver diseases, including 55 patients with alcoholic cirrhosis and 25 patients with alcoholic hepatitis.

Results

The relative concentration of LDH 1 isoenzyme is decreased in alcoholic hepatitis, but LDH 4 and LDH 5 isoenzymes are increased. In addition, the relative level of LDH 5 isoenzyme is increased in alcoholic cirrhosis. The concentrations of intermediate isoenzymes (LDH 2 and LDH 3) are reduced in alcoholic cirrhosis. There were also an increase of liver ALP isoenzyme and parallel decrease in its bone isoenzyme in alcoholic hepatitis and cirrhosis. The severity of liver cirrhosis, expressed as a Child-Pugh score, didn't affect total activity of both enzymes but effects the relative concentration of LDH 2, LDH 3 and LDH 4 isoenzymes.

Conclusions

The serum profile of lactate dehydrogenase and alkaline phosphatase isoenzymes alters in alcoholic liver diseases and can be explained by the tissue prevalence of isoenzymes and metabolic preferences of isoenzymes.

doi: 10.1016/j.cca.2022.04.513

T269

Comparison of indocyanine green tests in between 44 Korean institutions

S.Y. Moon [c], J. Lee [c], K. Shin [c], S.M. Lee [d], S. Yu [b], K.R. Jun [b], C.M. Park [a]

[a] *Department of Laboratory Medicine, Dongnam Institute of Radiological and Medical Sciences, Busan, South Korea*
[b] *Department of Laboratory Medicine, Inje University Haeundae Paik Hospital, Busan, South Korea*
[c] *Department of Laboratory Medicine, Pusan National University Hospital, Busan, South Korea*
[d] *Department of Laboratory Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*

Background-aim

Indocyanine green (ICG) test is a classical spectrophotometric test for evaluating the hepatic uptake function before liver resection. ICG test requires a self-production of calibrators, IV injection, numerous timed samples, and statistical estimation for interpretation of results, which increase the variation between different institutions. Here we produced plasma based materials and performed inter-institution comparison in Korea tertiary hospitals.

Methods

ICG was spiked into anticoagulated plasma derived from discarded fresh frozen plasma after 22 um filtration. Four levels of materials assuming before, 5, 10 and 15 minutes after injection was grouped as one set. Homogeneity and short term stability in refrigerator was evaluated according to Korean External Quality Assessment Service (KEQAS) guideline. Over 50 sets of materials were sent to each institutions performing ICG tests. Absorbance, concentration, R15, K, and half-life was required to be filled in KEQAS website.

Results

By measuring absorbance, difference between samples were less than 1.5% of CV and stability for 24 days in refrigerator was less than 10% of bias. In total 44 tertiary hospitals reported the analysis result of one set, while absorbance, concentration, R15, and K was reported by 30~33, 27~30, 40, and 27 institutions, respectively. Outliers defined as Tukey method were observed in 1/8 of participated institutions in absorbance and 1/5 in concentration. After exclusion of 7 outliers, R15 showed mean 13.76 and standard deviation 2.99. After exclusion of 3 outliers, K showed mean 0.141 and standard deviation 0.011. To 11 institutions showed outlier values, individual interpretation for cause was delivered.

Conclusions

This is the first effort to compare ICG tests between tertiary hospitals in Korea. Based on different settings of sampling, measurement, and interpretation, various results with numerous outliers were reported. Further harmonization is needed in this classical test injecting dye to patients.

doi: 10.1016/j.cca.2022.04.514

T270

Simple noninvasive tests of liver fibrosis in patients with chronic Hepatitis B and C in an adult Sub-Saharan African population

B.E. Edinga Eyebe Epse Melenge M

Department of Biochemistry and Serology, Centre Pasteur of Cameroon, Yaoundé, Cameroon

Background-aim

Background:

Hepatitis is an inflammation of the liver. The condition can be self-limiting or can progress to fibrosis, cirrhosis or liver cancer. Hepatitis viruses are the most common cause of hepatitis in the world but other infections, toxic substances such as alcohol, certain drugs, and autoimmune diseases can also cause hepatitis. Viral hepatitis B and C are a major public health problem in the world. Because of the burden of illness and death, they cause and the potential for outbreaks and epidemic spread. Hepatitis viruses B and C also lead to chronic disease in hundreds of millions of people in the world and, together, are the most common cause of liver cirrhosis and cancer.

It is a necessity to stage liver fibrosis when the diagnosis of chronic hepatitis is made in order to determine therapeutic decisions and to detect complications such as cirrhosis and hepatocellular carcinoma. Liver fibrosis is an abnormal accumulation of the extracellular matrix

which is rich in fibrillary collagens. This fibrosis develops any time that there is prolonged aggression of a liver. It is the main complication of chronic liver disease. Its evolution leads to cirrhosis. Liver histology is the gold standard to predict fibrosis in patients with chronic hepatitis. Liver histology is invasive which limits its use in common practice. Non-invasive fibrosis tests have been developed. Fibrotest® (it is a mathematical synthesis of the results of the combination of alpha2 macroglobulin, total bilirubin, haptoglobin, apolipoprotein A1, gamma glutamyl-transferase adjusted with age and sex) is the most used non-invasive fibrosis test, particularly for chronic hepatitis C. However, its high cost is still a limit for the management of many patients. Especially in low and –middle-income countries.

Simple and Inexpensive noninvasive tests have therefore recently been proposed as an alternative for low incomes countries for assessing fibrosis in patients with chronic hepatitis. Amongst them we have: The Aspartate Amino-transferase to platelet ratio (APRI), the combined platelet count, Alanine aminotransferase, Aspartate Aminotransferase and age (FIB-4) and the Gamma Glutamyl-Transferase to platelet ratio (GPR). These tests have the advantage to combine routine biochemical and hematological markers for the monitoring of chronic hepatitis.

Aim:

The aim of this study was to evaluate the correlation between APRI, FIB4 and GPR scores in the staging of liver fibrosis compared to Fibrotest® in chronic hepatitis B or C in Cameroon.

Methods

Methods:

To do this, we conducted a prospective and cross-sectional study at the Center Pasteur of Cameroun (CPC). The study population was made of patients with chronic hepatitis B or C who came to perform the Fibrotest®. Measurement of the enzymatic activities of Aspartate Aminotransferase (ASAT), Alanine aminotransferase (ALAT), and Gamma Glutamyl-Transferase was carried out in the CPC's Biochemistry Laboratory using the dried chemistry measurement principle. The full blood count used to obtain the platelet ratio was performed in the CPC's Hematology laboratory using the principle of impedance measurement. The APRI, FIB-4, and GPR scores were then calculated according to the formulas proposed by the authors. The Fibrotest® was performed at the CERBA laboratory in France. The statistical analysis of the data collected was carried out by SPSS software version 20.0. The Spearman correlation test was used to study the association between two quantitative variables. The result was significant when $p < 0.05$.

Results

A total of 52 participants were selected; including 27 (51.9%) with chronic viral hepatitis B virus and 25 (48.1%) with chronic viral hepatitis C. In chronic hepatitis B, the correlations between Fibrotest® and FIB-4 were positive and significant ($p = 0.003$). The three evaluated scores (APRI, FIB-4, and GPR) were positively and significantly correlated with Fibrotest® ($p < 0.001$ for each score) in chronic hepatitis C.

Conclusions

At the end of this study, we found a good correlation between the three scores (APRI, FIB-4, and GPR) with Fibrotest® in patients with Hepatitis C. However in chronic hepatitis B only the FIB-4 was positively correlated with the Fibrotest® score.

doi: 10.1016/j.cca.2022.04.515

T271

Bile acids imbalance induced by post-hepatic Jaundice due to benign obstruction

E. Danese [a], M. Pucci [a], S. De Nitto [a], D. Negrini [a], D. Ambrogi [b], G. Lippi [a], G.L. Salvagno [a]

[a] *Clinical Biochemistry section, Department of Neurological, Biomedical and Movement Sciences, University of Verona, Italy*
[b] *Waters S.p.a. Sesto San Giovanni, Milano, Italy*

Background-aim

Bile acids (BAs), the amphipathic primary end-products of cholesterol metabolism, help the digestion process and participate in signal transduction in several hepatic and enteric pathways. Characterization of plasma BAs profile abnormalities induced by jaundice is necessary for accurate interpretation of data obtained in studies evaluating the role of BAs in hepatobiliary cancers.

Methods

A pool of plasma samples drawn from 8 patients with obstructive jaundice due to gallstones in common bile duct (total bilirubin, 290 mol/L; conjugated bilirubin, 190 mol/L) was diluted with a pool of plasma samples taken from healthy subjects ($n = 10$; total bilirubin, < 18 mol/L). The quantification of 16 primary (p-BAs) and secondary (s-BAs) BAs was performed with a newly developed and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS: TqS micro, Waters) technique. Spearman's correlation was used to assess the strength of association between bilirubin and BAs plasma values.

Results

Colic acid (CA) and chenodeoxycolic acid (CDCA) were directly ($r = 0.95$, $p < 0.0001$) and inversely correlated ($r = -0.77$, $p = 0.0053$) with bilirubin values, respectively. Free sBAs (DCA and UDCA) values decreased with bilirubin levels ($r = -0.95$ and $r = -0.94$, $p < 0.0001$ for both), indicating that microbial transformation from p-BAs to s-BAs may be influenced by jaundice. Such impairment in p-BAs transformation was comparable for CA and CDCA (correlation between CA/DCA and CDCA/UDCA, $r = 0.93$; $p < 0.0001$). Conjugated BAs from both p-BAs (CA) and s-BAs (DCA, LCA and UDCA) were highly correlated with their precursors ($r > 0.86$, $p < 0.0005$ for all), indicating that conjugation is not influenced by jaundice. The ratios between free and conjugated BAs were highly conserved for both pBAs and sBAs (CA/TCA + GCA and CDCA/TCDCA + GCDCA: $r = 0.93$; DCA/TDCA + GDCA and UDCA/TUDCA + GUDCA:$r = 0.93$, $p < 0.0001$ for both).

Conclusions

Post-hepatic jaundice due to benign obstruction alters BAs profile, seemingly depending on imbalance in production/accumulation of p-BAs CA and CDCA, and on their transformation in s-BAs by gut microbiota. Further studies, considering different types of intra- and post-hepatic jaundice, shall be carried out for better characterizing the modification of BAs composition in benign and malignant hepatobiliary diseases.

doi: 10.1016/j.cca.2022.04.516

T272

Features of the manifestation and compensatory mechanisms of MGUS in patients with liver cirrhosis

S. Kazakov [b c], O. Rukavitsin [a,c]

[a] *Burdenko main military clinical hospital of Ministry of defense, Moscow, Russia*
[b] *Russian Medical Academy of Continuing Professional Education of Ministry of Health, Moscow, Russia*
[c] *Burdenko main military clinical hospital of Ministry of defense, Moscow, Russia*

Background-aim

It is known that Monoclonal Gammopathy of Undetermined Significance (MGUS) is a pre-disease and is closely associated mainly with oncohematological (myeloma) and some systemic (amyloidosis) diseases. At the same time, MGUS is detected in some chronic diseases, such as bronchial asthma, and in our opinion is a manifestation of hyperactivation of the B-cells link of the immune system. We have not found data in the literature on the incidence and possible mechanisms of MGUS development in patients with liver cirrhosis. At the same time, we identified cases of MGUS in patients with cirrhosis of the liver, which indicates the features of this pathology and compensatory capabilities of the body.

To study clinical and laboratory data and to carry out differential diagnostics in patients with cirrhosis of the liver on the subject of MGUS and multiple myeloma.

Methods

Two patients in the serum and urine of immunofixation and nephelometry methods have been identified in the serum of abnormal paraproteins (heavy and light, free light chains of immunoglobulins). The patient performed complex studies, including electrophoresis of serum proteins, determination of the number of plasma cells in the bone marrow, concentration of immunoglobulins A, M. G, E, CRP, IL-6, ® 2-microglobulin, ESR, and other hematological and biochemical studies.

Results

In the study of protein electrophoresis of the serum revealed a compensatory redistribution of protein fractions in a considerable increase in ©-fractions till 52,04 and 47.4 g/L, while reducing the amount of albumin to the levels of 30.94 and of 19.85 g/l, against the background of increasing amounts of total protein in the serum to values till 95 and 84 g/L. At least one patient has an increased release of Bens-Jones protein in the urine, in the amount of 68.56 % of the total protein in the urine. Immunofixation revealed the presence of pathological paraproteins in the blood serum, represented in one case – PigG|, PIgA_, and in the second – PigG| and free (_ and |). When immunofixation of urine was determined - in one patient – PigG|, PIgA_ and free |and in the second - PIgG|. The study of the number of free (_ and) revealed their increased amount in the blood serum _- 44.98 and 85.63 mg/l and |-120.04 and 118.69 mg/l and in the urine _-16.6 and 50.8 mg/l and |-60.68 and 147.04 mg/l. The number of plasma cells in the bone marrow was 3% and 2%. Elevated serum levels of CRP, IL-6, and ®2-microglobulin were detected, including increased amounts of ®2-microglobulin in the urine. No clinical or radiological data were found for myeloma.

Conclusions

Data on the detection of MGUS in patients with liver cirrhosis are presented for the first time. Among the main mechanisms of production of elevated pathological paraproteins, we can consider compensatory production of B-lymphocytes of paraproteins in response to a decrease in albumin production that occurs when the liver is affected and its synthetic function is reduced, which allows maintaining oncotic pressure and reduces for a while the occurrence of edema characteristic of late-stage cirrhosis.

doi: 10.1016/j.cca.2022.04.517

T273

Serum transferrin isoforms in autoimmune cholestatic liver diseases

L. Chrostek [a], A. Bauer [b], E. Gruszewska [a], B. Cylwik [c]

[a] *Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok, Poland*
[b] *Department of Biochemistry and Molecular Biology, Centre of Postgraduate Medical Education, Warsaw, Poland*
[c] *Department of Pediatric Laboratory Diagnostics, Medical University of Bialystok, Bialystok, Poland*

Background-aim

The aim of the study was to compare the serum profile of transferrin isoforms in autoimmune cholestatic liver diseases: primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) with the possibility of their use in differential diagnostics.

Methods

The study groups consisted of 76 patients with PBC, aged 30 to 77 years (68 women and 8 men) and 100 patients with PSC, aged 26 to 75 years (79 men and 21 women). The patients were treated in the Department of Gastroenterology, Hepatology and Clinical Oncology of the Centre of Postgraduate Medical Education in Warsaw. The control group consisted of 40 healthy subjects from the Occupational Medicine Clinic, aged 21-54. Capillary electrophoresis was used to determine the profile of transferrin isoforms.

Results

There were no differences in the concentrations of transferrin isoforms in PSC patients but the changes in PBC patients were demonstrated. The mean relative concentrations of disialotransferrin and trisialotransferrin were significantly lower in PBC patients (mean: 0.34 ± 0.22% and 2.78 ± 1.37%, respectively) than those in the controls (mean: 0.71 ± 0.47% and 3.61 ± 1.16%, respectively). Only the concentration of disialotransferrin differs significantly between tested group and it was significantly lower in PBC group than that in PSC group ($P < 0.001$). This difference was confirmed by the analysis of ROC curves, in which the area under the ROC curve for disialotransferrin in PBC patients (AUC = 0.819, SE = 0.056) was significantly higher than the AUC for PSC patients (AUC = 0.572, SE = 0.072) ($P < 0.001$).

Conclusions

The obtained results indicate the changes in the serum profile of transferrin isoforms in autoimmune cholestatic liver diseases and the possibility of using disialotransferrin for laboratory differentiation of PBC from PSC.

doi: 10.1016/j.cca.2022.04.518

Clinica Chimica Acta 530 (2022) S193–S197

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Immunodeficiency

T274

Natural killer cell subsets and phenotypic expression of markers of activation, maturation and associated with slow progression of HIV in HAART naïve HIV patients in Addis Ababa, Ethiopia

H. Tegared

Armauer Hansen Research Institute (AHRI), Ethiopia

Background-Aim

Natural killer cells are crucial effector cells of the immune response to viral infections including HIV. It mediates cytotoxicity and cytokine production which have anti-HIV activities. Besides, HIV provides evading strategies such as by expansion of rare NK cell subsets and alteration of surface NK cell markers. However, limited number of studies are done on inhibitory/activating receptors, markers of activation, maturation and delayed HIV progression in Africa particularly Ethiopia.

Methods

A cross sectional study was conducted in 15 HAART naïve HIV patients and 15 HC using convenient sampling technique. Whole blood was collected and stained with CD3, CD16/CD56, CD16, CD56, KIR3DL1/S1, CD8, NKG2C, CD2, CD7, CD57 and HLA-DR. The sample was acquired on a FACS Canto II flowcytometry and analyzed by Flow Jo software. Mann-Whitney U-test with JMP software was used for statistical analysis of the data. Statistical significance was set at P value < 0.05.

Results

The median fluorescent intensity of CD16/56 on NK cells were significantly decreased in HAART naïve HIV patients compared to HC. The frequency of CD8 + NK cells were also reduced and are inversely correlated with HIV viral load. From lymphocytes, CD56bright and CD56dim NK cell subsets were significantly reduced in HAART naïve HIV patients, respectively. Within total NK cells, CD56dimsubsets are still reduced and the CD56-ve subsets are expanded in HAART naïve HIV patients. The CD56bright subsets further showed lower frequency in CD8 and NKG2C expression among HAART naïve HIV patients relative to HC. However, the frequency of KIR3DL1/S1 + CD56bright and HLA-DR +CD56bright NK cell subsets were expanded in HAART nave HIV patients compared to HC.

Conclusions

This study showed that HIV infection is associated with altered subsets distribution, varied expression of markers associated with slow progression, activation, and high potency particularly on CD56bright NK cell subsets. However, further follow-up and functional study need to be conducted.

doi: 10.1016/j.cca.2022.04.086

T275

Arginase activity and frequency of low-density granulocytes in the blood of visceral leishmaniasis treatment naïve, treated and follow up visceral leishmaniasis/HIV co-infected patients

A. Sefew [b], Y. Teferi [c], E. Abate [a]

[a] *Ethiopian Public Health Institute, Ethiopia*
[b] *Immunology and Molecular Biology, Department of Medical Laboratory Sciences, College of Medicine, Debre Berhan University, Ethiopia*
[c] *Leishmaniasis Research and Treatment Center, University of Gondar, Ethiopia*

Background-Aim

Background: Main mechanisms of how visceral leishmaniasis and HIV co-infection cause profound suppression of the immune response is not well investigated. Arginase enzyme has been significantly shown to play a key role of suppressing the cellular branch of immune response in patients with VL, HIV and other diseases. Understanding the Immunologic role of arginase in the disease progression, severity and its association to the increased relapse in patients with VL/HIV will give information on future therapeutic approaches for treatment of patients with VL/HIV co-infection.

Objective: It is to measure the level of arginase activity and frequency of low-density granulocytes in the blood of visceral leishmaniasis treatment naïve, treated, and follow up VL/HIV co-infected patients.

Methods

Methods: Crosses sectional study of VL/HIV co-infected patients from University of Gondar Hospital was recruited. Arginase activity and the phenotype of arginase expressing cells were measured in the plasma and peripheral blood mono nuclear cells respectively in the blood of VL treatment naïve, treated and follow up VL/HIV co-infected patients.

Results

Results: The frequency of LDGs was significantly higher in the treatment naïve patients compared with non-relapsed follow up patients. Furthermore, there was a significant increase in the level of arginase activity in the plasma of treatment naïve patients compared with non-relapsed follow-up patients. Moreover, activation marker of LDGs

(CD63) was significantly increased in treatment naive patients compared with non-relapsed follow up patients. However, expression level of CD3 + in CD4+ and CD8+ T-cells had no significant difference in all groups.

Conclusions

Conclusion: The results suggest that the increased frequency of LDGs and arginase activity in the plasma of treatment naïve and relapsed follow up patients might contribute to the poor treatment outcome and increased rate of relapse of VL in VL/HIV co-infected patients.

doi: 10.1016/j.cca.2022.04.087

T276

Natural killer cell of subsets and phenotypic expression of markers associated with maturation, slow progression and activating receptors among HAART naïve HIV patients in Addis Ababa, Ethiopia

A. Henok [d], K. Mulugeta [d], A. Derbie [c], G. Alene [e], K. Amare [b], W. Meseret [b], L. Mulualem [b], H. Rawleigh [a]

[a] *Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia*
[b] *Department of Immunology, School of Medical Laboratory Science, College of Medicine and Health Science, University of Gondar, Gondar, Ethiopia*
[c] *Department of Medical Laboratory Science, Arba Minch College of Health Science, Arba Minch, Ethiopia*
[d] *Department of Medical Laboratory Science, College of Medicine and Health Science, Debre Tabor University, Debre Tabor, Ethiopia*
[e] *Department of Medical Laboratory Science, College of Medicine and Health Science, Woldia University, Woldia, Ethiopia*

Background-Aim

Natural killer cells are crucial effector cells in the immune response to viral infections including HIV. They lyse infected cells, and produces cytokines which have anti-HIV activities. Altered surface receptor expression and expansion of subsets of NK cells with sub-optimal functional activity have been described previously; however, no studies on NK cells have been reported in Ethiopia among HIV and healthy controls. We therefore investigated NK cell subsets and expression of multiple markers including KIR3DL1/S1, CD8, NKG2C, CD2, CD7, CD57 and HLA-DR in HAART naïve HIV patients and healthy controls.

Methods

A cross sectional study was conducted in 15 HAART naïve chronically infected HIV patients and 15 HC using convenient sampling. Whole blood was collected and stained with antibodies to CD3, CD16, CD56, KIR3DL1/S1, CD8, NKG2C, CD2, CD7, CD57 and HLA-DR. The sample was acquired on a FACS Canto II flowcytometry and analyzed by Flow Jo software. Mann-Whitney U-test with JMP software was used for statistical analysis of the data. Statistical significance was set at P value < 0.05.

Results

The median fluorescent intensity of CD16+56 on NK cells was significantly decreased in HAART naïve HIV patients compared to HC. The frequency of CD8+ NK cells was also reduced and was inversely correlated with HIV viral load. From lymphocytes, CD56bright and CD56dim NK cell subsets were significantly reduced in HAART naïve HIV patients. Within total NK cells, the CD56dimsubsets was diminished and the CD56-ve subsets was expanded in HAART naïve HIV patients. The CD56bright subsets among HAART naïve HIV patients showed reduced expression of CD8 and NKG2C, but increased expression of HLA-DR and KIR3DL1/S1.

Conclusions

Our data showed chronic HIV infection is associated with altered distribution of subsets, and dysregulated expression of surface markers. In particular, the CD56bright in Ethiopian HIV patients showed many changes in cell surface marker expression, a finding not appreciated in other previous studies.

doi: 10.1016/j.cca.2022.04.088

T277

Trends in transmitted drug resistance in a cohort of art-naive HIV-1 infected individuals in Ethiopia

M. Kiros [a], W. Erku [b], A. Mihret [a], E. Geberekidan [c], D. Hailu [a], A. Mulu [a]

[a] *Bacterial and Viral Disease Directorate, Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia*
[b] *Department of Microbiology, Immunology & Parasitology, Faculty of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*
[c] *The Ethiopian Public Health Institute, Addis Ababa, Ethiopia*

Background-Aim

Transmitted drug resistance (TDR) is associated with suboptimal treatment outcomes and there are limited data from Ethiopia. The aim of this study was to assess HIV-1 genetic diversity and transmitted drug resistance mutations among ART-naive newly diagnosed asymptomatic HIV-1 infected individuals in Addis Ababa, Ethiopia.

Methods

This was a prospective study amongst 51 newly diagnosed ART-naive HIV-1 infected patients seen in our center in Addis-Ababa from June to December 2018. Partial HIV-1 pol region (PR) covering the complete protease and partial reverse transcriptase (RT) regions of blood samples were amplified and sequenced using in-house assay. Drug resistance mutations were examined using calibrated population resistance (CPR) tool version 6.0 from the Stanford HIV drug resistance database and the International Antiviral Society-USA (IAS-USA) 2019 mutation lists.

Results

Using both algorithms, 9.8% (5/51) of analyzed samples had at least one TDR Mutation. TDR mutations to Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) were the most frequently detected mutation (7.8% and 9.8%, according to the CPR tool and IAS-USA algorithm, respectively). The mutations observed by both algorithms were K103N (2%), Y188L (2%), K101E (2%), and V106A (2%) but only E138A (2%) was observed according to IAS-USA. Y115F and M184V (mutations that confer resistance to NRTIs) were detected according to both criteria's in a single study participant (1/51, 2%) who also had NNRTIs associated mutation (Y188L). Similarly, TDR mutation to protease inhibitors were found to be low (G73S; 2%) seen only with the CPR tool. Phylogenetic analysis showed that all 51/51 (100%) of the study participants were infected with subtype C virus.

Conclusions

This study showed significant polymorphism at the PR and RT regions associated with TDR and confirmed homogeneity in the circulating HIV-1 clade C. We will recommend routine baseline genotypic drug resistance testing in all newly diagnosed HIV infected patients before initiating treatment. This will aid selection of appropriate therapy in achieving 90% of patients having undetectable viral load in consonance with the UN targets.

doi: 10.1016/j.cca.2022.04.089

T278

Identification of HIV-specific CD8 + T cells in human duodenal tissues

A.E. Lakudzala

Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Malawi

Background-Aim

Human immunodeficiency virus (HIV)-1 disease is associated with extensive structural, immunological and microbial alterations in the intestinal microenvironment. Key to fighting HIV infection are CD8 + T cells, which carry out their function through cytokine production and cytolytic granule formation. We hypothesize that HIV specific CD8 + T cells exhibit poor cytolytic function, in turn, contributes to on-going viral replication in the duodenum in individuals on antiretroviral therapy (ART).

Methods

Paired peripheral blood and duodenal tissue biopsies samples were collected from HIV-uninfected, ART-naïve HIV-infected adults and HIV-infected individuals on ART. Flow cytometry-based immunophenotyping and intracellular cytokine staining were performed on peripheral blood mononuclear cells and duodenal cells. Fluorescent microscopy was used to define cell localization in formalin-fixed paraffin-embedded duodenal tissue.

Results

We observed that CD8 + T-cells in human duodenal mucosa display a tissue-resident phenotype which is characterized by co-expression of CD103 and CD69. Immunohistochemistry showed that a majority of the tissue resident CD8 + T cells reside within the epithelial lining of the duodenum. Tissue-resident CD8 + T cells exhibited low perforin, granzyme B expression. HIV-specific tissue-resident duodenal CD8 + T cells were seen to have high EOMES expression and low perforin and Granzyme B expression.

Conclusions

Duodenal-derived CD8 + T cells are distinct from those derived from peripheral blood. Our data show that HIV-specific duodenal CD8 + T cells in HIV-infected adults possess less cytolytic potential, and that duodenal CD8 + T cells are mostly not co-localised with CD4 + T cells. Poor CTL activity and localisation of CD8 + T cells away from HIV replication sites could explain the high permissiveness of the gut to HIV infection

doi: 10.1016/j.cca.2022.04.090

T279

Assessment of some reproductive hormones and inflammatory cytokine levels in HIV infected females on hormonal contraceptives in Nnewi, Nigeria

N.R. Ukibe [a], V.O. Agu [a], C.C. Onenekwe [a], S.N. Ukibe [b], C.J. Awalu [a]

[a] *Department of Medical Laboratory, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, PMB 5025, Anambra State, Nigeria*

[b] *Department of Medical Microbiology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, PMB 5025, Anambra State, Nigeria*

Background-Aim

This is a case-controlled, observational and retrospective study designed to assess the levels of some reproductive hormones, immunoglobulin and cytokines in HIV infected females on contraceptives in Nnewi, Nigeria, using purposive sampling technique.

Methods

A total of 118 premenopausal females on regular menstrual cycle aged (17-49) years were recruited based on their menstrual cycle phase (follicular (7-13th) and luteal (21-23rd day) with aid of questionnaire. 58 out of them were HIV seropositive females [29 on hormonal contraceptive (A), 29 not on hormonal contraceptive (B)], while 60 were HIV seronegative females (controls) [30 on hormonal contraceptives (C) and 30 not on hormonal contraceptive (D)]. Reproductive hormones [FSH, LH (iu/ml), prolactin (ng/ml), progesterone (ng/ml), estradiol (pg/ml)] and cytokines [TNF-⟨ (pg/ml), IL-2 (μg/ml)] were assayed using enzyme-linked immunosorbent assay kits (ELISA).

Results

TNF-⟨ was significantly increased in HIV seropositive females on contraceptives compared with their counterparts not on contraceptives and controls on/not on contraceptives at both phases of menstrual cycle (P 0.000). IL-2 was significantly decreased in HIV seropositive females on/not on contraceptive compared with control females not on contraceptive but significantly increased in HIV seropositive on contraceptive compared with their counterparts not on contraceptive at follicular phase of menstrual cycle (P 0.002; 0.005 respectively). Progesterone and Estradiol were significantly decreased in HIV seropositive females on contraceptives compared with their counterparts not on contraceptives and controls on/not on contraceptives at both phases of menstrual cycle (P 0.000, 0.001 respectively).

Conclusions

This study showed significant decrease in ovarian hormones which is an evidence of hypogonadism. The significant elevation in TNF-⟨ in HIV infected females on contraceptives indicates active inflammation which

was more marked at follicular phase of menstrual cycle. The active inflammation may be provoked by the progesterone component of the hormonal contraceptive and the hypogonadism observed may be linked to exacerbated inflammatory reaction.

doi: 10.1016/j.cca.2022.04.091

T280

The prevalence of vitamin D deficiency in human immunodeficiency virus (HIV) infections

U.S. Intansari, Y.W. Subronto, U. Sukorini, A.Z. Arjana

Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada, Indonesia

Background-Aim

Vitamin D play a important role as affects as an immunomodulator. Research on the association of vitamin D and immune activation and immunodeficiency is limited, especially in Indonesia which is a tropical country with sufficient sunshine. This study is aim to know the prevalence of vitamin D deficiency in human immunodeficiency virus (HIV) infection.

Methods

This was an observational study with cross sectional design. Subjects were taken consecutively in the ward or in the clinic of Dr. Sardjito. Subjects of this study were the rest of the blood sample of the study subjects whom include in HATI study. Inclusion criteria include adult patients, male and female, and diagnosed with HIV infection, either with or without antiretroviral therapy. Patients who are pregnant and not willing to follow the study were excluded. and exclusion criteria.

Results

Sixty subjects followed this study with various clinical stage. Twenty three subjects (38.3%) were vitamin D deficient with cut off value 20 ng/mL. Higher proportion was subjects with severe infection (stage 3 or 4). The proportion of subjects with normal level of vitamin D was higher in mild groups (stage 1 or 2).

Conclusions

Patients with severe infection of HIV tend to be vitamin D deficient

doi: 10.1016/j.cca.2022.04.092

T281

Evaluation and comparison of Dengue NS1, Chikungunya and HCV: Fluorescence immunoassay with rapid immunochromatographic test

M.S. Islam

Hokkaido University, Reproductive and Developmental Biology, Japan

Background-Aim

Dengue viral infections are one of the most important mosquito-borne diseases in the world. HCV is most commonly spread by direct contact with infected blood and blood products. The present study aimed to evaluate the fluorescence immunoassay test for detection of dengue NS 1 antigen, Chikungunya and HCV.

Methods

This study was a case control rapid diagnosis test of the Dengue NS1, Chikungunya IgG/IgM and HCV antibody. For the study we collected 15 Dengue NS1, 15 Chikungunya and 15 HCV previous confirmed (ELISA) patients and 15 control of NS1, Chikungunya and HCV from Virology laboratory of Bangabandhu Sheikh Mujib Medical University (BSMMU). The specificity and sensitivity was observed with rapid Immuno chromatographic test (ICT) and Fluorescence Immunoassay (FIA) F200 analyzer. The data were then analyzed with Statistical Package for Social Sciences (SPSS) and IMAGE J software.

Results

The 20 Dengue NS1, 20 Chikungunya and 20 HCV sample were tested by ICT and FIA to compare the two diagnosis method. The mean intensity of the rapid ICT test was observed. STANDARD F200 Analyzer device can perform qualitative and quantitative analysis on infections, respiratory diseases and chronic diseases where the rapid ICT card gives only qualitative band intensity. The FIA diagnostic test has 93% sensitivity in NS1, 100% in Chikungunya and HCV test, where the rapid ICT found 86% sensitivity in Dengue NS1, 93% in Chikungunya and HCV test results.

Conclusions

For Diagnostic accuracy one can use FIA test over Rapid ICT for early diagnosis of Dengue NS1, Chikungunya and HCV infection.

doi: 10.1016/j.cca.2022.04.093

T282

Evaluation of Dried Blood Spots (DBS) for human immunodeficiency virus (HIV-1) drug resistance testing in Zimbabwe… (Preliminary results)

A. Makura [a], J. Manasa [a], K. Mhandire [b], J. Mutsvangwa [a], A. Manjoro [a], H.T. Matarira [b]

[a] *Biomedical Research and Training Institute ,10 Seagrave, Avondale, Harare, Zimbabwe*
[b] *University of Zimbabwe, College of Health Sciences, Department of Chemical Pathology, Zimbabwe*

Background-Aim

Sub-Saharan Africa (SAA) alone has nine out of every 10 children living with HIV globally.

Treatment monitoring and management remains poor, even as these children grow older.

Most HIV-infected children are growing towards adolescence (over 2.1 million), with the potentials to reach adulthood.

Even with an overall reduction in HIV-related mortality, there are increasing rates of morbidity and mortality among children and adolescents living with HIV (ADLHIV). Pediatric ART success might be quickly jeopardized, with possible HIV-1 drug-resistance (HIVDR) emergence.

Plasma is specimen of preference for HIVDR testing , but its limitations include: requires maintenance of a cold-chain short transit times to preserve the integrity of the viral RNA prior to PCR amplification.

DBS are an alternative specimen for HIVDR testing in resource limited settings.

It is also important to assess baseline characteristics before doing HIVDR.Paucity data exists on HIVDR genotyping comparison between DBS and plasma specimens in children and adolescence in Zimbabwe

Methods

300 participants were recruited at baseline
270 were suppressed
270 were suppressed & excluded, 30 were eligible for HIVDR,24 had VL > 1000
6 pending VL confirmation
24 paired DBS and plasma have been successfully amplified and now pending sequencing (Targeted sample size = 97).

Results

Results (General participant characteristics)
68% of the participants were male adolescents
Mean age 15.7 years (0.8) and 40% were females
Duration on ART-Median duration 5.9 years (3.5yrs-11.1yrs)
Viral Load
Median recording 73 886copies/ml (29 499copies/ml-110 788-copies/ml)
Male adolescents had the highest VL values
Viremia in children (< 10 years) increased with shorter duration on ART

Conclusions

Male adolescents had the highest VL values and therefore there is need to monitor them and HIVDR should be effectively monitored in this group.

doi: 10.1016/j.cca.2022.04.094

T283

NK cells displayed lower frequency of CD8 expressing cells diminished intensity of CD7 and higher HLA-DR expression among HIV Infected HAART naïve Ethiopians

H. Andualem [b], M. Lemma [c], M. Workneh [c], B. Ayelign [c], M. Zewudie [a], A. Mehert [a], R. Howe [a]

[a] *Armauer Hansen Research Institute, Ethiopia*
[b] *Debre Tabor University, Ethiopia*
[c] *University of Gondar, Ethiopia*

Background-Aim

Natural killer (NK) cells are crucial effector cells of the innate immune response to viral infections including HIV. They lyse infected cells and produce cytokines that have anti-HIV activities. Altered receptor expression and expansion of subsets of NK cells with sub-optimal functional activity have been described previously. However, no previous report on either NK cell subsets or expression of multiple markers have been reported among HIV patients or healthy controls (HC) from Ethiopia.

Methods

We recruited 15 treatment naïve HIV patients and 16 healthy controls. Whole blood samples were stained with fluorochrome conjugated antibodies and analyzed by flowcytometry.

Results

The frequency of circulating CD56bright and CD56dim NK cell subsets were significantly lower among the HIV group compare to HC. The CD56negative subsets was higher in HIV patients, but this was only apparent when gated among total NK cells not total lymphocytes. NK cells among HIV participants also showed a lower and higher frequency of CD8+ cells and HLA-DR+ cells, respectively. CD7 median fluorescent intensity was significantly lower in HIV patients, whereas, a distinct population of KIR3DL1/S1 frequencies was unexpectedly higher in HIV patients.

Conclusions

Our data indicated that chronic HIV infection is associated with altered distribution of subsets, and dysregulated expression of surface markers in a fashion typical of NK cell described in other settings. However, the observed downregulation of CD7 and enhanced KIR3DL1/S1 within the CD56 bright subsets have not been widely reported among HIV patients are merit further research.

doi: 10.1016/j.cca.2022.04.095

Clinica Chimica Acta 530 (2022) S198–S203

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Infection Control

M079

Evaluation of a new accurate and reliable particle enhanced immunoturbidimetric assay for the detection of Procalcitonin in the management of sepsis

T. Masetto [b], C. Kochem [a], D. Ziska [a], S. Tölke [c], A. Bäcker [a], I. Delseith-Hermsdorf [a], M. Grimmler [c]

[a] *DiaSys Diagnostic Systems GmbH, Alte Str. 9, 65558 Holzheim, Germany*
[b] *Institut für Molekulare Medizin I, Heinrich-Heine-Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany*
[c] *Institute for Biomolecular Research, Hochschule Fresenius, Limburger Str. 2, 65510 Idstein, Germany*

Background-aim

The Third International Consensus Definitions for Sepsis and Septic Shock redefined sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis is considered as a major public healthcare issue affecting more than 30 million people worldwide, associated with a mortality of potentially 6 million people and accounts for almost $24 billion in health care expenditures only in the US, annually.

Rapid and accurate sepsis management is an absolute prerequisite to reduce mortality and economic burden. Therefore, reliable, fast and easy-to-use assays are needed for laboratory routine use. Procalcitonin (PCT) has revealed to be an indispensable tool for the management of sepsis in clinical chemistry.

Methods

We report a new polyclonal based particle enhanced immunoturbidimetric assay (PETIA) for the detection of PCT which can be applied to all the most commonly used high throughput clinical chemistry instruments. We present the method evaluation of the DiaSys PETIA Procalcitonin FS on Cobas c501 (Roche).

Results

Reagent stability was evaluated in terms of 37 °C stress stability (ε 19 days), on board stability (12 weeks) and calibration stability (12 weeks).

Precision was evaluated in terms of intra-day precision (CV at cut-off 0.5 ng/mL = 6.22 %) and inter-day precision at different concentrations (CV at cut-off 0.5 ng/mL = 6.15 %).

Limit of blank (LOB) and limit of quantitation (LOQ) were 0.03 ng/mL (NaCl) and 0.2 ng/mL, respectively.

The reagent displayed a linear recovery in the range between the LOQ up to 50 ng/mL. No Prozone effect was detected at least up to 1000 ng/mL.

No interferences were detected for the reagent in the tested ranges of the following interferent materials: Bilirubin, Rheumatoid Factors, Hemolysis, Triglyceride, Ascorbate, Calcitonin, ⟨-CGRP, ®-CGRP, Imipenem, Cefotaxime, Noradrenaline, Dobutamine, Furosemide, Vancomycin and Dopamine.

The Passing and Bablok regression versus the reference test showed an excellent comparison between the two methods (slope = 0.92, intercept = 0.04, R = 0.983).

Conclusions

The present work proves that the new DiaSys PETIA reagent Procalcitonin FS has superior performances, comparable to the reference test in the relevant analytical range.

doi: 10.1016/j.cca.2022.04.097

M080

Tinea unguium, tinea cruris and tinea corporis caused by trichophyton rurbum in HIV patient: A case report

Y. Merad [b], M. Belkacemi [a]

[a] *hemobiology department, Abdelkader Hassani Hospital, Algeria*
[b] *Parasitology-Mycology department, Abdelkader Hassani Hospital, Algeria*

Background-aim

Dermatophytes are a type of fungi that infect keratinized tissues. These infections are very common and categorized by their anatomical location and typically caused by one of three main species: Microsporum, Epidermophyton, or Trichophyton. Trichophyton rubrum is an anthroophilic, cosmopolitan, fungal complex that cause numerous forms of dermatophytosis. The disease in immunocompromised hosts is more varied and often more severe than in immunocompetent hosts. We report on an atypical clinical presentation with multiples locations on skin (neck, face, inguinal), and nails.

Methods

The clinical presentations, though very typical of tinea infection, samples (nail an skin scraping) were collected and examined by direct microscopy and culture on Sabouraud's medium for at least 4 weeks

Results

The cultures revealed the presence of Trichophyton rubrum. After few weeks of oral terbinafine treatment, the cutaneous lesions disappeared.

Conclusions

There have been few reports of generalized dermatophytosis infection in HIV patients. Thus, it is important to be aware of their transmissibility and complications in HIV patients.

doi: 10.1016/j.cca.2022.04.098

M081

Evaluation of acid-fast microscopy, mycobacterial culture and genexpert for the detection of pulmonary and extrapulmonary tuberculosis: The good, better and best

S. Tummidi [a], S. Prashant [a], R. Ashok [b], D. Deepa [b]

[a] *AIIMS, Mangalagiri, India*
[b] *Pathkind Labs, India*

Background-aim

The GeneXpert MTB/RIF real-time polymerase chain assay is being increasingly used to complement acid-fast microscopy and culture for faster diagnosis of pulmonary tuberculosis. However, few studies have compared its performance in pulmonary specimens with extrapulmonary samples. This study addresses this comparison.

Methods

The performance of acid-fast microscopy, culture and GeneXpert was evaluated in 455 pulmonary and 69 extrapulmonary specimens. Their sensitivity, specificity, predictive values, accuracy, area under the curve (AUC) and kappa were estimated. Using bootstrapped receiver operating characteristic cut-offs, GeneXpert cycle thresholds (Ct) were also evaluated for their utility as surrogate classifiers of smear positivity and time to culture positivity in various sample subsets

Results

With culture as gold standard, GeneXpert was significantly more sensitive (100% vs. 84.4%; $p < 0.001$) and nearly as specific (94.8% vs. 96.7%; $p = 0.344$), with a greater AUC (0.97 vs. 0.91; $p = 0.002$), than acid-fast microscopy. It showed higher accuracy (96.2% vs. 93.4%; $p = 0.003$) and agreement (kappa: 0.91 vs. 0.83) with culture than microscopy. It was equally sensitive and specific for pulmonary (100%; 94.1%) and extrapulmonary (100%; 96.5%) samples, and 100% sensitive for sputum, bronchoalveolar lavage, lymph node aspirates, pleural fluid, pus and urine. It also picked up smear negative but culture positive specimens with 100% sensitivity. GeneXpert Ct were inversely correlated with acid-fast microscopy grades (> -0.67; $p < 0.001$) and positively with time to culture positivity (> 0.69; $p < 0.001$). This yielded a mean Ct cut-off of 21.4 for identifying positive smears with 81.4% sensitivity and 79.2% specificity (AUC 0.85; $p < 0.001$), and further classified pulmonary (21.4) from extrapulmonary (23.4) samples ($p < 0.001$), various acid-fast microscopy grades and time to culture positivity. Rifampicin resistance was seen in 17% samples.

Conclusions

GeneXpert can be a reliable first line diagnostic for both pulmonary and extrapulmonary samples. By providing surrogate classifiers for smear positivity and time to culture positivity, its Ct cut-offs can give early indication of bacterial burden, disease severity, infection control risk and response to therapy.

doi: 10.1016/j.cca.2022.04.099

M082

Investigating the sensitivity of influenza A virus specific T cells

E. Chimbayo

Malawi Liverpool welcome trust, Queen Elizabeth Central Hospital, Blantyre, Malawi
University of Glasgow, University Avenue, Glasgow U, United Kingdom

Background-aim

The rapid annual incidence of influenza A infection (IAV) poses a significant threat to human health globally. The virus evolves rapidly, changing the protein targets of neutralizing antibodies. As a result, seasonal antibody-based vaccines are designed yearly and provide limited protection to new strains. Memory T cells that recognize conserved regions of internal viral proteins provide a potential for a universal protection. The ability of IAV specific memory CD4 + and CD8 + T cells to produce robust pro inflammatory cytokines such as Interleukin-2 (IL-2), Interferon -gamma (IFN-□) and Tumor necrosis factor alpha (TNF□) is key in the control of viral infections. However, little is known about the sensitivity of IAV specific T cells in anti-influenza A virus immunity. We aimed to characterize IAV specific T cells ex vivo to question whether memory IAV specific CD4 and CD8 T cells are more sensitive to antigen-driven activation than primary responding T cells.

Methods

We utilized a flow cytometry and an IAV infection mouse model to examined whether memory IAV specific CD4 and CD8 T cells are more sensitive to peptide and antigen stimulation than primary responding T cells. C57BL/6 (B6) mice were infected intranasally with 200-300 PFU of WSN strain of influenza a virus. Spleen tissues were collected 8- or 30-days post infection and co-cultured in vitro with peptide pulsed Bone marrow derived dendritic cells (bmDCs). Cells were then stained with appropriately titrated concentrations of anti-CD4, anti-CD8 and anti-CD44 antibodies for surface markers. Cytokine expression profiles were determined by intracellular staining with anti-IL-2, anti-IFN-□□and anti- TNF for proinflammatory markers. Samples were then acquired on flowcytometry and analyzed on flowjo software.

Results

We show that CD4 T cell cytokine responses are dose-dependent in both the primary and memory response in comparison to CD8 T cells. Additionally, memory CD4 T cells are able to produce cytokine response at a lower concentration of peptide stimulation than primary responding cells. This indicates that IAV specific CD4 memory T cells have increased sensitivity compared to primary responding T cells. Furthermore, in contrast to CD8, IAV specific CD4 T cells are more likely to produce multiple cytokines at both primary and secondary and their responses are dose-dependent. However, both CD4 and CD8 multifunctional memory T cells produce a more robust cytokine response per cell than single cytokine producers.

Conclusions

Collectively, these findings in part reveal the functional superiority of IAV specific cytokine producing memory T cells over primary effectors. These data may support the advent of T cell-based vaccines that

offer universal protection for heterosubtypic IAV strains and reduce the need for annual reformulation of anti-Influenza A vaccine.

doi: 10.1016/j.cca.2022.04.100

M083

Why do we have to reduce the use of mobile phones in hospitals

C. Grigore [a], S.V. Cristea [a], M. Totan [b], N. Grigore [b]

[a] *Laboratory Department, Academic Pediatric Hospital, Sibiu, Romania*
[b] *University Lucian Blaga, Sibiu, Romania*

Background-aim

Healthcare workers frequently use mobile phones during their work. Because these devices are seldom cleaned and often touched during or following the examination of patients without hand washing, they are known as a media of spreading germs.

Each hospital have to be aware about the flora mobile phones can spread and settle rules for their use in the hospital, in order to control healthcare associated infections.

Methods

Our study was made on 200 mobile phones belonging to our staff, devided into 4 categories: A (doctors), B (nurses), C (cleaning staff), D (kitchen staff). An epidemiology assistant took swabs from the surfaces of the phones, transported them into the laboratory where were planted on a plate with chromogenic agar and putted into the thermostat at 37 degrees Celsius for 24 hours. The microbiologist made the identification of the grown colonies on a Vitek 2 automated system.

Results

From the 200 plates only on 28 (14%) were identified pathologic bacteria:12 Enterococcus spp, 8 Staphylococcus aureus, 6 Acinetobacter, 2 Staphyloccocus aureus methicilin resistant.

The percentages varied with the group. In group A (54 Doctors), we found 10 positives (18.5%) , in group B (72nurses) we found 12 positives (16.6%), in group C (48 cleaning staff) we found 6 positives (12.5%) and in group D (26 kitchen staff) we found 0 positives.

Conclusions

The mobile phones of our hospital healthcare workers carry pathological germs and because they represent a potential way of transmitting health care associated infections we have to reduce their use in the hospital. The regulations have to focus on doctors hand hygiene rules, because these category have the highest rate of contamination on their devices.

doi: 10.1016/j.cca.2022.04.101

M084

Rotavirus vaccine shedding is associated with increased frequency of circulating class-switched memory B-cells in Malawian infants

E. Thaulo

Malawi Liverpool Wellcome Trust Clinical Research Programme, Malawi

Background-aim

Rotavirus causes severe gastroenteritis amongst infants and young children. Malawi introduced Rotarix rotavirus vaccine (RV1) in 2012, and despite experiencing significant impact in reduction of mortality and severe diarrhea rates, the vaccine effectiveness is lower compared to high income countries. The factors contributing to the sub-optimal effectiveness and immunogenicity of RV1 in Malawi are still unknown. We investigated changes in viral shedding and memory B-cell subsets induced following RV1 vaccination in Malawian infants as potential correlate for vaccine take and immune activation, respectively.

Methods

Peripheral blood and stool samples were collected 4 days post-first dose and week 14 of age (4 weeks post-second dose). Flow cytometry-based immunophenotyping was used to characterize the circulating B-cell subsets in blood samples. Rotavirus-specific VP6 quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and Rotarix-specific NSP2 RT-PCR assays were used to determine vaccine shedding in stool samples.

Results

The frequency of class- switched and non-switched memory B-cells was similar between vaccine shedders ($n = 10$) and non-shedders ($n = 10$) (class switched 0.21-0.18, $p = 0.58$; non-class switched 2.01 vs 2.5, $p = 0.09$) four days post-first dose. However, four weeks post-second dose, the frequency of class-switched memory B-cells was higher in vaccine non-shedders than shedders (0.66 vs 0.4, $P = 0.048$), while the non-switched memory B-cells were similar between the two groups (3.21 vs 3.18, $p = 0.86$).

Conclusions

Rotarix vaccine shedding is associated with a change in the frequency of circulating class-switched memory B cells four weeks post second dose. Further ongoing work will characterize maturation profiles and functional capacity of RV1-specific memory B-cells in Malawian infants.

doi: 10.1016/j.cca.2022.04.102

M085

Determinants of systemic immunity to invasive salmonella infection in endemic settings

L. Preciado-Llanes [a], T. Nyirenda [b]

[a] *Medical Research Council (MRC) Human Immunology Unit, Weatherall Institute of 10 Molecular Medicine, University of Oxford, United Kingdom*
[b] *University of Malawi, College of Medicine, Malawi*

Background-aim

Invasive non-typhoidal Salmonella(iNTS) and Salmonella Typhi are the commonest causes of invasive bloodstream infections in sub Saharan Africa. The controlled human infection model (CHIM) has been used to study cross-reactive clades for Salmonella Typhi and iNTS. Investigating these immune responses in individuals with or without natural Salmonella disease in an endemic setting would support our understanding of cross-protective efficacy of novel vaccine candidates of invasive Salmonella infection.

Methods

Objective: To determine cellular immunity conferred by an episode of invasive Salmonella exposure in an endemic setting.

Methods: We conducted a prospective observational case-control study in Blantyre Malawi. We recruited individuals (aged 2 to 60 years) with blood culture confirmed Salmonella Typhi infection and their age-matched healthy controls (HIV-ve, HIV + ART + /-). We characterized systemic IFN-gamam responses following invasive Salmonella Typhi infection using ELISpot of peripheral blood mononuclear cells. IFN-gamma responses to Salmonella specific peptides: cytolethal distending toxin subunit B homolog(CdtB), hemolysin E(HlyE), PhoN peptides, and influenza were evaluated.

Results

Preliminary results: We found that invasive Salmonella Typhi cases had significantly higher IFN-gamma producing cells specific for CdtB and PHoN peptides compared to all controls ($p = 0.0308$ and $p = 0.0043$ respectively). There was no significant difference between cases and controls in IFN-gamma producing cells specific for HlyE. In addition, we found that invasive Salmonella Typhi cases had significantly higher IFN-gamma producing cells specific for PhoN peptides compared to HIV + ART + and HIV + ART- controls ($p = 0.0282$ and $p = 0.0382$ respectively). Interestingly, there has been evidence of generalized proinflammatory phenotype with raised IFN-gamma production in HIV + ART- individuals, which is reversed on ART.

Conclusions

Our findings are important for development of Salmonella Typhi diagnostics and vaccines. We have shown that invasive Salmonella infection in endemic settings induce immune cells to generate IFN-gamma producing cells specific to candidate vaccine antigens CdtB and PhoN. Previous T-cell clade work in the CHIM suggests that PhoN might be cross protective against iNTS. This deserves further investigation among iNTS cases in this endemic area.

doi: 10.1016/j.cca.2022.04.103

M086

Screening of HIV infection: Five years experience of a tertiary care Greek hospital

A. Karyoti [a], E. Myserli [a], C. Zarras [a], K. Mpani [a], E. Papadimitriou [b], D. Rousis [b], D. Chatzidimitriou [b], E. Vagdatli [a]

[a] *Microbiology Laboratory, Hippokration General Hospital of Thessaloniki, Greece*
[b] *National AIDS Reference Centre of Northern Greece Medical School University of Thessaloniki, Greece*

Background-aim

Laboratory diagnosis of Human immunodeficiency virus (HIV) is essential for HIV infection control and prevention. Fourth-generation HIV Ag/Ab Combo immunoassays, detecting HIV antibodies and p24 antigen, have higher analytical sensitivity and they have significantly reduced the diagnostic window period. The aim of the study is to evaluate the effectiveness of this screening tool for the laboratory diagnosis of HIV infection.

Methods

This study is a five years retrospective analysis retrieved from database of the results of HIV screening at Hippokration General Hospital of Thessaloniki. 64088 samples were tested using Architect HIV Ag/Ab Combo assay in Architect i2000. Specimens with signal to cutoff (S/CO) ratio less than 1.00 were considered non reactive while greater or equal to 1.00 were reanalyzed in duplicate. Repeatedly reactive specimens were confirmed at National AIDS Reference Centre of Northern Greece.

Results

118 samples were found repeatedly reactive (0.184%). 53 were confirmed as true positives while 65 as false positives. S/CO values of true positive results ranged between 31.95-1368.65, whereas S/CO values of false positives ranged between 1.01-6.48. Positive Predictive Value (PPV) was 44,9%. Among samples with true positive results, 4 samples were tested as negative or intermediate with confirmatory tests, meanwhile HIV viral load tests were positive. The combination of a reactive HIV Ag/Ab Combo test, a non reactive HIV-1/HIV-2 antibody immunoassay result and a reactive HIV viral load, indicates laboratory evidence for acute HIV-1 infection.

Conclusions

Fourth-generation screening assays are accurate and reliable in detecting HIV early infection. However they may yield to nonspecific reactions, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be verified with confirmatory tests. The choice remains between eliminating all false negative results and accepting false positives. The primary goal of HIV screening tests is to detect HIV infection as soon as possible. In our opinion high sensitivity is desirable for early detection and should be a priority. In conclusion, fourth generation HIV Ag/Ab Combo assay is an effective tool for the laboratory diagnosis of HIV infection.

doi: 10.1016/j.cca.2022.04.104

M087

Positive hepatitis E virus infections

I. Ivanova [b], B. Atanasova [a]

[a] *Clinical Laboratory Department, Medical University – Sofia, UMHAT "Alexandrovska" – Sofia, Bulgaria*
[b] *Clinical Laboratory Department, Medical University – Sofia, UMHAT "St. Ivan Rilski" – Sofia, Bulgaria*

Background-aim

According to the World Health Organization hepatitis E virus (HEV) infection has a large distribution worldwide. Europe, East Asia and the Americas are endemic areas with anti-HEV prevalence up to 10%. The aim of this study is to present retrospective data for the number of anti-HEV positive cases in our practice.

Methods

A total of 125 patients were examined for anti-HEV IgM and anti-HEV IgG in the Clinical Laboratory Department of the University Hospi-

tal "St. Ivan Rilski", Sofia, Bulgaria, for a period of 2 years (January 2018 – January 2020). The mean age of the patients was 50 ± 15 years with the male/female ratio 68/57 (54.0% / 46.0%). Serum samples are examined for anti-HEV antibodies by Enzyme Linked Fluorescent Assay using miniVidas automated system, Biomerioux.

Results

No HEV infection was determined in 101 patients (80.8%); 13 patients were with positive anti-HEV IgG (past infection); 2 cases was with positive anti-HEV IgM only (acute phase of infection - onset) and 9 cases were with positive results both for anti-HEV IgM and IgG. Totally 24 patients (19.2%) were detected with HEV infection in our routine practice for 2 years period.

Conclusions

The latest data show that HEV infection is the most common cause of acute viral hepatitis worldwide. Usually the infection is self – limiting but in cases with other causes of hepatitis (viral, toxic, autoimmune, steatosis, Wilson disease, and immunocompromised patients) it could be a reason for the worst course of the disease. The incidence of HEV positive patients in our practice over a one year period is approximately 9.6%, i.e. – less than 10% of all tested individuals. Our observations are in content to other epidemiology data in the literature for this geographic region.

doi: 10.1016/j.cca.2022.04.105

M088

Epidemiological study of toxigenic clostridioides difficcile by PCR-ribotipification: Detection of the first strain RT027 in a university hospital of the city of Buenos Aires, Argentina

C. Braeckman [b], S. Chalabe [b], S. Luna [b], P. Uchima [b], N. Azula [a], V. Romano [c], J. Smashevsky [a]

[a] *Bacteriology Area, Clinical Analysis Department, CEMIC University Hospital, Buenos Aires, Argentina*
[b] *Clinical Biochemistry Residence, University Institute CEMIC (IUC), Buenos Aires, Argentina*
[c] *Virology Area, Clinical Analysis Department, CEMIC University Hospital, Buenos Aires, Argentina*

Background-aim

Clostridioides difficile is a sporulated anaerobic gram-positive bacillus, cause of nosocomial diarrhea. Its pathogenesis is due to the production of toxins that are stimulated by the tcdR gene and repressed by the tcdC gene. Numerous hospital outbreaks have been described worldwide due to hypervirulent strains (HV) associated with toxigenic hyperproduction, binary toxin (CDT) production and/or deletion in the tcdC gene. Molecular typing by PCR–ribotyping allows knowing the ribotypes (RT) and identifying those HV, as strain RT027, known for producing outbreaks. Objetives: Characterize toxigenic C. difficile isolates by PCR-ribotyping by capillary electrophoresis (EC). Know the circulating RT in our institution. Evaluate the presence of RT HV and/or possible HV.

Methods

Thirty strains of toxigenic C. difficile recovered from 27 patients of our institution, between the years 2017 to 2018. They were previously characterized by toxigenic culture, immunochromatography and qPCR. qPCR detects the tcdC gene and is capable of detecting HV strains with deletion in the tcdC gene. The strains were replicated in sheep blood agar in anaerobiosis and DNA extraction was performed. For ribotyping, the intergenic spacer (igs) region between the genes encoding the 16S rRNA was amplified by conventional PCR. The products of the PCR were analyzed by capillary electrophoresis and WebRibo software, allowing defining the RT.

Results

The 30 isolates were grouped into 17 RT, the ones with the highest prevalence being RT106 (n = 6) and RTAI-82/1 (n = 3). The remaining 5 RTs were not previously reported. Three RT were HV, detecting the first RT027 isolation in our institution. Four were potential HVs, since they presented CDT and deletion in the tcdC gene, but outbreaks for these RTs have not yet been reported. Ten RT were not HV. We identify 3 cases of recurrences.

Conclusions

A great diversity of toxigenic C. difficile was detected in our institution, no outbreaks have occurred in the study period. PCR-ribotyping is a useful method to study the epidemiology of C. difficile, it helps improve preventive strategies. It allows to detect the appearance of HV strains and to alert them. RT027 associated to worldwide outbreaks was first detected in our institution.

doi: 10.1016/j.cca.2022.04.106

M089

Viral serology interpretation pitfall following IVIg therapy

B. Kyle, J. Suresh

Saskatchewan Health Authority, Canada

Background-aim

We describe a case whereby an intravenously-administered immunoglobulin (IVIg) therapy led to a series of clinical false positives in viral serology, inconsistent with the patient history.

Methods

Blood was drawn for viral serology testing that included cytomegalovirus (CMV), hepatitis, and HIV investigations. All serologies were performed on an automated Roche Diagnostics e601 platform analyzer according to manufacturer's instructions.

Results

The patient presented to hospital with petechiae-type bleeding rashes and was investigated for thrombocytopenia after initial blood investigations indicated very low platelets.

Subsequent testing of the potential causes for low-platelet involved several viral serology investigations, including hepatitis, cytomegalovirus (CMV) and human immunodeficiency virus (HIV). Initial testing indicated patient exhibited negative status for all viral antibodies and antigens (except immunity for hepatitis B surface antigen antibody).

As part of the thrombocytopenia treatment, IVIg therapy was administered, and subsequent viral serology was ordered. These investigations indicated a positive status for several hepatitis antibodies as well as CMV.

Conclusions

This case study illustrates the potential for improper diagnosis of previous or ongoing infection status in patients administered IVIg therapy.

doi: 10.1016/j.cca.2022.04.107

M090

Interleukine 6 is associated with severity and poor outcome in ICU-patients with SARS-CoV-2 infection

N. Mario [a], J. Lavillegrand [c], M. Garnier [f], A. Spaeth [a], T. Urbina [c], Y. Chantran [b], M. Fartoukh [e], B. Guidet [c], H. Ait-Oufella [d], M. Vaubourdolle [a]

[a] *Département de Biochimie, Hormonologie et Suivi Thérapeutique, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France*
[b] *Département d'Immunologie Biologique, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France*
[c] *Service de Médecine Intensive-Réanimation, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France*
[d] *Service de Médecine Intensive-Réanimation, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France et Inserm U970, Cardiovascular Research Center, Université de Paris, France*
[e] *Service de Médecine Intensive-Réanimation, Hôpital Tenon, Assistance Publique-Hôpitaux de Paris, Paris, France*
[f] *Service d'Anesthésie-Réanimation, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France*

Background-aim

SARS coronavirus 2 (SARS-CoV-2) is responsible for high morbidity and mortality worldwide, mostly due to the exacerbated inflammatory response observed in critically ill patients. However, little is known about the kinetics of the systemic immune response and its association with survival in Covid-19 patients admitted in ICU

Methods

We performed a retrospective multicenter study including all patients with SARS-Cov-2 infection admitted in 3 ICUs between March 1st and April 15th 2020, with at least 2 measurements of Interleukin 6 (IL6) in 4 days (baseline and day 3-4). Patients who received immunomodulatory treatment were excluded. IL6 was measured on serum by ELISA (Quantikine R&D Systems) and results were expressed at median [25th – 75th percentile]. The relationship between IL6 and CRP, organ failure severity (SOFA score) or in-ICU mortality was analyzed.

Results

From the 140 patients admitted in the 3 ICU for SARS-Cov2 infection (PCR diagnosis), 101 patients were included, the mean age was 59 ± 11 years with a high proportion of men (82%). Patients had severe respiratory disease with media SOFA score of 4 [3-7] and 83 required endotracheal intubation/mechanical ventilation at baseline. An increase of SOFA score between baseline and day3-4 was observed in 32 patients (worsening group). Baseline measurements were done 14 days [11-20] after onset of symptoms. At the end of the study, on April 15th 2020, 47 patients had been discharged from ICU, 35 were still in ICU, and 19 had died in ICU.

Baseline IL6 concentrations were positively associated with SOFA score. Moreover, baseline IL-6 and CRP concentrations were significantly higher in the worsening group vs the non-worsening: 278 [70-622] vs 71 [29-153] pg/mL ($P < 0.01$) for IL6 and 178 [100-295] vs 100 [37-213] mg/L ($P < 0.05$) for CRP. However, IL6 concentrations were not correlated with CRP.

Il6 and CRP concentrations were higher in non-survivors at baseline and at day 3-4. CRP significantly decreased in survivors (190 [80-248] to 108 [45-185], $P < 0.05$) whereas IL6 decreased in both groups.

Conclusions

In this multicenter cohort of ICU patients with SARS-CoV-2 infection, we found that Il6 was associated with organ failure severity, worsening and poor outcome.

doi: 10.1016/j.cca.2022.04.108

Clinica Chimica Acta 530 (2022) S204–S213

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Infiammation, Vascular DiseaseT284–T303

T284

Haptoglobin phenotype 2-2 is involved in oxidative stress

V. Manolov [b], S. Hadjidekova [e], V. Vasilev [a], Z. Gramatikova [h], R. Emilova [c], O. Georgiev [g], I. Petrova [f], B. Bogov [d], E. Hadjiev [d], V. Pencheva-Genova [g], G. Tzvetkova [d], G. Angov [f], V. Spasova [e], T. Kunchev [f], K. Tzatchev [b], M. Karadjova [f], L. Traykov [f]

[a] *Clinical Laboratory, "Aleksandrovska" hospital, Sofia, Bulgaria*
[b] *Dept. of Clinical Laboratory, Medical University, Sofia, Bulgaria*
[c] *Dept. of Immunology, NCIPD, Sofia, Bulgaria*
[d] *Dept. of Internal Diseases, Medical University, Sofia, Bulgaria*
[e] *Dept. of Medical Genetics, Medical University, Sofia, Bulgaria*
[f] *Dept. of Neurology, Medical University, Sofia, Bulgaria*
[g] *Dept. of Propaedeutics of Internal Diseases, Medical University, Sofia, Bulgaria*
[h] *R.E.D. Laboratories N.V./S.A., Zellik, Belgium*

Background-aim

Human gene of haptoglobin is presented by two alleles. Haptoglobin types are 1-1, 1-2 and 2-2. Different studies shows role of type 2-2 in cardio-vascular disease occurrence during diabetes. Haptoglobin type 1 is known to suppress haemoglobin based oxygenation of HDL and LDL, acting like antioxidant.

Methods

We aimed that Bulgarian population is haptoglobin 2-2 type, which causes frequent morbidity by systematic diseases, such as atherosclerosis, diabetes, diabetic nephropathies, gestational diabetes, anaemia, etc. 79 volunteers were included, age 32.3 ± 5.1. IMT, ABI, CBC, iron homeostasis, hsCRP and haptoglobin type were evaluated.

Results

Higher serum hepcidin concentrations were established in volunteers with haptoglobin phenotype 2-2 (29.6 ± 3.1 μg/L) compared to other types: 2-1 (22.9 ± 4.6 μg/L) and 1-1 (15.4 ± 2.7 μg/L), $P < 0.005$. In haptoglobin type 2-2 was found strong positive correlation between serum hepcidin levels and higher IMT and ABI ($r = 0.845$, $r = 0.871$, resp.; $P < 0.05$). Volunteers with haptoglobin type 2-1 and 1-1 had lower IMT (0.33 ± 0.05, 0.29 ± 0.03) and ABI (1.09 ± 0.05, 1.05 ± 0.05) parameters; $P < 0.005$.

Conclusions

The main reason for acute coronary thrombosis is atherosclerotic plaque rupture. Extra-vascular haemoglobin plays role as start mechanism for inflammation in the plaques. Important contra-active mechanism is played by haptoglobin. Thus, it prevents kidney injury from free haemoglobin. Released iron from destructed erythrocytes forms reactive oxygen radicals through Fenton's reaction. Hepcidin regulates iron homeostasis by its interaction with intracellular iron exporter ferroportin.

Acknowledgements: This project is sponsored by MU-Sofia, as part of Grant Д-213/2018.

doi: 10.1016/j.cca.2022.04.110

T285

Oxidative insult induced senescence in retinal pigment epithelial cells: A possible mechanism for age-related macular degeneration

H.I. Ahmad Mulyadi Lai [b], S.H. Chiou [a]

[a] *Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan*
[b] *Institute of Pharmacology, National Yang-Ming University (NYMU), Taipei, Taiwan*

Background-aim

Oxidative damage to retinal pigmented epithelial (RPE) cells has been implicated in the pathogenesis of aged macular degeneration (AMD), which is known to cause irreversible central vision loss. A multifactorial disease that includes advanced age, smoking, obesity and low dietary intake of antioxidant as the main risk factors. Recently, review highlight the relationship between cardiovascular disease (CVD) and overproduction of reactive oxygen species (ROS) as an important role in CVD pathophysiology. ROS can be secreted from nicotinamide adenine dinucleotide (phosphate) oxidase, mitochondria, or the uncoupling of nitric oxidase synthase in vascular cells. Therefore, in this study, we sought to investigate the effects of sodium iodate ($NaIO_3$) as an oxidative compound to induced oxidative stress, which could elicit a senescence response in cultured RPE cells.

Methods

MTT assay was used to evaluate the cell viability of exposed human RPE cell line (ARPE-19) to a variety of $NaIO_3$ concentrations (1-20 mM) for 24 hours. Immunofluorescence and western blot analysis were used to investigate tight junction, adherent junction, and senescence marker. qPCR used to quantify the absolute amount of cDNA generated from the mRNA of the two classical cadherins. Senescence-associated-ß-galactosidase (SA-ß-Gal) assay used to detect galactosidase activity.

Results

Structural changes appearing in senescent cells causes changes in the shape and size of RPE cells insulted with $NaIO_3$. RPE cells integrity was determined by immunofluorescence analysis of tight junction protein zonula occludens-1 (ZO-1), revealed that expression of ZO-1 was dose-dependently inhibited with increasing concentration of $NaIO_3$. Additionally, we evaluated ©-H2AX immunofluorescence to assess the impact of $NaIO_3$ on DNA integrity and these cultures showed immunofluorescent ©-H2AX nuclear foci, demonstrating the presence of an active DNA damage response. We quantified the absolute amount of cDNA generated from mRNA of the two classical cadherins, E-cadherin and N-cadherin on oxidative insulted RPE. The expression of adherent junction mRNA was also significantly decreased in the treated cells. These results indicate that $NaIO_3$ – induced oxidative stress, cause significant changes inside RPE cells at the molecular and structural levels, although RPE morphology looks grossly normal. Further analyzed the ability of $NaIO_3$ oxidative insult by senescence induction as shown by positive senescence associated-ß-galactosidase (SA-ß-Gal) staining, and p16 and p21 protein upregulation. Senescent cells upregulated the proinflammatory cytokine IL-6 and IL-8, the main markers of the senescence-associated secretory phenotype.

Conclusions

Our results support the hypothesis that $NaIO_3$ insulation plays a role in the induction and progression of AMD. Moreover, they would also explain the striking association of AMD with age-associated functional losses due to the accumulation of reactive oxygen and nitrogen species-induced damage along with lifestyle habits, cigarette smoking.

doi: 10.1016/j.cca.2022.04.111

T286

Phytochemical, mineral analysis and antioxidative effect of methanolic extract of ocimum gratissimum in hypertensive male wistar rats

E.O. Iloh [d], O.B. Onyema-Iloh [b], S. Meludu [a], I. Onuorah [c]

[a] *Basic Medical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*
[b] *Chemical Pathology Department, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria*
[c] *Chemical Pathology Department, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*
[d] *Industrial Chemistry Department, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria*

Background-aim

Hypertension is a serious public health problem associated with oxidative stress which can damage cells and may also play a role in development of neurodegenerative diseases. Ocimum gratissimum is a medicinal plant with useful phytochemicals and minerals. Antioxidative effect of methanolic extract of Ocimum gratissimum (MEOG) in tissue samples of hypertensive male wistar rats was investigated.

Methods

Forty wistar rats (100-110)g were assigned to five groups of eight rats each. Group 1-5 constitutes the normal, hypertensive group, MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks. At 8weeks, the tissue samples (liver, kidney, testes and heart) were excised out and homogenized for analysis. Phytochemical, mineral and oxidative stress markers analysis was done using standard methods.

Results

The mean level of antioxidant enzymes on tissue samples of liver, kidney, testes and heart showed significant ($P < 0.05$) increase at different doses when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant increase when compared to 200mg/kg/bwt of MEOG. In lisinopril group and untreated group, there were significant decrease ($P < 0.05$) when compared with the control group at 8weeks. The mean level of Malondialdehyde (MDA) on tissue samples of liver, kidney, testes and heart showed significant ($P < 0.05$) increase in untreated group, lisinopril group and 200mg/kg/bwt of MEOG when compared with the control group. Moreover, at higher doses of 400mg/kg/bwt of MEOG, there was significant ($P < 0.05$) decrease when compared with the control group.

Conclusions

Ocimum gratissimum possesses antioxidative effect which may be linked to the rich phytochemicals and minerals resident in the plant. Therefore, may be beneficial in ameroliating the effect of oxidative stress in hypertensive condition.

doi: 10.1016/j.cca.2022.04.112

T287

The change for qualitative balance of vascular endothelial growth factor-a may contribute to therapeutic efficacy for an anti-neutrophil cytoplasmic antibody associated vasculitis subtypes

R. Kikuchi [c], T. Naotake [e], S. Maruyama [d], T. Murohara [a], T. Matsushita [b]

[a] *Department of Cardiology, Nagoya University Graduate School of Medicine, Aichi, Japan*
[b] *Department of Clinical Laboratory, Nagoya University Hospital, Aichi, Japan*
[c] *Department of Medical Technique, Nagoya University Hospital, Aichi, Japan*
[d] *Department of Nephrology, Nagoya University Graduate School of Medicine, Aichi, Japan*
[e] *Department of Nephrology, School of Medicine, Fujita Health University, Aichi, Japan*

Background-aim

Anti-neutrophil cytoplasmic antibodies (ANCA) are a group of autoantibodies that cause systemic vascular inflammation by binding to target antigens of neutrophils. The purpose of this study was to elucidate whether the change of circulating total vascular endothelial growth factor-A (VEGF-A) and anti-angiogenic isoforms VEGF-A (VEGF-A165b) levels contribute to therapeutic efficacy for ANCA-associated vasculitis (AAV).

Methods

We analyzed newly diagnosed AAV (n = 172) patients who were subjected to the Remission Induction Therapy in Japanese patients with ANCA-associated vasculitis (RemIT-JAV) study, which is a prospective cohort study. Circulating total VEGF-A and VEGF-A165b levels were measured using enzyme-linked immunosorbent assay.

Results

We measured circulating total VEGF-A and VEGF-A165b levels in patients with various type of renal disease. Circulating total VEGF-A and VEGF-A165b levels were significantly increased in patients with AAV as compared to control subjects. To assess whether VEGF-A or VEGF-A165b are associated with AAV, we evaluated circulating total VEGF-A and VEGF-A165b levels before and after treatment in identical AAV patients from RemIT-JAV cohort. Comparison analysis for AAV serum samples before and after treatment in identical AAV patients demonstrated that circulating total VEGF-A and VEGF-A165b levels were significantly decreased in response to the after treatment. We also analysed circulating total VEGF-A and VEGF-A165b levels among AAV disease subtypes of eosinophilic granulomatosis with polyangiitis (EGPA), granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). As a result, levels of VEGF-A165b were significantly reduced after treatment with EPGA, as well as total VEGF-A levels were significantly reduced after treatment with MPA. These results indicated that the distinct specificities of VEGF-A and VEGF-A165b for AAV disease subtypes.

Conclusions

Evaluations of total VEGF-A and/or VEGF-A165b in circulation have potential for useful markers to assess therapeutic efficacies in AAV subtypes.

doi: 10.1016/j.cca.2022.04.113

T288

Some oxidative stress biomakers and antioxidant genes in diabetic male rats

S. Meludu [c], O. Chikezie [b], C. Obi-Ezeani [a]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Federal Medical Centre, Umuahia, Abia State, Nigeria*
[c] *Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

Background-aim

Diabetes mellitus is a group of metabolic disorder in which there are high blood sugar levels over a prolonged period. This study assessed the effects of diabetes on levels of some oxidative biomarkers and pattern of antioxidant genes expression in the peripheral blood cell of diabetic male rats.

Methods

This is an experimental study that involved 40 adult male albino rats (wistar strain) which were randomly assigned to five groups (A, B, C, D and E) of eight (8) animals each. Group A (Normal Control, sacrificed after 72 hours), Group B (Diabetic rats of 72 hours post diabetes induction), Group C (metformin treated diabetic rats), Group D (Diabetic Control untreated), and Group E (Normal Control, sacrificed after 3 weeks). Five milliliters of fasting blood sample was collected, and serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), vitamin C and vitamin E as well as peripheral blood antioxidant genes (CAT and CU-ZnSOD) were analyzed using standard methods.

Results

The mRNA of CAT and Cu–ZnSOD genes were up-regulated 72 hour post diabetes induction and down regulated 3 weeks after confirmation of diabetes ($P < 0.05$). The mean values of GPx, CAT, SOD, VIT C and VIT E were significantly higher in the treated diabetic group when compared with untreated diabetic control ($P < 0.05$) while MDA was significantly lower in treated diabetics when compared with the untreated diabetic control ($P < 0.05$). Furthermore, blood mean levels of GPx, CAT, VIT C and VIT E were significantly lower in the diabetic groups (treated and untreated) when compared with non diabetic control ($P < 0.05$) while MDA was significantly higher in the diabetic groups (treated and untreated) when compared with non diabetic control ($P < 0.05$). Additionally, there was significant negative relationship of blood glucose with GPx in the untreated group ($P < 0.05$).

Conclusions

The study suggests that hyperglycemia could cause expression of mRNA of CAT and Cu–ZnSOD genes on the peripheral blood cell in acute condition and significant alterations of oxidative stress biomarkers, but metformin treatment has showed not only hypoglycemic effect, but also anti-oxidant properties.

doi: 10.1016/j.cca.2022.04.114

T289

Effect of reproductive tract infection on oxidative stress parameters, sperm DNA fragmentation, and semen analysis in male infertility

L. Kangsheng

Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, Jiangsu 210029, China

Background-aim

Recent years have seen a rise of male infertility mostly caused by the decline of sperm quality. The ratio of infertile males to infertile females has escalated from 3:7 in 2013 to today's 5:5, turning human infertility into the research focus of reproductive medicine. This article aimed to clarify the effect of reproductive tract infection by ureaplasma ure-

alyticum(UU) and chlamydia trachomatis (CT) on the DNA integrity and routine parameters of infertile males' sperm.

Methods

The data of 259 infertile males treated in the Andrological Laboratory Examination and Reproductive Medicine Center in our hospital were analyzed. QRT-PCR was used to examine the infection by CT and UU. We evaluated the semen parameters and biochemical data of 253 men who fulfilled the eligibility criteria. According to PCR results, all the subjects were divided into four groups: group I (CT positive, 63 cases), group II (UU positive, 60 cases), group III (CT positive and UU positive, 62 cases), and group IV (no infection, 68 cases). Examinations were conducted in these four groups to test DNA fragmentation index (DFI), sperm count, vitality and morphology, elastinase level, seminal plasma malondialdehyde (MDA), and total antioxidant capacity (TAC).

Results

A retrospective study was performed. Compared to group IV, the other three groups (group I, group II and group III) showed difference in semen volume, proportion of sperm with normal morphology, sperm motility, progressive motility, and vitality ($P < 0.05$). Compared to group IV, group II and group III showed difference in DFI ($P < 0.05$). Compared to group IV, group II and group III showed difference in elastase level ($P < 0.05$).

In group II, UU infection significantly increased the level of seminal leukocytes, but no obvious change was found in the other three groups, indicating that UU can increase the level of seminal leukocytes. Compared with the group of normal leukocyte level, there were statistically significant differences in total motility, forward motility and normal sperm ratio between the two groups. The proportion of sperm with abnormal morphology (mostly in the head) showed obvious difference between the groups of high and normal seminal leukocytic level.

Conclusions

Infection caused by UU, CT, or the combination of the two can decrease sperm quality and lead to male infertility.

doi: 10.1016/j.cca.2022.04.115

T290 Association between lipid peroxidation and antioxidant enzymes in rheumatoid arthritis patients

S. Sobki

Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Background-aim

Rheumatoid Arthritis (RA) is an autoimmune disease that most commonly affects the joints of the hands, feet, wrists, elbows, knees and ankles. RA can affect body systems including the skin, eyes, lungs, heart and blood vessels. RA is characterized by Chronic Hypertrophic synovitis leading to destruction of connective tissue and functional damage of cartilage and bone structures. Recent studies have implicated oxygen derived free radicals in the Pathogenesis of RA. The current study was undertaken to determine the level of Lipid Peroxidation and Antioxidant Enzymes in RA patients and their correlations with the severity of the disease.

Methods

Total of (34) RA female patients diagnosed according to standard Criteria of American Rheumatism Association and (17) matched controls were included in the study. The RA patients were graded into (3) categories (mild, moderate, severe) on the basis of their joint and functional scores and the levels of C - reactive protein and Erythrocyte Sedimentation rate. Blood was collected via venipuncture and the plasma was separated for the analysis of thiobarbituric acid reactive substances (TBARS) and Vitamin E, whereas antioxidant enzymes Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) were analyzed in the haemolysates.

Results

The results showed a significantly higher levels of plasma (TBARS) in RA patients (1.006 ± 0.06 nmol/ml) as compared to normal controls (0.582 ± 0.04 nmol/ml), with a direct correlation (r = 0.67) to severity of RA. The level of plasma vitamin E in RA patients (22.49 ± 0.86 umol/L) was not found to be significantly different from the control group (20.91 ± 1.0 umol/L). No significant change in the activities of Erythrocyte SOD (2011.4 ± 67.5 vs 1918.6 ± 91.7 U/g Hb) and GSH – Px (12.3 ± 0.15 vs 11.3 ± 0.36 U/g Hb) in RA patients versus normal controls.

Conclusions

The findings demonstrate increased Lipid Peroxidation with no change in the antioxidant enzymes in blood of RA patients. Studies on the analysis of Synovial fluid with blood may provide better information about free radical – mediated biochemical alterations in the joints of RA patients.

doi: 10.1016/j.cca.2022.04.116

T291

Total oxidant and antioxidant status in primary open angle glaucoma

K. Sarici [c], P. Sultan [b], A. Koro [a], H. Aral [a]

[a] *University of Health Sciences, Istanbul Training and Research Hospital, Department of Medical Biochemistry, Istanbul, Turkey*
[b] *University of Health Sciences, Istanbul Training and Research Hospital, Department of Ophthalmology, Istanbul, Turkey*
[c] *University of Health Sciences, Kanuni Sultan Süleyman Training and Research Hospital, Department of Ophthalmology, Istanbul, Turkey*

Background-aim

In both normal pressure and high pressure glaucoma patients, the presence of endothelial dysfunction has been shown. Chronic endothelial dysfunction results in an excess production of superoxide and endothelin levels and a decrease in nitric oxide levels that are associated with an augmented oxidative stress. We aimed to investigate the total antioxidant status (TAS) and total oxidant status (TOS) in the plasma of primary open angle glaucoma (POAG) patients and to compare it to that of the control group.

Methods

Plasma samples were obtained from 39 advanced stage POAG patients (Group 1), 26 early stage POAG patients (Group 2) and 37 glaucoma free controls (Group 3) of matching age, sex and ethnicity. TAS and TOS levels were determined by spectrophotometric methods (Rel Assay Diagnostics, Gaziantep, Turkey).

Results

TOS mean (SD) levels were 5.14 ± 4.38 μmol/L in Group 1, 4.37 ± 3.56 μmol/L in Group 2, and 4.05 ± 1.36 μmol/L in the controls. There was no statistically difference between the values (p = 0.187). TAS mean (SD) levels were 1.83 ± 0.29 mmol/L in Group 1, 1.75 ± 0.26 mmol/L in Group 2, and 1.76 ± 0.30 mmol/L in the controls; again TAS values did not differ between the groups (p = 0.605).

Conclusions

The role of oxidative stress and antioxidant defense mechanisms is not understood clearly in glaucoma pathophysiology. We believe that future high-volume molecular studies will elucidate the pathophysiology of the disease, and possible role of antioxidants in prevention and treatment of POAG.

doi: 10.1016/j.cca.2022.04.117

T292

Obesity adolescents: novel targets for increased cardiovascular risk and antioxidant parameters

D. Pap [a], V. Jurisic [b]

[a] *Students Health Protection Institute Novi Sad, Department for laboratory diagnostics, Serbia*
[b] *University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia*

Background-aim

The aim of this study was to gain an insight into student's health, nutrition habits and general lifestyle with conducting the survey, specific anthropometric measurements, analyses of antioxidative enzymes, to established novel targets for the prevention of obesity, type 2 diabetes and cardiovascular diseases.

Methods

Study were included 660 students, both sexes, age matched. According to the body-mass index (BMI) δ 25 kg/m2 and waist circumference (WC) δ 94 cm (80 cm for females) two groups were formed : the control group- 293 students and the risk group -367 students and examinations of the antioxidant protection and general biochemical tests for assessing the cardiovascular risk and diabetes were performed.

Results

The activities of the antioxidant enzymes were significantly lower among students in the high risk group -obese students compared with the control group. Significantly negative correlations were obtained between antioxidant and anthropometric parameters in risk obesity students, and significantly positive correlations were obtained between BMI, WC and positive family anamensis and fasting glucose, postprandial glicemia, HbA1c status,microalbumin in urine.The results showed significantly positive correlation between physical activity and glutatione peroxidase (GSH-Px) and total antioxidative status (TAS) ($p < 0.05$) and negative correlation for smoking and activity of GR, SOD-1, GSH-Px and TA ($p < 0.01$).Activity of TAS and SOD showed significantly positive correlation of weekly consumation of fish and drinking red wine ($p < 0,05$) and as well as suplementation of omega -3-fatty acids in the risk student population. Fasting glucose, postprandial glicemia, HbA1c status and microalbumin in urine and hsCRP were significantly higher in the high risk group. In risk group we discovered 2.33% students with type 2 diabetes with average values of glucose 9.36 ± 1.38 mmol/l, postprandial glicemia 10.87 ± 1.28 mmol/l ,HbA1c status 7.49 ± 0.71% vs.57 ± 7.71mmol/mol, microalbumin in urine 26.50 ± 5.68mg/L and hsCRP 1,23 ± 0.71mg/dL.

Conclusions

These data can provide a good basis for taking the primordial and primary prevention through the changes and promotion of a healthy lifestyle "eat less and exercise more" and the modifications of the risk factors for obesity and type 2 diabetes.

doi: 10.1016/j.cca.2022.04.118

T293

Retrospective study of hyperproteinemia and hypergammaglobulinemia in French Guiana

G. Pasquier, F. Boukraa, I. Jday, B. Toulet, M. Dueymes, M. Pierre-Demar, H. Zouaghi

Centre hospitalier de Cayenne, French Guiana

Background-aim

Hyperproteinemia and hypergamma peaks are frequently observed in inflammatory process. Nethertheless, hyperproteinemia with high level gamma peaks remains scarce. We retrospectively explore this phenomemon in French Guiana.

Methods

We present the retrospective results (July to December 2019) of serum electrophoresis (capillaris Sebia) and colorimetric assessment of total protids g/L (Cobas 6000) in 63 consenting patients. Group A (n = 32, 13 male, 43 + /-21 yrs) inclusion criteria were total protids > 80 g/l with hypergamma > 30 g/l. A subdivision was made between HIV + patients (A1, n = 17) and HIV negative (A2, n = 15). Group A was paired with a control group B (n = 31, 15 male, 53 + /-14 yrs) with normal proteinemia and electrophoresis profile. The statistical analyzes using student t-Test were made at BiostaTGV site with $p < 0.05$.

Results

Compared to the B group, A group showed a significant increase ($p < 0.05$) of both total protids, Alpha-1Globulines (A1G), Beta-2 Globulins (B2G) and Gamma Globulins and a decrease of Albumin. While no significance for both Alpha-2 and Beta-1 Globulins was found. The comparison between patients A1 (HIV +) and patients A2 (various diseases HIV –) showed no significance.

The absence of any difference between the patient HIV + and the patients HIV- does not support the hypothesis and practice of proteins Electrophoresis as a biological monitoring of ART.

On another hand Albumin a binding protein with high capacity and low affinity for ligands was found strongly diminished in both A1 and A2 groups (42.37g +/- 33.24g) -the 21.54 % loss of Albumin.

Nevertheless the increase of Alpha-1 globulins from 2.99 g/L to 3.88 g/L is comforted by the rise in this inflammatory disease of the positive Acute phase reactants proteins (APRP) e.g.Alpha-1 Antitrypsin or Alpha Glycoprotein Acid and perhaps Corticosteroid Binding Globulin (H Zouaghi et al, Clin Chem 30 :1984). The same for the increase of B2G which would be accompanied by some proteins changes: e.g. Haptoglobin, Alpha-2 macroglobulin, C protein and Thyroxin Binding Globulin which binds thyroxin with a high affinity.

Conclusions

Taken together our results remains to be enhanced by the number of patients (since 2015) and confirmed by the determinations of some Acute Phase Reactants especially some high affinity binding proteins.

doi: 10.1016/j.cca.2022.04.119

T294 A novel model for age-related macular degeneration: oxidative insulted retinal pigment epithelial conditioned media induced macrophage polarization

H.I. Ahmad Mulyadi Lai [c], C. Shih-Hwa [b], R.I. Ahmad Mulyadi Lai [a]

[a] *Clinical Biochemistry & Immunology, Geneflux Bioscience Sdn Bhd, Selangor, Malaysia*
[b] *Department of Medical Research, Taipei Veterans General Hospital, Taiwan*
[c] *Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan*

Background-aim

Macrophages play an important role in the development of age-related macular degeneration (AMD). The precise roles and impacts of macrophages in AMD are remaining unclear and debated. Macrophage populations are heterogeneous and display different phenotypes that may explain why macrophages can be both protective and harmful to local tissue in AMD. To our knowledge, there are not many studies that highlighted the conditioned medium in regulating the functional polarization of macrophages. The purpose of this study was to investigate and clarify the interactions between conditioned media system of oxidative stress retinal pigmented epithelium cells (RPE) in the polarization of macrophages.

Methods

THP-1 were grown in RPMI-1640 and differentiate into a macrophage-like state with phorbol 12-myristate 13-acetate (PMA). Adherent macrophage without lipopolysaccharide (LPS) or TNF-a stimulation were cultured with conditioned media from hydrogen peroxide insulted oxidative stress retinal pigmented epithelium cells. qPCR used to quantify the absolute amount of cDNA generated from the mRNA of the M1 and M2 marker genes. MTT assay was used to evaluate the cell viability of exposed human RPE cell line (ARPE-19) to a variety of H_2O_2concentrations (100-1000 mM) for 24 hours. Immunofluorescence and western blot analysis were used to investigate tight junction and senescence markers.

Results

Structural changes appearing in senescent cells causes changes in the shape and size of RPE cells insulted with H_2O_2. RPE cell integrity was determined by immunofluorescence analysis of tight junction protein zonula occludens-1 (ZO-1). Additionally, we evaluated ©-H2AX immunofluorescence to assess the impact of H_2O_2 on DNA integrity and these cultures showed immunofluorescent ©-H2AX nuclear foci, demonstrating the presence of an active DNA damage response. Monocytes, M1 and M2 macrophage morphology were stained with Wright-Giemsa and observed under an inverted microscope. CD206 immunofluorescence was performed to detect macrophage mannose receptor (MMR) on the mature macrophage, revealed CD206 stains mature macrophages polarized towards M2 phenotypes and does not stain monocytes and M1-polarized macrophage. We quantified the absolute amount of cDNA generated from mRNA on THP-1 macrophage after 24 hours of polarization cultured with condition medium from oxidative insulted RPE. Condition medium from RPE induced oxidative stress shown activation and polarization of M2 macrophage, by significantly upregulated M2 marker gene, MRC-1, dectin-1, CD206, Fibronectin, IL-10, CCL18, and CCL22. These results indicate that RPE insulted with H_2O_2 – induced oxidative stress condition medium able to polarized macrophage into M2 instead of M1 phenotypes.

Conclusions

This is the first report to demonstrate the effect of conditioned medium from oxidative stress RPE able to induce macrophages polarization. Conditioned medium encompasses proteins shed from the cell surface and intracellular proteins released through non-classical secretion pathway or exosomes, and able to cause polarization of macrophages by regulating cell-to-extracellular matrix interactions. The suggested approaches might be successfully applied for other cell types if their conditioned medium was shown to be promising for application in the disease model. Moreover, our result suggests that the interplay between perturbed RPE homeostasis and activated macrophages influences key features of AMD development.

doi: 10.1016/j.cca.2022.04.120

T295

Clinical and laboratory efficiency criterion of non-surgical treatment of periodontitis

M. Myandiev [e], V. Nelyubin [a], V. Mudrov [b,d], S. Kazakov [b,c], S. Ivanov [e]

[a] *Evdokimov Research Medical and Dental Institute Moscow State Medical University, Moscow, Russia*
[b] *Russian Medical Academy of Continuing Professional Education of Ministry of Health, Moscow, Russia*
[c] *Burdenko Main Military Clinical Hospital of Ministry of Defense, Moscow, Russia*
[d] *9 Medical and Diagnostic Center of Ministry of Defense, Moscow, Russia*
[e] *Sechenov First Moscow State Medical University of Ministry of Health, Moscow, Russia*

Background-aim

Inflammatory periodontal diseases are the most difficult problem of practical dentistry. In the pathogenesis of periodontal diseases, the periodontopathogenic microflora is directly involved. A.actinomycetem-

comitans, P.gingivalis, P.endodontalis, P.intermedia, T.denticola, T.-forsythia, F.nucleatum, are the most aggressive, but the drugs used in therapy are not always effective, because their action is directed only to the singular bacterial component. The introduction of immunotropic drugs in the treatment of periodontitis can increase the efficiency of elimination of pathogenic microflora. At the same time, an important aspect of the diagnosis and treatment of periodontitis is the development of the effectiveness criterion of treatment for these diseases.

The aim of the study was to develop effectiveness criterion of non-surgical treatment of inflammatory periodontal diseases using vascular endothelial growth factor (VEGF).

Methods

36 patients aged 30-75 years with a diagnosis of chronic generalized periodontitis of mild and moderate severity were examined. Non-surgical treatment was performed using a drug containing endothelial growth factor. The periodontal health index, tooth mobility, and the depth of the periodontal pocket were used as clinical indicators. Before and after treatment, periodontopathogenic microflora and VEGF were determined.

Results

The obtained data showed that the combination of the etiological and immunotropic approach in the treatment of periodontitis has a pronounced positive effect. Comparison of the data of the quantitative determination of microorganisms and clinical criteria showed that the reduction ratio of the number of P.endodontalis, P.intermedia, F.nucleatum, VEGF and the depth of the periodontal pocket are the optimal parameters for evaluating the effectiveness of non-surgical treatment of periodontitis.

Conclusions

These indicators reflect the dynamics of the inflammatory process and can serve as criteria for evaluating the effectiveness of periodontitis therapy.

doi: 10.1016/j.cca.2022.04.121

T296

The impact of LDL-apheresis on antioxidant defense parameters in serum and red blood cells

E. Colak [b], K. Lalić [a], N. Rajković [a], A. Đorđević [c], T. Stanojević [c]

[a] *Clinic of Endocrinology, Diabetes and Metabolic disorders, Clinical Center of Serbia and School of Medicine, University of Belgrade, Serbia*
[b] *Deprtment for Scientific Research and Education, Clinical Center of Serbia, Belgrade, Serbia*
[c] *Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia*

Background-aim

LDL-apheresis is an advanced and established extracorporeal therapy for patients with primary hypercholesterolemia. There is evidence that such pathologic condition is followed by increase oxidative stress. In conditions of increase cholesterol levels, lipoprotein particles, especially LDL-particles are susceptible to oxidative modification. The aim of this study was to determine the oxidative stress status via the levels of antioxidant defense parameters in patients with the primary hypercholesterolemia undergoing LDL-apheresis

Methods

23 patients with diagnosed primary hypercholesterolemia treated by LDL-apheresis twice a month were inroled in this study. The following antioxidant parameters were determined in red blood cells and serum before and after LDL-apheresis: Zn,Cu-superoxide dismutase (SOD), Se-dependent glutathione peroxidase (GPx), glutathione reductase (GR) and total antioxidant status TAS). SOD and GPx were determined in red blood cells while GR and TAS in serum of tested patients obtained after blood centrifugation at 3000 rpm, for 15 minutes.

Results

Significaltly increased SOD and GPx and decreased TAS values ($p < 0.05$) were obtained after LDL-apheresis in patients with primary hypercholesterolemia.

Conclusions

Our study showed that LDL-apheresis is a suitable therapy for normalizing antioxidant status in patients with primary hypercholesterolemia.

doi: 10.1016/j.cca.2022.04.122

T297

Levels of HBA1C, URIC ACID, cholesterol and components of complete blood count in stroke patients

R.K. Dubey [a], A. Paudel [a], A. Jayan [a], A. Shahi [b], R.K. Kc [c]

[a] *Department of Biochemistry, Universal College of Medical Sciences, Bhairahawa, Nepal*
[b] *Department of Internal Medicine, Universal College of Medical Sciences, Bhairahawa, Nepal*
[c] *Department of Neurosurgery, Universal College of Medical Sciences, Bhairahawa, Nepal*

Background-aim

Stroke is the major cause of mortality and morbidity in Asian countries. Certain risk factors have consistently been identified as significant predictors of stroke outcome like age, hypertension, smoking, alcohol intake, diabetes, blood viscosity. But the role of uric acid, cholesterol level as a risk factor for stroke is controversial. Thus we aimed to find out the relation of glycated hemoglobin (HbA1c), uric acid, cholesterol, components of complete blood count (CBC) and whole blood viscosity (WBV) in stroke patients and to know different risk factors associated with stroke.

Methods

All the stroke patients admitted in the hospital identified by computed tomography (CT) scan were included in this hospital based cross-sectional study. Total 50 patients were recruited in this study. Patients were categorized into ischemic stroke and hemorrhagic stroke. Different biochemical and hematological parameters were analyzed. Data were analyzed by statistical package for social sciences (SPSS) and compared between ischemic and hemorrhagic stroke group.

Results

Out of 50 stroke patients, ischemic stroke was more common (60%) with male preponderance (64%) and 58% of patients were above 60 years. Uric acid level (mg/dl) was greater in ischemic (5.33 ± 1.70, p = 0.002) than in hemorrhagic stroke (3.90 ± 1.11) and its concentration correlated positively with LDLc (r = 0.37, p = 0.04). HDLc was significantly lower in ischemic stroke than in hemorrhagic stroke. Hypertension was the most common (64%) risk factors associated with stroke followed by alcohol intake, smoking and diabetes in both the stroke group.

Conclusions

Concentration of serum uric acid was greater, however within normal range, in ischemic stroke than in hemorrhagic stroke. This study recommends that the level of serum uric acid should be measured in healthy subjects at regular interval and its value should be maintained at low level to prevent the risk of developing ischemic stroke. Its concentration should be monitored more thoroughly in stroke patients, particularly in ischemic stroke to reduce mortality and morbidity associated with it. Result of this study demands a prospective study design with large sample size to examine the biochemical role of uric acid in pathogenesis of ischemic stroke.

doi: 10.1016/j.cca.2022.04.123

T298

Serum myeloperoxidase as predictive marker in different stages of chronic kidney disease leading to end stage renal disease

S. Shafique [c], A.R. Khan [b], S. Ahmad [a]

[a] *Department of Nephrology, SRHU, Swami Rama Nagar, Doiwala, Dehradun, Uttrakhand, India*
[b] *SRHU, Swami Rama Nagar, Doiwala, Dehradun, Uttrakhand, India*
[c] *Senior Resident Biochemistry, SRHU, Swami Rama Nagar, Doiwala, Dehradun, Uttrakhand, India*

Background-aim

To study serum myeloperoxidese level in different stages of chronic kidney disease leading to end stage renal disease.

Methods

Myeloperoxidase in the samples was estimated by a sandwich enzyme linked immmunosorbent assay (ELISA), based on principle of competitive binding.

The ELISA test provides a quantitive in vitro assay for human autoantibodies of IgG class against myeloperoxidase (MPO) in serum.

Results

Results: On comparison of the level of serum MPO (values in Mean ± SD) between the cases (18.661 ± 13.088) and control subjects (61.761 ± 24.840) a highly significant difference was noted (P-value δ0.01).

A highly significant difference was also found on comparison of the levels of serum MPO (values in Mean ± SD) in various stages of CKD [Stage 1 (n = 6) 103.422 ± 7.043], [Stage 2 (n = 6) 26.862 ± 2.136], [Stage 3 (n = 6) 14.698 ± 3.796], [Stage 4 (n = 6) 9.325 ± 1.717] and [Stage 5 (n = 6) 4.068 ± 1.302] in the cases (P-value δ0.01).

Conclusions

In our study there was high prevalence of atherosclerotic cardiovascular disease in patients with chronic kidney disease including ESRD.

We also found that there was a strong independent correlation between serum MPO level and stages of CKD, the level was found lowest in stage 4 and stage 5, emphasizing that MPO plays a mechanistic role in pathogenesis of endothelial dysfunction leading to cardiovascular disease in these patients.

There was rapid decline in serum MPO level with advancing chronic renal failure which appeared to be caused by a synergism of different mechanism such as inflammation, oxidative stress and genetic components.

doi: 10.1016/j.cca.2022.04.124

T299

IL-6, IL-8 and serum cystatin c levels in obese patients on alternative day fasting with low carbohydrate diet

Y. Bansal [b], N. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences Bhopal MP, India*
[b] *Department of Biochemistry, Maharishi Markandeshwar University Ambala, India*

Background-aim

Weight management is the practice of aiming for and achieving a healthy body weight, which is a major tool in the prevention and treatment of chronic diseases & markers related to depression, anxiety, and cardiovascular disease that cannot be stay ignored in an obese person. So the present study planned to measure the levels of markers of depression, anxiety and cardiovascular disease in obese patients with and without alternative day fasting with a low carbohydrate diet.

Methods

In this cross-sectional study,100 obese patients were selected as per guidelines mentioned in NIH & NHLBI where Group A included 50 obese patients and Group B included 50 obese patients on an alternate day fasting combined with a low carbohydrate diet. We measured the serum concentration of IL6, IL8, TGF B, and cystatin C by using an Enzyme-linked immunosorbent assay (ELISA) technique for the prediction of depression, anxiety and risk of cardiovascular disease among groups.

Results

In Group B serum levels of IL8 (P = 0.001) and serum cystatin C (P = 0.001) were significantly decreased whereas the serum levels of IL6 (P = 0.002) & TGF B were significantly increased as compared to group A obese persons. In addition, a statistically significant decrease in the levels of C- reactive protein (P = 0.005) was in obese patients with alternate-day fasting with a low carbohydrate diet.

Conclusions

This finding suggests that levels of selected markers can act as pre-indicator for the onset of depression, anxiety, and cardiovascular disease among obese people and alternative day fasting with a low carbohydrate diet in obese people is a promising therapy for preventing them from life-threatening diseases.

doi: 10.1016/j.cca.2022.04.125

T300

Chronic stress causes significant alteration to testicular cells in male albino rats: a comprehensive histological, immunohistochemical and biochemical study

P. Potaliya [a], S. Kataria [b], S. Ghatak [a], A. Choudhary [a]

[a] *All India Institute of Medical Sciences, Jodhpur, India*
[b] *Dr. S. N. Medical College, Jodhpur, India*

Background-aim

Stress triggers divergent behavioral responses and precise activation of the hypothalamic-pituitary-adrenal (HPA) axis. Chronic stress can have detrimental outcome on the various organs of the organism. The present study aimed to highlight the various molecular, immunological and histological changes in adult albino rat's testis exposed to chronic stress.

Methods

Hundred adult male albino rats were used in this study. They were allocated groups as: Control group (positive and negative controls with equal numbers); stress group (exposed to restraint stress in specialized apparatus for 4-hour duration followed by noise stress for 30 minutes for four weeks. Collection of blood samples and testicular tissues were done and examined for histological, immunohistochemical and biochemical alterations.

Results

The results of the present study showed that there were deteriorating alterations in spermatogenic and Sertoli cells of experimental group and exhibited substantial reduction in height of germinal epithelium, protein Ki-67 (marker for cell proliferation) and vimentin (intermediate filament protein in Sertoli cells of testis) immunoexpression. Also, chronic stress markedly elevates the serum levels of malondialdehyde, and levels of total antioxidant capacity were found to be reduced.

Conclusions

The present study validates that chronic stress is responsible for causing imbalance in oxidant-antioxidant coordination and has disastrous apoptotic influence leading to testicular damage.

doi: 10.1016/j.cca.2022.04.126

T301

Therapeutic potential of visnagin against liver inflammation mediated through oxidative stress: application of inflammatory mediators for the physiological functions

D.K. Patel [a], K. Patel [b]

[a] *Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India*
[b] *Krishnarpit Institute of Pharmacy, Rewa Rd, Prayagraj, Uttar Pradesh 212111, India*

Background-aim

Visnagin is a furanochromone class chemical found to be present in the Ammi visnaga used for the treatment of oxidative stress, angina pectoris and gall bladder. Visnagin have neuroprotective and anti-inflammatory activity. Liver inflammation induced by oxidative stress is main causative factors of various types of hepatic disorders and complications. Medicinal importance of plants and their derived active phytoconstituents are the leading areas of research for the treatment of hepatic disorders.

Methods

In order to determine the physiological role of visnagin in the hepatic disorders and complications induced through oxidative stress, present research work summarizes literature data's analysis of visnagin for their therapeutic role in the hepatic disorders. However other important pharmacological activities of visnagin have been also included in the present study in order to support the data's of the present work. Data analysis of the presented data has been done through different literature database search through in-vitro and in-vivo experiments. Biological importance of nuclear factor kappa B and soluble epoxide hydrolase have been investigated through literature data analysis to know the molecular mechanism. Effect of visnagin in the oxidative stress level was also analyzed through literature data analysis and co-related for the development of better molecule against hepatic disorders.

Results

In the present study, literature data analysis of the scientific work revealed the importance of visnagin in the liver inflammation and oxidative stress. Beneficial effect of visnagin for the treatment of hepatic disorders has been justified through these experimental works. Effect of visnagin on various form of liver cell injury have been investigated in the literature work and found to be significant and played important role in the prevention and treatment of hepatic inflammation. Molecular study supports various in-vivo and in-vitro scientific data of visnagin for their effectiveness against hepatic disorders.

Conclusions

Presented information of this work will be beneficial to the community and researchers to know the therapeutic role of visnagin against hepatic disorders and complications in the future.

doi: 10.1016/j.cca.2022.04.127

T302

Correlation between the serum levels of procalcitonin and CRP in patients with odontogenic and non-odontogenic abscesses in maxillofacial surgery

Y. Yankov [a b], Y. Bocheva [c]

[a] *Clinic of Maxillofacial Surgery, St. Marina University Hospital, Varna, Bulgaria*
[b] *Medical University - Varna, Bulgaria*
[c] *Dept. of Clinical Laboratory, MU-Varna, Bulgaria*

Background-aim

Abscesses are the most common emergency diseases in maxillofacial surgery. The ratio between odontogenic and non-odontogenic abscesses is about 2:1. Caused by bacterial endotoxins they both lead to acute phase response from the immune system, mediated by a production of acute phase proteins such as procalcitonin (PCT) and C-reactive protein (CRP). The aim of the study is to determine the correlation between the levels of PCT and CRP in patients diagnosed with odontogenic and non-odontogenic abscesses in maxillofacial surgery.

Methods

The study group consists of 79 patients: 50 patients - 28 males (56%) and 22 females (44%) - with odontogenic, and 29 patients – 20 males (69%) and 9 females (31%) with abscesses, hospitalized in the University Hospital “St. Marina”, Varna, Bulgaria between July and December 2021.CRP and PCT are both determined by an immunoturbidimetric assays, adapted on ADVIA 1800.

Results

The serum levels of CRP in the group with non-odontogenic abscesses are significantly lower than the levels of the marker in the group with odontogenic abscesses (37,5 ± 56,2mg/l vs 104,9 ± 110 mg/l, p = 0,0017), while the levels of PCT in the non-odontogenic group are higher than those for the odontogenic group (1,3 ± 1,55 vs 0,84 ± 1,0.1 ng/ml), but the correlation is not significant(p = 0.07). Analyzing the correlations inside the groups, depending on the gender, we found that the levels of PCT in women in the non-odontogenic group are significantly higher than those for the odontogenic group (1,37 ± 1,07 versus 0,74 ± 0.8 mg/l; p = 0.04), for men there is no significant difference. For CRP we found significant difference between the two groups of odontogenic versus non-odontogenic patients for men (112.7 ± 125.3mg/l versus 50.4 ± 63mg/l.3, p = 0.), and for women (95.03 ± 87.5 versus 8.8 ± 7.8 mg/l, p = 0.0039). Also there is a significant difference between men and women inside the non-odontogenic group (p = 0.034).

Conclusions

According to our results, we suggest PCT might be a potentially useful laboratory marker for diagnosing and prognozing the outcome in patients with inflammatory disease in maxillofacial surgery, but the CRP is still better corresponding with the clinical diagnosis. The correlations between CRP and PCT need further evaluation in this specific clinical area.

doi: 10.1016/j.cca.2022.04.128

T303

A comparative study of correlations with multiple inflammatory biomarkers in patients with elevated serum amyloid A

H.N. Kim, Y. Lee, H.Y. Chi

Samkwang Medical Laboratories, Seoul, Republic of Korea

Background-aim

Serum amyloid A (SAA) is one of the sensitive acute-phase protein and has been reported usefulness in various inflammatory condition, infections, and even in neoplasms. This study aimed to find correlations between SAA and the other inflammatory biomarkers in patients with elevation of SAA level.

Methods

In July 2021, a total of 75 sera samples for routine clinical chemistry analysis were collected and there were 47 sera samples with elevated SAA level (Serum Amyloid A Kit, Medical System Biotechnology, Ningbo, China, cutoff; 11.0 mg/L). C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), and procalcitonin (PCT) level were additionally measured for further comparison analysis. The cut-off level of CRP and PCT were as follows; (CRP, 0.5 mg/dL), (PCT, 0.5 ng/mL). Statistical analysis was performed using MedCalc Statistical Software (version 15.8, MedCalc Software, Mariakerke, Belgium). P values less than 0.05 were considered statically significant.

Results

The agreement between SAA and CRP was substantial (kappa = 0.654, 95% CI; 0.489 – 0.820), and with PCT was fair (kappa = 0.304, 95% CI; 0.152 – 0.457). Among the elevated SAA level samples, median values of each analyte were as follows; (SAA, 193.7 mg/L, IQR: 32.68 - 403.50), (CRP, 3.67 mg/dL, IQR: 0.48 - 9.57), (hs-CRP, 43.23 mg/L, IQR: 4.63 - 114.03), (PCT, 0.34 ng/mL, IQR: 0.06 - 1.84). SAA level showed substantial correlation with CRP (r = 0.778, P = 0.0001), and hs-CRP (r = 0.786, P < 0.001), however, there was slight positive correlation with PCT (r = 0.231, P = 0.118). There were 7 (9.3%) discordant results between SAA and other biomarkers; SAA(+) /CRP(-) / PCT(-).

Conclusions

The elevated SAA levels shown comparable correlation with other inflammatory biomarkers. There were discrepant results in which SAA was sole positive, therefore, further research is needed to determine whether SAA can detect inflammation status earlier than other inflammatory biomarkers.

Clinica Chimica Acta 530 (2022) S214–S221

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Inherited Disease, Metabolic Disorder

W001

Evaluation of serum levels of adiponectin and fasting blood glucose in pre-eclamptic women visiting obstetrics and gynaecology unit in Nauth, Nnewi Anambra State Nigeria

W. Nnaemeka [a], I.P. Ezeugwunne [b], I. Maduka [a], A.J. Onuegbu [b]

[a] *Biochemistry Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*
[b] *Chemical Pathology Department, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

Background-Aim

Complications of pre-eclampsia remain a major cause of maternal morbidity and mortality especially in developing countries. It is characterized by hypertension, proteinuria, and oedema. Despite considerable research, the cause or causes of pre-eclampsia remain unclear and there are no clinically useful screening tests to identify women in whom it will develop.This study was designed to evaluate the role and relationship between adiponectin and glucose alteration in the development of pre-eclampsia.

Methods

A total of 35 pre-eclamptic subjects (20-40weeks gestation age) aged between 18-40years participated in this study. For comparative assessment 34 aged matched healthy pregnant women with same gestational age was used as control. Serum adiponectin levels and plasma fasting blood glucose of the subjects respectively were monitored.

Results

The serum adiponectin levels lowered significantly (957.86 ± 86 to 1123.91 ± 248.3 $p < 0.05$)) and that of fasting blood glucose was higher significantly in pre-eclamptic subjects (6.04 ± 1.75 to 4.85 ± 0.62 $p < 0.05$).

Conclusions

Adiponectin plays a vital role in the pathophysiology of pre-eclampsia. Higher Glucose levels, lower adiponectin resistance and high blood pressure are associated with the development of pre-eclampsia.

doi: 10.1016/j.cca.2022.04.131

W002

Do prematurity and gestational age affect dried blood spot reference interval of TSH and 17- hydroxyprogesterone?

L. Jafri, A. Habib, I. Muhammad, I. Nisar, A. Nizar, F. Jehan

Aga Khan University, Karachi, Pakistan

Background-aim

"Guthrie card" is the colloquially described dried blood spot (DBS) collection technique which underpins newborn screening programs worldwide. Reference intervals are used to determine screen positivity, which triggers a cascade of diagnostic testing. However there are gaps in available neonatal reference intervals for use in Pakistan, where DBS testing is still not standard of care. The impact of common conditions such as low birth weight and prematurity is also unknown. The objective of this study was to determine reference norms for nTSH and 17-hydroxy progesterone using DBS from a cohort of neonates enrolled in the AMANHI (Alliance for Maternal and Newborn Health Improvement) Biorepository at the Aga Khan University, Pakistan.

Methods

Sampling was done from Nov2017- Feb 2019. in peri-urban communities of Ibrahim Hyderi & Ali Akbar Shah, Karachi located along the Arabian Sea coast covering an area of 6 sq. km with a population of 187 357. After written informed consent from parents (4359-Ped-ERC) demographics, birth history and clinical history were noted. Blood samples from neonates were obtained from the middle part of the heel within 24-72 hours of birth on Whatman 903 Protein Saver filter paper. Samples were kept at -80° C and transported to University of Iowa for analysis in dry ice. nTSH and 17 hydroxy progesterone were done on Perkin Elmer Victor (semi quantitative enzymatic assay), while were analyzed on Perkin Elmer Auto Delfia. All newborns with high 17OHP (>33nmol/L) or high nTSH (above 10 uIU/ml) were excluded. Reference range was calculated before and after adding small for gestational age (SGA) newborns and premature (<37 weeks). The CLSI recommended method was used for the determination of upper and lower end points covering 95% of the reference values of each analyte with respective 90% Confidence intervals (CI).

Results

Out of the total 319 neonates 171 (53%) were females. Mean gestational age of mothers at the time of delivery was 38.4 ± 1.5 weeks. Mean

doi:10.1016/j.cca.2022.04.131

birth weight was 2789.9 ± 468.3 gm, 107 (34%) neonates being LBW and 45 (14%) being premature. GALT was normal in all, however 17OHP and nTSH were high in 2 (0.63%) and 20 (6.27%) neonates respectively and these neonates were excluded from study.

The reference interval for nTSH calculated in 163 neonates was 2.2 - 8.1 uIu/mL with 90%CI of 2.2-2.2 and 7.2-9.9 uIu/mL respectively. The reference interval for 17OHP in 163 neonates was 1.1-8.9 nmol/L (95% CI 0.6-1.6 & 8.4-10.4 nmol/L respectively). Reference interval was recalculated after including all SGA and premature babies and no significant difference was found between the reference ranges (p value > 0.05). The reference interval for nTSH in 297 neonates was found to be 2.2-8.1 uIu/mL with CI of 2.2-2.2 and 7.2-8.8 uIu/mL respectively. The reference interval for 17OHP in 294 neonates was 1.2 - 9.4 nmol/L (90%CI 0.1-1.5 & 8.5-10.3 nmol/L respectively).

Conclusions

No effect of SGA and prematurity was seen in newborns' reference interval for 17OHP and nTSH using DBS.

doi: 10.1016/j.cca.2022.04.132

W003

Status of vitamin d level in type II diabetic patients and healthy control

N. Bhandari

Modern Technical College, India

Background-aim

The prevalence of type II Diabetic Mellitus is increasing in an alarming rate in recent decades among the Asian population. This study aimed to correlates the status of vitamin D level in Diabetic patients and healthy control.

Methods

In this case-control cross-sectional study, 200 participants were enrolled from the OPD of star hospital, Sanepa. Among which 100 were diagnosed cases of Diabetes Mellitus and 100 were healthy controls. Venous blood by venipuncture was taken for the determination of the concentration of vitamin D. The serum concentration of 25-OH vitamin D were determined by competitive ELISA. Serum 25-OH D concentration of < 20ng/ml, (20-29)ng/ml, and > 30ng/ml were defined as vitamin D deficiency, Vitamin D insufficiency, and Vitamin D sufficiency respectively.

Results

Out of 200 Participants, 50% male and 50% female were enrolled in the case group while 44% female and 56% male were included in the matched control group. The mean serum concentration of vitamin D was significantly low in the case group as compared to healthy control with P-value 0.001 (16.3 ± 6.9 vs 24.7 ± 10.1). The prevalence of vitamin D deficiency, Vitamin D insufficiency, and vitamin D sufficiency in type II diabetic patients was 83%, 15% and 2% respectively. According to Pearson's correlation analysis, there was a significant positive correlation between age group and vitamin D level, with a correlation coefficient (r = 0.209 and P = 0.037)

Conclusions

The prevalence of vitamin D deficiency in diabetic patients and control is 83% and 46% respectively. The mean value of vitamin D in Diabetic patients is significantly lower than in control. There was a significant positive correlation between age and vitamin D level in Diabetic patients.

doi: 10.1016/j.cca.2022.04.133

W004

Characterization of a series of patients with cystathionine beta synthase deficiency

S. Abdul Mateen, H. Majid, A. Jamil, L. Jafri, A.H. Khan

Department of Pathology and laboratory Medicine, Aga Khan University, Pakistan

Background-aim

Cystathionine beta synthase (CBS) deficiency, is recognized as the most common inborn error of sulfur amino acid metabolism, which metabolizes homocysteine into cystathionine, leading then to the synthesis of cysteine. Aim was to characterize a series of patient's with CBS deficiency presenting to a biochemical genetics laboratory (BGL) in Pakistan.

Methods

A cross-sectional study was performed at the BGL, AKU. Plasma Amino acid (PAA) analyzed at BGL by high performance liquid chromatography and Plasma homocysteine (tHcy) levels analyzed by chemiluminescence immunoassay, from January 2013 to Dec 2019 were included. The CBS deficiency is diagnosed based on increased levels of Methionine, decreased cystine on PAA and high levels of plasma tHcy. Demographic, clinical and biochemical details were extracted from BGL history form. Data was analyzed by Microsoft Excel 2010.

Results

Over a 7 years period, 7259 patients were tested for PAA, of those 33 (0.4%) CBS deficiency diagnosed in patients based on PAA were included in the final analysis. The median (Q3-Q1) age of patients was 6.3 years (5-9), withthe majority presenting after 4 years of age.The male to female ratio was 1:2. Parents of 67% (n = 22) patients had consanguineous marriage. Median Plasma methionine, plasma homocysteine levels were 399 μmol/L (568-83) and 190 μmol/L (224.5-162.3) respectively.

Most common clinical feature was hypotonia/lethargy 27% (n = 9), followed by seizures and developmental delay in 21% (n = 7) and 54% (n = 18), mental retardation 30% (n = 10), eye lesions 15% (n = 5), ectopia lentis 24% (n = 8) patients respectively.

Follow-up plasma tHcy levels were reduced to < 50 μmol/L in 30% (n = 10) patients only and average time taken to achieve this level was 126 days (325-65).

Conclusions

In the present case series one-fourth of the CBS patients presented with ectopia lentis while plasma tHcy levels were decreased to < 50

μmol/l in one-third of the patients. In this context large scale awareness campaigns at both primary and tertiary care level and implement of newborn screening as public health policy are dire need of time.

doi: 10.1016/j.cca.2022.04.134

W005

A case series of patients presenting with biotin responsive multiple carboxylase deficiency: A single center study

M. Masood, H. Majid, A.H. Khan, a. Jamil, L. Jafri

Department of Pathology and laboratory Medicine, Aga Khan University, Pakistan

Background-Aim

Biotin responsive multiple carboxylase deficiency (MCD) is characterized by deficiency of a cofactor biotin, required by 4 carboxylase enzyme complexes; Acetyl CoA carboxylase, Propionyl CoA carboxylase, 3-methylcrotonyl CoA carboxylase and pyruvate CoA carboxylase.

Aim of this study was to determine the clinical spectrum and biochemical findings on urine organic acids (UOA) in MCD patients presenting to biochemical genetics laboratory (BGL).

Methods

A cross-sectional study was performed at the BGL, AKU. The UOA of patients reported as MCD, from January 2015-July 2019 were included. The UOA were analyzed by gas chromatography mass spectrometer.

Diagnosis was based on peaks of 3-hydroxy isovalerate (3OHIVA), 3-hydroxy propionate (3OHPA), 3-methyl crotonyl glycine (MCC), Tiglylglycine (Tig) and methyl citrate (MC) on UOA. Demographic, clinical and biochemical details were extracted from BGL history form. Data was analyzed by Microsoft Excel 2010.

Results

During a period of 55 months 5826, UOA were performed and 2.2% (n = 129) patients were reported as MCD. The median (Q3-Q1) presenting age of patients was 180 days (373 - 120), with Male to female ratio was 73:56. Parents of 76.6% (n = 95 out of 124) patients had consanguineous marriage, while data for 5 patients was not available. Four patients were from Afghanistan, whereas 26.4% (n = 33) 32.8% (n = 41), 40% (n = 50) were from Sindh, Punjab and KPK provinces respectively.

The main presenting features were neurological including seizures 48.8% (n = 63), hypotonia 48.8% (n = 63) and developmental delay 48% (n = 62). Median Blood pH, Ammonia, Lactate levels were 7.32 (7.42-7.2), 69.3ug/dl (105.25-49.7) and 549 mmol/l (12.65-3.01). On UOA 3OHIVA, 3OHPA, MCC, Tig and MC were observed in 100% (n = 129), 74.4% (96), 65.1% (n = 84), 24.03% (n = 31) and 81.3% (105) respectively

Conclusions

The incidence of biotinidase deficiency is high (2.2%) in Pakistani population. Considering this high incidence it is recommended to include this disorder in newborn screening programs.

doi: 10.1016/j.cca.2022.04.135

W006

Neurological deficit at the time of presentation in patients with maple syrup urine disease: A single centre point prevalence study

H. Majid, L. Jafri, S. Ahmed, A.H. Khan

Department of Pathology and laboratory Medicine, Aga Khan University, Pakistan

Background-aim

Maple syrup urine disease (MSUD) is characterized by deficiency of an enzyme complex, branched chain alphaketoacid dehydrogenase, causing elevated plasma levels of Leucine, Isoleucine and Valine. Aim of this study was to determine the frequency of MSUD patients presenting with Neurological deficit at the time of presentation

Methods

A multidisciplinary descriptive, cross-sectional study was performed at the biochemical genetics laboratory (BGL), AKU. Plasma Amino acid (PAA) analyzed at Biochemical Genetics Laboratory (BGL) by high-performance liquid chromatography, from January 2013 to Feb 2019 were included. Analysis of patients reported as MSUD based on increased levels of Leucine, Isoleucine, and Valine on PAA was performed. Demographic, clinical and biochemical details were extracted from BGL history form. Neurological deficit was labeled if the history of hypotonia, lethargy, developmental delay, mental retardation, seizures, encephalopathy or coma was present. Data were analyzed by Microsoft Excel 2010

Results

Total of 48 MSUD patients diagnosed based on PAAwere included in the final analysis. The median (Q3-Q1) age of patients was 28.5days (292.5-10.25), with only 6 presenting after 1 year of age. The male to female ratio was 1.3. Parents of 71% (n = 34) patients had a consanguineous marriage.

Median blood pH, ammonia, lactate, SGPT and random glucose levels were 7.4 (7.47-7.3), 141ug/dl (166.5-78.9), 3.2mmol/l (13.70-1.30), 20IU/l (60-18) and 73mg/dl (120-51.75) respectively. While median Leucine, Isoleucine and Valine levels were1964mmol/L (2802-1446), 450mmol/L (578.5-243) and 636 mmol/L (769.5-476.5) respectively. Neurological deficit was observed in 85% (n = 28) patients, with most common clinical feature was hypotonia/lethargy followed by seizures and developmental delay in 75% (n = 25), 30% (n = 10) and 21% (n = 7) patients respectively. While encephalopathy, coma and mental retardation was observed in 24% (n = 8), 18% (n = 6) and 15% (n = 5) patients respectively

Conclusions

The MSUD is an important cause of the neurological deficit and should be looked into in patients presenting with intractable seizures, unexplained hypotonia, developmental delay, mental retardation or recurrent coma. Physicians should be made aware of the possible cause of the neurological deficit, is a dire need of time.

doi: 10.1016/j.cca.2022.04.136

W007

Confirmation of positive neonatal screening data by biochemical and/or molecular tests

Y. Shin [b], T. Podskarbi [a]

[a] *Molecular Genetics and Metabolism Laboratory, Germany*
[b] *University Childrens Hospital, Munich, Germany*

Background-aim

Recently various inherited diseases have been successfully investigated by routine newborn screening program in many countries. Through this program many patients have been discovered and some have been treated to a various degree. However, there are several pitfalls due to false positive or false negative results. We describe here the accurate methods to validate the neonatal screening results.

Methods

The positive cases are mostly from Germany, Switzerland, Austria, Greece and other Western Europeans as well as Arabian countries, Egypt and Turkey.

For enzyme analyses of lysosomal disorders such as gangliosidosis, mucopolysaccharidosis, sphingolipidosis and others, the fluorometric procedure using methylumbelliferyl substrates has applied . For galactosemia tests radioisotopic substrates and mini ion-exchange columns are used.

Results

Among positive lysosomal disorders less than 10% of the cases could be confirmed to suffer from the suspected diseases, when the family history is negative. The percentage was much higher, up to 50%, with a positive family history. In the case of MCAD, almost 70% of the postive cases could be confirmed to carry MCAD by the gene analysis. Concerning galactosemias, among high galactose cases about 30-40% suffer from GALT deficincy, variants, compound heterozygotes and heterozygotes, about 2-3% galactokinase deficeincy, about 1-2% epimerase deificiency and a few cases hyper galactokinase syndrome.

Conclusions

We conclude that most positive cases by newborn screening, especially lysosomal disorders, should be confirmed by follow-up studies.

doi: 10.1016/j.cca.2022.04.137

W008

Improve the utility of immediate HbA1c testing in the management of diabetes

C. Kao, Y. Chang

Department of Laboratory Medicine, St. Martin De Porres Hospital, Taiwan

Background-aim

Diabetes mellitus (DM) is a common chronic metabolic disorder. In 2011 the WHO advocated the use of HbA1c for the diagnosis of type 2 DM. HbA1c is also an important indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding 8 to 12 weeks. However, in most hospital settings, the clinical process to assess patients with suspected DM or monitor the treatment of DM patients typically involves at least two appointments with physician; blood samples were taken during the first visit and 1-2 weeks later results being discussed with the patient after laboratory analysis, adding to the length of time taken to reach a diagnosis or monitoring control of blood glucose. Why are HbA1c results not available during their outpatient visits? The purpose of this study was to assess the performance of an immediate HbA1c testing workflow and endorse the new approaches based on patient values in our 554-bed regional hospital.

Methods

We relocated the HbA1c measurement system from the serology department to the hematology workstation, switched to an immediate HbA1c testing process with a goal of reporting STAT HbA1c within 30 minutes to enable therapeutic decisions be made at the earliest possible opportunity, resulting in fewer patient visits in January 2011, and then implemented an auto-verification middleware for automated selection and reporting results to continuously improve the turnaround-time (TAT) in June 2015. Recently, our central laboratory was equipped with two ARKRAY HA-8180v HbA1c analyzers to analyze about 150 outpatient samples every morning in March 2019, with a goal of TAT within 20 minutes since June 2019.

Results

We evaluated the outpatient's HbA1c testing TAT from 2011 to 2019 (108 months). The overdue rate per month on average was found to range from 0.24% to 7.65%, and the mean was 1.88%. It was almost that 97.7% of HbA1c tests take less than 20 minutes to complete in 2019.

Conclusions

This study showed and verified the performance of the new continuously improved workflow at our institution, to get the HbA1c result in 20 minutes. We suggest changes in the traditional practice and provide a recommendation for responsible offering clinicians and patients the immediate HbA1c results.

doi: 10.1016/j.cca.2022.04.138

W009

Pay attention to peripheral smear in patients with methylmalonic academia combined homocystinuria

L. Pang, B. Jin, J. Guan, N. Duan, Y. Xing, C. Huang, J. Du, H. Li

Department of Clinical Laboratory, Peking University First Hospital, Beijing, China

Background-aim

Schistocytes are fragments of red blood cells produced by extrinsic mechanical damage and a diagnostic feature of hemolytic uremic syndrome (HUS). Methylmalonic academia combined homocystinuria, which is cobalamin C defect and MMACHC-related, is the most common inborn error of cobalamin metabolism and it is a rare cause of HUS.

Methods

This study is a case report. A 16-year-old boy presented to the emergency department with 5 months of weakness and fatigue. Routine laboratory tests were performed. Peripheral smear, renal pathology and genetic analysis were also performed.

Results

Laboratory evaluation showed thrombocytopenia (platelet count, 58 × 109/L) and hemolytic anemia (hemoglobin, 7.7 g/dL; reticulocyte, 199.9 × 109/L; lactate dehydrogenase, 609 IU/L). Additional laboratory tests showed elevated creatinine level of 2.6 mg/dL and 24 h proteinuria was 5.66 g/24h. Wright-stained peripheral smear showed schistocytes (panels A and B; original magnification × 1000). Level of factor H and ADAMTS13 activity were normal. Anti-factor H antibody and Coombs test were negative. Renal pathology suggested that there were severe thrombotic microangiopathy. By screening for metabolic diseases, we found that the concentration of urinary methylmalonic acid and blood propionyl carnitine were increased obviously. Both serum and urinary homocysteine were increased. Further genetic analysis revealed a compound heterozygous MMACHC mutation (c.80 A>G and c.1A>C).

Conclusions

The patient was diagnosed as atypical hemolytic uremic syndrome induced by methylmalonic academia combined homocystinuria. Careful examination of peripheral smear is important for the patients with cobalamin C defect.

doi: 10.1016/j.cca.2022.04.139

W010

Amino acids profile in girls with turner syndrome during growth hormone therapy

J. Bugajska [a], J. Berska [a], M. Wójcik [b], J.B. Starzyk [b], K. Sztefko [a]

[a] *Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland*
[b] *Department of Pediatric and Adolescent Endocrinology, Institute of Pediatrics, Jagiellonian University, Medical College, Krakow, Poland*

Background-aim

Turner syndrome (TS) is the most common chromosomal disorder causing short stature in females. These patients are commonly treated with recombinant human growth hormone (GH) to improve their final height. It is not known if GH replacement therapy, which is important for proper protein anabolism, leads to changes in free amino acids (AAs) in girls with TS. The aim of this study was to assess the influence of GH replacement therapy on AAs profile in patients with TS.

Methods

The study group included 36 girls with TS: 28 are treated with GH (group GH+), mean age 12.5 ± 0.7 years, and 8 girls with no GH treatment (group GH−), mean age 9.2 ± 1.3 years. The control group consisted of 18 healthy girls, mean age 14.7 ± 0.9 years. Standardised body mass index in group GH+, group GH− and in control did not differ significantly (0.25 ± 0.17; 0.27 ± 0.32; 0.24 ± 0.21, respectively). Free plasma AAs were measured by the LC/MS/MS (Agilent Technologies, Jasem). The following plasma AAs were measured: aspartic acid, glutamic acid, serine, asparagine, glycine, glutamine, taurine, histidine, citrulline, threonine, alanine, arginine, proline, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, tryptophan, ornithine and lysine. Basic biochemistry and lipid profile (triglycerides, total cholesterol) were routinely measured.

Results

The mean plasma concentration of glutamine, threonine were significantly higher in the group GH+ than in the group GH− ($p<0.05$ in both cases) . The mean plasma level of tryptophan was significantly lower in the group GH+ than in the control ($p<0.003$). In girls with TS, both in group GH+ and in group GH− the mean concentration of lysine, ornithine, histidine, asparagine, serine, threonine, methionine, phenylalanine were significantly lower than in the control group ($p<0.04$–$p<0.0002$). In addition in group GH− the mean values of glutamine, alanine, isoleucine were also significantly lower than in the control ($p<0.05$–$p<0.008$), but the mean value of glutamic acid was significantly higher in the group GH− than in the control ($p<0.03$). Biochemistry and lipids profile were normal in all studied girls.

Conclusions

Amino acids profile in girls with Turner syndrome might be characteristic for the disease but also depends on GH treatment.

doi: 10.1016/j.cca.2022.04.140

W011

Total and exchangeable copper assay by inductively coupled plasma-mass spectrometry and establishment of a pediatric reference interval

J. Yim [b c], Y. Cho [a], S.B. Kwon [d], J.S. Han [d], J. Kim [a], S. Lee [a], E.H. Lee [d]

[a] *Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, South Korea*
[b] *Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, South Korea*
[c] *Department of Laboratory Medicine, College of Medcine, The Catholic University of Korea, Seoul, South Korea*
[d] *GC laboratories, Yongin-si, South Korea*

Background-aim

Recently, direct assay of exchangeable copper (CuEXC) has been suggested as a robust and feasible diagnostic tool for Wilson's disease (WD). Currently, few data are available regarding the status of copper in children. WD is a disorder that requires life-long treatment and monitoring, so we evaluated the performance of an exchangeable copper assay using inductively coupled plasma-mass spectrometry (ICP-MS) and established a reference interval for total and exchangeable copper in the pediatric population.

Methods

Serum samples were analyzed for 122 (1-5 yr), 125 (6-12 yr) and 120 (13-18 yr) children. Total and CuEXC concentrations were directly mea-

sured by ICP-MS, and relative exchangeable copper (REC) was calculated. Reference intervals were determined based on the 2.5th and 97.5th percentiles of the data, with 90% confidence intervals.

Results

Method validation study showed robust analytical performance of copper assay. There were significant differences in the median concentration of total copper and REC among the age groups. Reference intervals determined for total copper were 82–167 g/dL for children aged 1-5 yr, 75–139 g/dL for those 6-12 yr, and 64–133 g/dL for those aged 13-18 yr. The reference intervals for REC were 3.18-8.93% for individuals in the 1-5 yr age group, 3.81-8.56% for those in the 6-12 yr age group, and 3.50-10.51% for those in the 13-18 yr age group, respectively. Non-parametric analysis of these data suggested no significant differences in CuEXC concentrations among age groups. Reference intervals for CuEXC were 4.29–9.79 g/dL for 1-5 yr old, 4.02–9.09 g/dL for 6-12 yr old, and 3.55–8.25 g/dL for 13-18 yr old children, respectively. To evaluate the potential clinical usefulness of the results regarding free copper, specimens were obtained from 11 patients with suspected WD. Among these patients, three were diagnosed with WD. All three patients diagnosed with WD had a REC values beyond the upper limit of reference interval. In contrast, only one of eight patients who did not diagnosed with WD had a REC value (14.40%) over the upper reference limit (10.51%), but lower than the cut-off value (18.5%) previously reported.

Conclusions

Determination of CuEXC levels and calculation of REC is a new tool that can be used to diagnose WD. It was found to be an analytically reliable and robust method for the diagnosis and treatment monitoring of WD. The reference intervals for pediatric patients derived in the present study are expected to be useful for the diagnosis, differential diagnosis, and treatment monitoring of pediatric patients who are the primary targets for early diagnosis of WD.

doi: 10.1016/j.cca.2022.04.141

W012

Novel FANCA mutations in three families with Fanconi anemia

L. Qin

Department of Pathology and Lab Medicine, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Tianjin, PR China
Tianjin Sino-US Diagnostics Co., Ltd., Tianjin, PR China

Background-aim

Fanconi anemia (FA) is the most common inherited bone marrow failure syndrome that is caused by DNA repair deficiency. It is characterized by progressive bone marrow failure, multiple congenital malformations, and high predisposition to hematological and solid malignancies. To date, pathogenic variants in at least 22 genes have been identified to be associated with FA, with FANCA gene account for ~70% of the cases. Among over 400 pathogenic alterations reported from HGMD database, most of the FANCA alterations are gross deletions, missense/nonsense mutations, small deletions/insertions and splicing variants. In the current study we aim to report the novel causative mutations in three Chinese FA patients.

Methods

Whole-exome sequencing (WES) was performed in three clinically suspected FA patients to identify the pathogenic mutations, followed by Sanger sequencing of their parents to investigate the inheritance.

Results

Patient 1 was identified to be a compound heterozygote for FANCA mutations c.189 + 2T > A and c.15G > A (p.W5X) that was inherited from asymptomatic mother and father, respectively. Patient 2 was identified to be homozygous of FANCA mutation c.1A > T (p.M1L). Patient 3 was found to be hemizygous for FANCA mutation c.3348 + 1G > A that was inherited from asymptomatic mother, and WES data implicated an absence of heterozygosity (AOH) at chr16:89788910-89882399 for this patient.

Conclusions

In summary, we have identified two novel muations c.189 + 2T > A and c.3348 + 1G > A of FANCA gene that was not reported in FA patients previously. Our results suggested that molecular diagnosis by next-generation sequencing (NGS) is a fast, economic, and accurate way to detect and identify pathogenic alterations of inherited diseases, highlighting the potential usage of NGS in clinical practice.

doi: 10.1016/j.cca.2022.04.142

W013

Communicating extreme results in routine clinical biochemistry aids diagnosing inborn errors of metabolism (IEM) in critically ill patients

C. Backman Johansson [a], H. Von Horn [b], R. Zetterström [a]

[a] *Centre for Inherited Metabolic Diseases, Karolinska University Hospital, Stockholm, Sweden*
[b] *Division of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden*

Background-aim

IEM are rare genetic diseases due to defects in specific proteins causing block in cell metabolism. Onset is often in early infancy with severe and life-threatening symptoms. Rapid diagnosis is important since life-saving treatments are available in many cases.

Diagnostics of IEM is time consuming and only made in a few specialized laboratories, hence results from automated routine biochemistry is of utmost importance.

Methods

Case 1: Three-day old girl with acute onset of therapy resistant epilepsy. Blood gases showed high lactate. On MRI extensive symmetrical supratentorial abnormalities were seen and on MRS high lactate. Routine biochemistry was normal. Newborn screening turned out negative, but a review indicated unspecific signs of critical illness. Sulfite dip-stick was slightly positive and ruled out as inconclusive. Results from metabolic testing eventually showed high urine excretion of xanthine and undetectable urate indicating a molybdenum cofactor deficiency.

Review of biochemistry revealed an unmeasurable homocysteine level – an obvious indication of molybdenum cofactor deficiencies and sulfite oxidase deficiency.

Case 2: Two-day old boy suddenly became lethargic with high respiration rate and progressive encephalopathy within a few hours. Blood gases showed severe acidosis. Routine biochemistry showed increasing liver parameters. Ammonium was not reported. Samples for newborn screening and metabolic testing were sent by courier for acute analysis.

After contact between metabolic center and local laboratory blood samples were diluted and ammonium analyzed. Concentrations were in the range of 1000 mmol/L leading to the conclusion of an acute onset urea cycle defect. The child was referred to a metabolic center and correct treatment initiated.

Results

In our experience hospital laboratories often discard erratic low or high analytical results. Automated production quality programs prohibit reporting extreme results hiding them behind technical limits or general comments about mistreated samples. Also reference intervals adjusted to common situations do not take extremal cases into account.

Conclusions

To ease the diagnostic process our goal is to facilitate communication between laboratory and referral clinician as well as continuous education about these rare and important cases.

doi: 10.1016/j.cca.2022.04.143

W014

About a case of rhabdomyolysis in a 7 year-old child secondary to a deficiency of very long-chain acyl-coa dehydrogenase (VLCAD)

M. Tber [a], O. Askander [c], M. Ztoute [b], M. Bensaad [b], S. Nader [b], H. Kabbaj [b], A. Bentahila [b], A. Guedira [b]

[a] *Biomedical laboratory, Cheikh Zaid Hospital, UIASS, Rabat, Morocco*
[b] *Biomedical laboratory, Cheikh Zaid Hospital, UIASS, Rabat, Morocco*
[c] *Genetic department, Cheikh Zaid Foundation, Rabat, Morocco*

Background-Aim

The aim of this study is to demonstrate the interest of biology and genetics in the diagnosis, monitoring and prevention of hereditary metabolic diseases such as VLCAD.

Methods

We report the case of a 7-year-old child who has been diagnosed with a VLCAD. He was hospitalized for one week at our hospital for an episode of severe rhabdomyolysis following intense physical exertion.

Result

Biochemical analyses showed a very high CPK level of 41362 U/L, hepatic cytolysis with high ASAT and ALAT levels (765 U/L and 194 U/L respectively) with an ASAT/ALAT ratio over 1 and uremia at 0.58g/L.

Regarding the genetic profile of the patient, the father has a frameshift mutation resulting in the early emergence of a stop codon at exon 10 and leading to a truncated mRNA and therefore to a non-functional enzyme.

The mother also has a frameshift mutation, not previously identified, located on exon 9, and giving rise to a stop codon.

The patient is therefore compound heterozygous for these two mutations with a non-functional enzyme.

Conclusions

The VLCAD is a rare metabolic disease whose genetic diagnosis remains complicated by the diversity of the mutations and the emergence of novel mutations not previously identified.

The main complication is rhabdomyolysis, however, other complications such as kidney failure or heart disease may also occur in patients with severe forms of the disease.

This pathology requires early diagnosis, regular monitoring and an appropriate management in order to decrease the frequency of rhabdomyolysis episodes and prevent cardiac, renal and hepatic manifestations.

doi: 10.1016/j.cca.2022.04.144

W015

Inborn errors of metabolism of purines and pyrimidines: Screening methods

s. Chellouai [a], z. Chellouai [a], s. Saadi-Ouslim [b]

[a] *Departement of Pharmacy, Faculty of Medecine, University of Oran, BP 1510 El M'Naouer31100 Oran, Algeria*
[b] *Faculty of Medecine, University of Oran, BP 1510 El M'Naouer31100 Oran, Algeria*

Background-aim

Purine and pyrimidine are important constituents of the cell, fulfilling various physiological functions: synthesis of nucleic acids, storage of energy, and intracellular signaling.

The aim of this poster is to present the analytical methods of screening for inborn errors of the metabolism of purine and pyrimidine.

Methods

A literature review was conducted by performing database research (via PubMed and Sciencedirect).

Result

Inborn errors affecting the metabolism (IEM) of purines and pyrimidines form a diverse group of disorders with a wide spectrum of clinical manifestations: a neurological, hematological, immunological and renal symptoms, therefore their diagnosis is a serious challenge for physicians.

Screening biological fluids such as blood, urine and cerebrospinal fluid to detect these anomalies is crucial; it includes the measurement of uric acid in blood and urine as a starting point.

However the determination of urinary excretion profile of purine and pyrimidine and their metabolites remains essential.

The analytical methods to assay urinary excretion profile have evolved over the years from thin layer chromatography (TLC) to high performance liquid chromatography (HPLC) with UV detection with diode arrays, , proton nuclear magnetic resonance (H-NMR), gas chromatography - mass spectrometry (GC / MS) and liquid chromatography

- tandem mass spectrometry (LC / MS / MS) and capillary electrophoresis. Further tests may be needed to confirm diagnosis like enzymatic or genetic test

Conclusions

Physicians' awareness of inborn errors of metabolism of purine and pyrimidine is necessary, as is to include screening for IEM of purine and pyrimidine metabolism in neonatal screening tests.

doi: 10.1016/j.cca.2022.04.145

W016

Hyperferritinemia in heterozygous H63D and hypertriglyceridemia

M. Molina-Arrebola [c], I. Portel-Rigo [b], C. Porrino-Herrera [b], Á. Alba-Sosa [c], A. Rayo-Bonor [d], M. Giménez-López [d], R. Pérez-Moyano [d], C. Avivar-Oyonarte [a]

[a] *Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*
[b] *Clinical Analysis Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*
[c] *Haematology and Haemotherapy Unit, Biotechnology Department, Poniente Hospital Public Health Agency, El Ejido, Almeria, Spain*
[d] *Haematology and Haemotherapy Unit, Biotechnology Department, Poniente Hospital Public Health Agency, Guadix, Granada, Spain*

Background-aim

Hereditary hemochromatosis (HH) is the most prevalent form of iron overload in Caucasians and comprises a group of disorders that lead to abnormalities in iron homeostasis, increasing intestinal absorption of iron and tissue deposition. If not treated, the accumulated iron can result in tissue damage such as liver cirrhosis, diabetes mellitus, arthropathy, myocardiopathy, endocrine disorders and hepatocarcinoma. Variants C282Y and H63D of the HFE gene (chromosome 6p21.3) are the two polymorphisms most frequently associated with HH. Studies of allele frequencies in northern European populations have reported 10–15% C282Y heterozygotes, 0.25–1.0% C282Y homozygotes, 20–30% H63D heterozygotes and 2% C282Y/H63D compound heterozygotes. The iron-loading risk profile for the possible genotypes is described: C282Y/C282Y > > > C282Y/H63D > C282Y/-, H63D/H63D, H63D/-. Non-C282Y homozygotes with significant iron loading should be investigated for rare iron-loading genotypes. Hypertriglyceridemia (HTG) is a common and important lipid abnormality in Western societies. Approximately 18% of adults have triglycerides over 200 mg/dl, defining HTG. The HFE locus is associated with primary HTG, and in approximately 5% of cases, the HFE genotype could play a role in their pathogenesis. It is supposed that increased iron deposits associated with certain HFE genotypes induce an overproduction of liver triglycerides in predisposed individuals. In one study, this synergic effect seems to be especially relevant in C282Y/H63D compound heterozygotes; subjects with this genotype are at low risk of iron overload in the general population but, in the presence of HTG, iron overload penetrance seems to be much higher. On the other hand, phlebotomies significantly decreased triglycerides, especially in subjects with basal HTG.

Methods

Decription of characteristics of a patient with hyperferritinemia, H63D heterozygous and hypertriglyceridemia and the evolution after a phlebotomy regimen.

Result

A 44-year-old male was referred to us because of hyperferritinemia. Body mass index 22.86, No toxic habits, including alcohol consumption. High blood pressure diagnosed at 35. Familiar hypertriglyceridemia. Nephritic colic, renal lithiasis and chronic uric acid nephropathy. He was taking carvedilol, manidipine, doxazosin, ezetimibe + atorvastatin, levetiracetam and omega 3. Abdominal ultrasound showed moderate hepatomegaly of left predominance, with no other abnormalities. Abdominal Angio-TC showed bilateral double renal artery.

Hemochromatosis HFE gene: C282Y negative, H63D heterozygous,

Since H63D heterozygous is not supposed to justify the intense hyperferritinemia, rare iron loading genotypes were investigated, showing negative results for SLC40A1, TFR2, HFE2 or HAMP mutations.

Hepatitis virus serology negative; autoimmune studies normal.

The outstanding values of the analysis, and the comparison before - after the 3 phlebotomies are (normal range in parentheses):

White-cell count (per mm3): 3,600 - 5,800 (4,200-10,500)
Haemoglobin (g/dl):14.3 - 15.1 (13.5 - 16.5)
Mean corpuscular volume (fl): 101.4 - 97.9 (80-101)
Platelet count (per mm6): 193 - 179 (130-450)
Alanine aminotransferase (U/l): 60 - 24 (1-50)
Aspartate aminotransferase (U/l): 74 - 29 (10-50)
Gamma-glutamyltransferase (U/l): 267 - 44 (1-55)
Iron (g/dl): 177 - 58 (53-167)
Transferrin (mg/dl): 201 - 395 (200-360)
Ferritin (ng/dl): 3,150 - 54 (20-500)
Transferrin saturation (%): 69.3 - 11.6 (17.1-30.6)
Total cholesterol (mg/dl): 134 - 262 (140-200)
Triglycerides (mg/dl): 415 - 168 (89-150)

Conclusions

H63D heterozygous patients, considered at low risk of iron overload in the general population, can express much higher iron overload penetrance in the presence of HTG.

Phlebotomies, even in a low number, led to normalization of the ferritin level, improved hypertriglyceridemia and the hepatic impact of the iron overload (leukopenia, macrocytosis, high liver enzymes) was normalized.

Along with the specific treatment, this case suggests that therapeutic phlebotomy could be a useful therapeutic approach in patients with HTG and iron overload.

doi: 10.1016/j.cca.2022.04.146

Clinica Chimica Acta 530 (2022) S222–S240

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Kidney Disease, Urinalysis

W017

Assessing iron status in end-stage renal disease using transferrin saturation and serum ferritin

S. Gwiliza

Department of Chemical Pathology, Dr George Mukhari Academic Laboratory, National Health Laboratory Service, Sefako Makgatho Health Sciences University, Pretoria, South Africa

Background-Aim

Background: Anaemia is common in end-stage renal disease (ESRD) and effectiveness of its treatment is reliant on the optimisation of the iron status. International guidelines on iron assessment in chronic kidney disease (CKD) recommend the use of transferrin saturation (TSAT) and serum ferritin despite their limitations and the availability of newer and more effective markers like reticulocyte Hb content (CHr) and percentage of hypochromic red cells (%HRC).

Aim: To establish the usefulness of transferrin saturation and serum ferritin in assessing iron status in ESDR.

Methods

Methods: A retrospective audit of the TSAT and serum ferritin requests from the Renal Unit at the Dr George Mukhari Academic Hospital was performed, for the period of June 2018 to March 2019. Results were extracted from the TrakCare® laboratory information system and patient profiles with concurrent eGFR (MDRD) <15 ml/min/1.73 m2, TSAT, ferritin, haemoglobin (Hb) and creatinine results, were selected. Iron status was classified based on the values of TSAT and serum ferritin and the following cut-offs were used: serum ferritin <100 μg/L and TSAT <20% for deficiency, serum ferritin >500 μg/L and TSAT>50% for overload and serum ferritin 100-500 μg/L and TSAT 20–50% for normal, based on the NKF-K/DOQI clinical practice guidelines for anaemia of CKD. Values not conforming to these groups were classified as indeterminate.

Results

Results: A total of 62 patient profiles were included in this audit. The majority were males (53%) with a mean age of 45 years. The mean TSAT and serum ferritin results were 33% and 919 μg/L, respectively. The iron status could not be established for the majority of the patients with 71% classified as indeterminate, while 13% were overload, 11% normal and 5% deficiency.

Conclusions

Conclusion: The TSAT and serum ferritin are not useful in assessing iron status in ESDR. The use of alternative markers is recommended.

doi: 10.1016/j.cca.2022.04.148

W018

The role of routine and novel biomarkers following flexible ureterorenoscopy for the treatment of kidney stones

C. Ellison [a], P. Ella-Tongwiis [b], S. Fon Hughes [b]

[a] *Keele University, United Kingdom*
[b] *North Wales Clinical Research Centre, United Kingdom*

Background-Aim

Kidney stones area painful disorder which is ubiquitous with significant morbidity but only low mortality. In the last 10 years, less invasive procedures have been developed to help reduce the impact on patients with kidney stones such as flexible ureterorenoscopy (FURS) with a holmium laser. Assessing patients after kidney stone removal procedures is important due to the potential side effects of the operation such as urinary tract infections and injuries to the bladder, urethra or ureters. Research pertaining to serum biomarkers in patients with kidney stones post flexible ureterorenoscopy is limited. This study aimed to determine if there are demonstrable changes in ferritin, ß2-microglobulin, neutrophil gelatinase-associated lipocalin (NGAL), cysteine rich protein 61 (CYR-61), C3, C4 and high sensitivity-CRP in patients who have undergone kidney stone treatment with FURS.

Methods

With local research ethics approval, analysis were performed using pre- and post-operative samples from patients attending Wrexham Maelor Hospital in Wales, United Kingdom for kidney stone treatment. The main goal was to assess whether the biomarkers being explored were clinically suitable as monitoring tools for FURS treated kidney stone patients with the aim to improve patient care. Fifteen patients underwent analysis for these 7 biomarkers in June 2019 (using: Thermo Fisher Konelab 20 clinical analyser, mini VIDAS® bioMérieux and Quantikine® Colorimetric Sandwich ELISA kits).

Results

The results from the small patient cohort were not normally distributed and based on the small numbers of patients, we suggest that the suggest that further studies conducted to assess these biomarkers using a larger patient population. Our initial data suggests that there may be some utility of these biomarkers.

Conclusions

Our initial data suggests that there may be some utility of these biomarkers.

doi: 10.1016/j.cca.2022.04.149

W019

Determination of total urinary proteins with sulfosalycilic acid turbidimetric method with or without using urine blank sample

S. Cekovska, I. Kostovska, K. Tosheska-Trajkovska, J. Brezovska-Kavrakova, S. Topuzovska

Department of Medical and Experimental Biochemistry, Medical Faculty, University Ss Cyril and Methodius, Skopje, Former Yugolav Republic of Macedonia, The

Background-Aim

Background: The method using sulfosalycilic acid reagent is simple and frequently used method in biochemical laboratories for total urinary proteins measurement. Due to the use of some medicines or presence of disease, certain percentage of urine samples are with specific colour. Because of the influence of urine colour on results, blank for each urine sample should be used.

Aim: The aim of the study was to make comparison of results for total urinary proteins concentrations obtained with or without urine blank sample for each urine included in examination.

Methods

Methods: In our examination total urinary proteins concentration was determined in 50 urine samples with light to dark brown colour due probably to excretion of some medicines or metabolities. For total urine protein concentration determination, turbidimetric method of Meuleman's with sulfosalycilic acid was used.

Results

Results: The results have shown that there was statistical significant difference ($p < 0.01$) between urinary proteins concentrations with or without urine samples as a blank, with higher results (from 10 to 50%) in seria without using urine blank, in which the absorbance due to the presence of dark colour of the urine was not obtained.

Conclusions

Conclusion: As a conclusion, urine blank sample should be used for each urine sample for obtain the more accurate results for urinary proteins concentration.

doi: 10.1016/j.cca.2022.04.150

W020

Performance evaluation and diagnostic accuracy of anti phospholipase A2 receptor (PLA2R) IGG for the diagnosis of primary membranous nephropathy – Experience from a clinical laboratory in Pakistan

R. Kausar, L. Jafri, A.H. Khan, S. Ahmed

Department of Pathology and Laboratory Medicine, The Aga Khan University, Karachi, Pakistan

Background-Aim

Membranous nephropathy (MN) is a rare disease in which immune complexes deposit at the glomerular basement membrane, leading to proteinuria. In approximately 70% of patients with primary MN (pMN), the immune complexes consist of autoantibodies against the podocyte protein M-type phospholipase A2 receptor (PLA2R). Our objective is analytical validation and evaluation of diagnostic accuracy PLA2R for the diagnosis of pMN against renal biopsy.

Methods

This cross sectional study was conducted at the Section of Clinical Chemistry, Aga Khan University, Karachi Pakistan from March-November, 2019. PLA2R on ETI-Max 3000 immunoassay using Euroimmun test kit (Medizinische Labordiagnostika AG). For analytical validation precision, verification of accuracy, linearity and analytical measurement range (AMR) was assessed using CLSI guidelines. Alternate assessment was performed by comparing results with peer laboratory using same methodology and Deming regression analysis was performed. Furthermore, diagnostic accuracy based on sensitivity, specificity, NPV and PPV was assessed. Concordance was calculated using Cohen's Kappa. SPSS version 21 and EP evaluator was used for statistical analysis.

Results

Precision study of level-1 and 2 were acceptable with a Mean ± SD of 2.0522 ± 0.0682 RU/mL (95% confidence for observed SD 0.0519-0.0996) and 105.538 ± 2.210 RU/mL (95% confidence for observed SD 1.681-3.228) respectively. The accuracy, AMR and linearity were analyzed by 5 calibrators over a measured range of 0.6-1500 RU/ml and were found acceptable. Mean recoveries were within the allowable systematic error. Alternate assessment with peer lab revealed good correlation with r: 0.99. Diagnostic accuracy (n = 20) taking renal biopsy as gold standard yield sensitivity, specificity, NPV and PPV of 87.5 %, 100%, 92.3 % and 100% respectively. Concordance showed Cohen's Kappa of 89.4%.

Conclusions

This study has validated the performance of a PLA2R IgG ELISA assay and found acceptable results. Detection of the serum PLA2R antibody yield a high specificity for diagnosing pMN and should be considered in all suspected cases of pMN instead of the highly invasive renal biopsy.

doi: 10.1016/j.cca.2022.04.151

W021

Evaluation of urine results in patients with stage 3B and stage 4 of chronic kidney disease

B. Ilkovska [b], B. Trifunova Kotevska [a], A. Dimovski [c]

[a] Department of Dermatology and Venereology, Acibaden City Clinic Tokuda Hospital Sofia, Bulgaria
[b] PHO Clinical Hospital Dr. Trifun Panovski Bitola Department of Medical Biochemistry, Former Yugolav Republic of Macedonia, The
[c] PHO Clinical Hospital Dr. Trifun Panovski Bitola Department of Radio Diagnostics, Former Yugolav Republic of Macedonia, The

Background-Aim

Chronic kidney disease (CKD) is defined as the presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73 mt2, persisting for 3 months or more, irrespective of the cause. The true incidence and prevalence of CKD are difficult to determine because of the asymptomatic nature of early to moderate CKD. The prevalence of CKD is around 10% to 14% in the general population. Similarly, albuminuria and GFR less than 60 ml/min/1.73 mt2 have a prevalence of 7% and 3% to 5%, respectively.

Methods

This prospective interventional study was performed between January 2019 and December 2019 in the PHO Clinical Hospital in Bitola. The study included 30 subjects - 12 females are with join pain and 18 males, age 44 to 84, mean 68, 9 years. We evaluate urine results in patients with stage 3b and stage 4 of chronic kidney disease

Results

We analyze results of urine: glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, leukocyte esterase, white blood cells, red blood cells, hyaline cast, triple phosphate crystals, mucus, bacteria, squamous epithelial cells, yeast, sodium, albumin, creatinine, albumin/creatinine ratio. Three patients have increased urine glucose. Bilirubin and ketone are negative in all patients. Eight patients have blood in urine. Nineteen patients have increased urine proteins and albumin.

Conclusions

Chronic kidney disease are serious public health concern because of the asymptomatic nature of this disease. This disease is chronic and leads to reduced quality of life. Its comorbidities such as hypertension and anemia severely impair working ability and frequent routine controls on urea and creatinine in healthy people are necessary to make an early diagnosis.

doi: 10.1016/j.cca.2022.04.152

W022

Are soluble erythropoietin receptor, IL-6 and ACE potential predictors of hypo responsiveness to erythropoiesis stimulating agents (ESA) treatment in maintenance hemodialysis (HD) Patients?

T. Cvetkovic [e], N. Stevic [d], N. Stefanovic [c], V. Cosic [a], M. Cvetkovic [b], V. Stoiljkovic [a], Z. Dimitrijevic [b], B. Mitic [b]

[a] Center for Medical and Clinical Biochemistry, Clinical Centre, Nis, Serbia
[b] Clinic of Nephrology, Clinical Centre, Nis, Serbia
[c] Department of Pharmacy, Medical Faculty, University of Nis, Serbia
[d] Institute of Biochemistry, Medical Faculty, University of Nis, Serbia
[e] Institute of Biochemistry, Medical Faculty, University of Nis, Center for Medical and Clinical Biochemistry, Clinical Centre, Nis, Serbia

Background-Aim

The treatment of anemia with erythropoiesis-stimulating agents (ESA) is the mainspring of improving the quality of life and reducing the need for red blood cell transfusions in patients with end stage renal disease (ESRD). Hyporesponsiveness to ESA treatment has been associated with iron deficiency, inflammation, oxidative stress, malnutrition, hyperparathyroidism, inadequate dialysis, and with numerous other factors. In this study we evaluate the influence of erythropoietin/soluble erythropoietin receptor ratio (Epo/sEpoR) as a potential predictor of hypo responsiveness to erythropoiesis stimulating agents (ESA) treatment in maintenance hemodialysis (HD) patients as well as correlations between Epo/sEpoR ratio, angiotensin-converting enzyme (ACE) and interleukin-6 (IL-6) concentrations, in order to interconnect those possible causes of Epo resistance.

Methods

We included 123 HD patients (102 patients who recived ESA tretman) and 61 individuals with preserved renal function on HD. Plasma Epo, sEpoR, IL-6 and ACE levels were evaluated using Elisa technique. We calculated ESA Resistence Index (ERI), defined as the weekly weight-adjusted ESA dose (U/kg/week) divided by hemoglobin level (g/dL).

Results

ACE concentrations correlated positively with IL-6 concentrations and negatively with Epo/sEpoR ratio. Ratio Epo/sEpoR correlated positively with Epo, ESA dosage and ERI, and negatively with sEpoR, IL-6 and ACE. sEpoR concentrations correlated positively with IL-6 concentrations and negatively with ESA weekly dosage and Epo/sEpoR ratio. In order to investigate possible influence of various parameters on Epo/sEpoR ratio in HD patients treated with ESA, we conducted univariate and multivariate regression analyses. The final multivariate model confirmed that ACE independently and negatively affected Epo/sEpoR ratio and ESA weekly dosage independently and positively affected Epo/sEpoR ratio in HD patients treated with ESA (R squared = 9.1%, $p = 0.012$).

Conclusions

Ratio Epo/sEpoR correlated not only with IL-6 and ACE, but also with ESA dosage and ERI. Epo/sEpoR ratio, as a measure of Epo availability, could be possibly used for identification of HD patients with potentially higher risk to develop Epo resistance.

doi: 10.1016/j.cca.2022.04.153

W023

Thrombospondin type-1 domain-containing protein 7A: Two positive cases of membranous nephropathy

Y. Ronchev [a], D. Terzieva [b]

[a] *Clinical Laboratory, University Hospital "Kaspela", Faculty of Medicine, Medical University, Plovdiv, Bulgaria*
[b] *Department of Clinical Laboratory, Faculty of Pharmacology, Medical University, Plovdiv, Bulgaria*

Background-Aim

Membranous nephropathy (MN) is defined as „unique glomerular lesion", which is associated with progressive impairment of renal function. It occurs in two forms: primary MN (PMN) and secondary MN (SMN). PMN is an organ-specific autoimmune disease. It is supposed that autoimmune mechanism of PMN is based on the production of autoantibodies against phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing protein 7A (THSD7A), transmembrane proteins which are expressed on the surface of the podocytes. Antibodies against PLA2R (anti-PLA2R1) can be detected in 70-80% of PMN patients while antibodies against THSD7A (anti-THSD7A) in 3-5% of anti-PLA2R negative PMN patients. The aim of our study is to determine the number of patients positive for anti-THSD7A in anti-PLA2R1 negative PMN patients.

Methods

The study included 52 patients with PMN, 32 men and 20 women. In these patients the serum concentration of anti-PLA2R1 was determined by ELISA kit (Anti-PLA2R ELISA, IgG, EUROIMMUN, Lübeck, Germany) using MR-96A microplate reader (MINDRAY). From the study group of patients with PMN, the number of negative for anti-PLA2R1 was 23. In these negative patients the serum anti-THSD7A was analyzed with indirect immunofluorescence method (Anti-THSD7A IIFT, IgG, EUROIMMUN, Lübeck, Germany) using epi-fluorescence microscope (OMAX, China). All data are presented as mean value ± standard deviation and percentage. Significance was defined as $P < 0.05$.

Results

The mean age of patients with PMN was 52.63 ± 13.81 years. There was no significant difference between mean age of males and females (52.06 ± 12.08 years vs 53.55 ± 15.58 years, $P = 0.710$). Ratio between males and females in PMN group was in favor of males (1.6:1). 29 (56 %) of patients with PMN were positive for anti-PLA2R1 and 23 of them (44%) - negative. This 23 patients were examined for anti-THSD7A antibodies and we found two positive results. It was found that anti-THSD7A was positive in 3.9 % of PMN patients and in 8.7 % of the anti-PLA2R1 negative group.

Conclusions

Our data shows that some patients were anti-PLA2R1 negative and positive for anti-THSD7A and together these two indicators may be used as diagnostics biomarkers.

doi: 10.1016/j.cca.2022.04.154

W024

Bubble cells in the urine sediment: A marker of tubular damage in patients with nephrotic syndrome

J. Poloni [b], F. Hickmann [a], K. Pêgas [a], E. Cambruzzi [a], E. Keitel [a], C. Garcia [a], L. Rotta [c]

[a] *Santa Casa de Misericórdia de Porto Alegre, Brazil*
[b] *Universidade do Vale do Rio dos Sinos, Brazil*
[c] *Universidade Federal de Ciências da Saúde de Porto Alegre, Brazil*

Background-Aim

Bubble cells (BCs) are bizarre, large cells that seem to contain intracellular clear vesicles and have been associated to tubular damage in the kidneys, but its origin remains unclear. BCs appearance are cited in various condition as ethylene glycol poisoning, acute tubular necrosis, osmotic nephrosis from mannitol and associated with intravenous immunoglobulin therapy, indeed of in experimental animal model (polyethylene-glycol-conjugated protein, iotrolan and nitrolotriacetate or sucrose treatment; glycogen nephrosis during diabetes). There have been rare reports of an atypical vacuolated cell in the urine sediment of patients with tubular injury.

Methods

Urine samples from 9 patients with nephrotic syndrome that presented BCs in the urine sediment and that also have a kidney biopsy performed were considered for analysis of the urine sediment characteristics and clinical picture.

Results

Urine sediment findings - BCs within urinary casts (4/9), oval fatty bodies (OFB - 9/9), free RTECs with preserved morphology (7/9) and various casts (RTECs: 5/9, fatty: 7/9, vacuolated: 7/9, granular: 8/9, waxy: 9/9 and white blood cell: 6/9) were observed. Serum creatinine values were altered in 8/9 patients. The kidney biopsies revealed at least one glomerulus sclerosed (8/9) and focal and segmental glomerulosclerosis (5/9), tubular atrophy (7/9), fibrosis (interstitial or cortical – 7/9) and arteriosclerosis (6/9).

Conclusions

The urinary BCs were found within urinary casts, showing that they derive from the kidneys; the BCs could be considered a marker of tubular damage because they are observed in the damaged tubular epithelium; BCs are typical and characteristic findings in nephrotic syndrome patients with tubular damage as are the OFB.

doi: 10.1016/j.cca.2022.04.155

W025

FGF 23 and NT-proBNP as predictive markers of mortality among chronic kidney disease patients

A. Ogunleye [a], M. Charles-Davies [a], Y. Raji [c], A. Akinbodewa [b], O. Adejumo [b], E. Agbedana [a]

[a] *Dept. of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria*

[b] Internal Medicine, University of Medical Science, Ondo, Nigeria
[c] Nephrology Unit, Internal Medicine, University College Hospital, Ibadan, Nigeria

Background-Aim

Mortality associated with cardiovascular diseases (CVD) remains the leading cause of death among chronic kidney disease (CKD) patients irrespective of stage of the disease. FGF 23 and NT-proBNP have been described as essential biomarkers of CVD. However, prognostic and possible therapeutic values of these biomarkers are yet to be ascertained.

This study aimed at investigating the predictive values of FGF23 and NT-proBNP in mortality among participants with CKD.

Methods

180 participants aged ε 18 years consisting of 60 CKD patients on conservative treatment (group 1) and 60 CKD patients on maintenance haemodialysis (group 2), age and sex matched with 60 apparently healthy individuals- (control) were enrolled into this study. Blood was collected from each participant after informed consent. Samples were analyzed for FGF23 and NT-proBNP using ELISA methods and participants were followed up for 12months. Data obtained were analyzed, Cox regression analysis was done and taken to be significant at $p < 0.05$.

Results

The mean age of the participants were 50 ± 13 (group 1); 47 ± 13 (group 2) and 48 ± 12 (control). At the end 12months follow up, 24 participants died in group 1 while 47 died in group 2. FGF23 and NT-proBNP were significantly higher among those who died than those who survived ($p = 0.049$). Higher FGF23 and NT-proBNP showed significant correlation with 6months mortality, it also showed that unit increase in FGF23 was associated with 0.3% likelihood of mortality ($p = 0.025$)

Conclusions

High FGF23 may predict 6months all-cause mortality among individuals with CKD irrespective of stage of disease or treatment modalities.

doi: 10.1016/j.cca.2022.04.156

W026

The diagnostic accuracy of a point of care urine albumin to creatinine ratio test, and urine dipstick analysis in a resource limited setting

S. Currin [a], J. Fabian [b], J. George [a]

[a] Department of Chemical Pathology, National Health Laboratory Service and University of Witwatersrand, Johannesburg, South Africa
[b] Department of Internal Medicine, University of Witwatersrand, Wits Donald Gordon Medical Center, Johannesburg, South Africa

Background-Aim

The prevalence of chronic kidney disease (CKD) is predicted to rise rapidly over the next few decades. In resource limited settings access to central laboratory services is limited, compounding the difficulty in detecting CKD at early stages. Point of care (POC) urine dipstick testing offers the potential to detect markers of kidney damage (albuminuria) as well as markers of other disease processes. Semi-quantitative results are preferable over visual dipstick analysis for proposing guidelines. We evaluated the diagnostic accuracy of the semi-quantitative albumin creatinine ratio (ACR) Sysmex UC-1000 POC urine dipstick system as well as the extent of other abnormal dipstick findings in urine.

Methods

700 participants from a rural area in South Africa were screened for albuminuria. A spot random urine sample was collected and analyzed using POC ACR as well as a central laboratory ACR (Roche Cobas 8000). We determined the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the POC ACR, and recorded dipstick parameters.

Results

The prevalence of albuminuria was 11.6% ($n = 81$) amongst the participants. Those with albuminuria had higher mean diastolic (82 vs 79, $p = 0.019$) and systolic (133 vs 128, $p = 0.002$) blood pressures and a higher proportion of diabetes mellitus (17.6% vs 4.9%, $p < 0.001$). The sensitivity of the POC ACR system was 0.79 (0.69 – 0.87), specificity 0.84 (0.80 – 0.86) and NPV 0.97 (0.95 – 0.98). The sensitivity improved to 0.80, 0.85 and 0.85 in those with elevated blood pressure, diabetes mellitus and HIV positive participants respectively. 240 (34.3%) of the samples had other abnormalities detected besides ACR. 88 (12.6%) were positive for haematuria, including 38 (46.9%) of those with albuminuria; 113 (16.1%) were positive for leucocytes; 66 (9.4%) were positive for nitrites; 23 (3.3%) were positive for both leucocytes and nitrites, 27 (3.9%) were positive for glucose. All parameters except for leukocytes 14.8% vs 16.3% were found with higher proportion in those with albuminuria.

Conclusions

Our study shows that the Sysmex POC ACR has good NPV and could be used to rule out albuminuria when screening for CKD. We also found a high proportion of participants had other urine abnormalities which may reflect kidney disease or co-morbid untreated genitourinary pathology such as urinary tract infections or endemic schistosomiasis.

doi: 10.1016/j.cca.2022.04.157

W027

The importance of cytokine concentration measurement in the assessment of allograft rejection in renal transplant patients

V. Stojiljkovic [a], T. Cvetkovic [b], V. Cosic [a], S. Stojiljkovic [a], L. Zvezdanovic [a], M. Cvetkovic [a], N. Stefanovic [c]

[a] Center for Medical and Clinical Biochemistry, Clinical Center, Nis, Serbia
[b] Center for Medical and Clinical Biochemistry, Clinical Center Nis, Medical Faculty, University of Nis, Serbia
[c] Medical Faculty, University of Nis, Serbia

Background-Aim

In the long term period after transplantation the most common causes of allograft damage are chronic allograft nephropathy, chronic

rejection, oxidative stress and vascular damage. In all of these conditions, a certain level of tissue inflammation and the production of proinflammatory cytokines, including TNF-, IL-1® and IL-18, can be found. The aim of our study was to determine the predictive value of TNF-, IL-1® and IL-18 for allograft damage in renal transplant patients, in the long-term postoperative period.

Methods

The study involved 65 patients, transplanted at least 12 months prior to our investigation, and 30 healthy volunteers, as a control group. Patients were divided into three groups, regarding the time passed between the operation and the beginning of our study (12-24, 24-48, and more than 48 months after transplantation). Concentrations of TNF-, IL-1® and IL-18 in the plasma of the subjects were measured using ELISA method.

Results

The TNF-concentration was statistically significantly higher in the first group of patients compared to the other two patient groups and the control group ($p < 0.05$). The IL-1® concentration was significantly lower in the third group of patients compared to the second ($p < 0.01$) and the control group ($p < 0.01$), while the IL-18 level did not show a statistically significant difference between the tested groups.

Conclusions

TNF-could be a useful marker for the monitoring and detection of subclinical damage to allograft in the second year of transplantation. The dynamics of the change in concentration, of both TNF- and IL-1®, may also have been altered by the components of the immunosuppressive protocols used in these patients, such as tacrolimus, which is a link that is yet to be examined.

doi: 10.1016/j.cca.2022.04.158

W028

Semi-quantitative analysis of the results of an image-based urine sediment analyzer considering the preanalytical factors

C. Eun-Jung [b], L. Eun Jin [b], H. Jungwon [b], L. Woochang [a], K. Hyun Soo [b], C. Sail [a], M. Won-Ki [a]

[a] *Asan Medical Center, University of Ulsan College of Medicine, South Korea*
[b] *Hallym University Dongtan Sacred Heart Hospital, Hallym University College of Medicine, South Korea*

Background-Aim

The automated microscopic platform is now used increasingly in the clinical laboratory due to time saving and rapid analysis. However, it is important to determine the reporting results quantitatively or semi-quantitatively because the automated platform has various techniques for analysis and sediment preparation methods. The results of cell counting using the on-screen image review program for the Cobas 6500 were different from manual review. The purpose of this study was to evaluate the concordance rates for the semi-quantitative assessment for reporting the white and red blood cells (WBC/RBC) count based on the difference in the sediment concentration.

Methods

This study was conducted using the freshly collected urine specimens of about 600 in or out-patients. All samples were analyzed using the Cobas 6500 instrument (Roche Diagnostics International) and manual microscopic analysis. WBC and RBC counts by Cobas 6500 were compared with manual methods.

Results

The semi-quantitative results of manual microscopy were graded as 0-2, 3-5, 6-10, 11-20, 21-30, many or numerous cells /high power field (HPF). We calculated the mean, SD, 95% confidence interval for RBC and WBC quantitative results of Cobas 6500, following comparing results according to the grading by the manual count. There was a difference of one grade in RBC and WBC count between the manual count and image analyses with relative consistency.

Conclusions

When reporting the results of the image analyses, RBC and WBC counts need to be raised to one grade in order to compensate for microscopic examination. Therefore, each laboratory should verify the results on-screen review of images corresponding to a field of microscopic view due to inter-laboratory variation in the interpretation and describing results.

doi: 10.1016/j.cca.2022.04.159

W029

Equations to estimate creatinine excretion rate (CER) modified for Korean population

T. Kim [b], I. Park [a]

[a] *Laboratory Medicine, Hanyang University/Kuri Hospital, Kuri, South Korea*
[b] *Laboratory Medicine, Hanyang University/Seoul Hospital, Seoul, South Korea*

Background-Aim

Researchers have tried to estimate individual patient's creatinine excretion rate (eCER) to determine whether an individual 24-hr urine volume is accurately collected or to estimate protein excretion rate (ePER) by multiplying with protein creatinine ratio (PCR). An equation using patient's age, sex, and weight as variable by Ix ($eCER_{Ix}$), and other equation using only age and sex by Ellam ($eCER_{Ellam}$) were recently developed and are used widely. We found, however, these equations were not fit to Korean patients. We changed the constants of the equations with Korean patients and compared the eCERs of original and modified equations with measured CER (mCER).

Methods

To acquire new constants we followed the same way to have derived the equations, so, linear multiple regression test was performed with variables such as age, gender, weight for $eCER_{Ix}$, and polynomial regression plot was performed with variable of age in each gender for $eCER_{Ellam}$ with 792 patients who had performed creatinine clearance test (Ccr). Another 426 patients who had performed Ccr and PCR within 1 day before or after Ccr test were subjects of application of the equa-

tions. Original $eCER_{Ix}$, $eCER_{Ellam}$ and modified $eCER_{Ix}$, $eCER_{Ellam}$ were calculated and compared with mCER. Accuracy was obtained by the percentage of individuals with mCER within 30% (P30).

Results

The modified equation we found were: $eCER_{Ix}$ (mg/day) = 698.24 + 11.87 x weight (kg) – 260.29 (in female) - 6.75 x age, and $eCER_{Ellam}$ (mg/day) = 1020.7 - 1.29 x Age - 0.056 x Age^2 for female, 1737.8 - 4.73 x Age - 0.089 x Age^2 for male. Median and quartile range of mCER, original $eCER_{Ix}$, and $eCER_{Ellam}$ and modified $eCER_{Ix}$, and $ePER_{Ellam}$ were 899 (670-1173) mg/day, 1134 (853-1420) mg/day, 1121 (988-1637) mg/day, 945 (717-1164) mg/day, and 867(708-1160) mg/day, respectively in order. P30 (confidence interval) value of original $eCER_{Ix}$, and $eCER_{Ellam}$ and modified $eCER_{Ix}$, and $eCER_{Ellam}$ were 61.5% (56.9-65.5), 42.9% (39.2-47.0), 79.2% (75.9-82.3), and 73.8% (70.3-77.4), respectively in order.

Conclusions

Original equations' estimation of CER were calculated too high for Korean population and modified equations' estimation were closer to the measured CER.

doi: 10.1016/j.cca.2022.04.160

W030

Assessment of URD parameter on the UF-5000 urine sediment analyzer in identifying glomerular hematuria

H. Cho [a], J. Yoo [a], H. Jang [b], K. Cha [b], H. Chae [a], Y. Kim [a]

[a] *Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, Seoul St. Mary's Hospital, Seoul, Republic of Korea*

Background-Aim

Most patients with microscopic hematuria present with an isolated hematuria. Therefore initial triage of patients according to the origin of hematuria is important for appropriate follow-up investigations and management. Microscopic examination of urinary sediments for erythrocyte casts and dysmorphic erythrocytes is an indispensible tool in differentiating glomerular from non-glomerular hematuria. However, it is time-consuming, labor-intensive and has difficulty in standardization. Therefore, efforts to detect glomerular hematuria with automated instrument for urine sediment analysis have been made. With this aim, we evaluated the URD parameter of an automated flow cytometry analyzer, UF-5000 (Sysmex, Kobe, Japan) for detecting glomerular hematuria.

Methods

Fresh urine samples from patients with hematuria (>20/L) were consecutively acquired and analyzed by UF-5000. Microscopic examination of sediment by light microscopy was also performed in parallel. The classification of the glomerular or non-glomerular hematuria was based on the clinical diagnosis of the patient as the gold standard. Independent samples t-test and ROC analysis were performed to analyze the difference in parameters according to the origin of hematuria and to determine the best cut-off for differentiation.

Results

A total of 377 urine samples were analyzed. Of the parameters derived with UF-5000, %URD, a parameter based on the proportion of small erythrocytes, differed significantly between glomerular and non-glomerular hematuria, with mean %URD of 62.1% and 28.7%, respectively ($P < 0.0001$). On ROC analysis, the area under curve was 0.814 and the ideal cut-off value was >24.9%. When using this cut-off value, the sensitivity and specificity for detecting glomerular hematuria was 97.0%, 54.6% respectively. In comparison, ROC analysis of dysmorphic erythrocytes showed an AUC of 0.683 and sensitivity and specificity of 61.5% and 73.9%, respectively.

Conclusions

%URD derived from UF-5000 could be an informative parameter to differentiate glomerular from non-glomerular hematuria that can also be easily obtained using an automated flow cytometry urine sediment analyser.

doi: 10.1016/j.cca.2022.04.161

W031

Biochemical alterations after increasing dialysate flow rate in chronic hemodialysis – One year follow-up

N. Trakas [a], A. Barbatsi [b], T. Hiras [b], J. Skarakis [c], A.P. Kakavelakis [d], M. Sonikian [b], E. Karakou [a]

[a] *Biochemistry Department , Sismanoglio General Hospital, Athens, Greece*
[b] *Nephrology Department, Sismanoglio General Hospital, Athens, Greece*
[c] *Research and Development Department, DEMO S.A. Pharmaceutical Industry, Athens, Greece*
[d] *Rural General Practitioner, Phthiotis Region, Greece*

Background-Aim

Hemodialysis (HD) is one of replacement therapies for end-stage kidney disease. HD adequacy is accessed by two indexes, urea reduction rate (URR) and single pool (sp) Kt/V. Increasing dialysate flow rate (DFR) is reported to ameliorate HD adequacy, but this is a matter of controversy. The effect of DFR increase on URR, Kt/V and various biochemical parameters was investigated.

Methods

23 patients, M/F = 20/3, aged 65(44-89) years, dialyzed thrice weekly for 50(6-274) months, using polysulfone low flux dialyzers, participated in an annual randomized cross-over study. Patients were dialyzed with DFR of 500 and 700ml/min for 6 consecutive months respectively, according to their usual HD prescription and with ultrafiltration volumes according to clinical need. Blood was sampled before and at the end of midweek sessions at the beginning of the 1st, 7th and 13rd month for serum urea, creatinine, potassium (K), sodium, albumin, total Ca, phosphate (sP). URR, spKt/V, corrected for albumin Ca (sCa) and sCaxsP product (CaxP) values were calculated.

Results

Under both 500 and 700ml/min DFRs used, the expected post-HD alterations were found: decreased values in urea (161.5 ± 38 to 49.9

±20.1-p < 0.001, 140.3 ± 30 to 56 ± 20.4mg/dl-p < 0.001), creatinine (10.2 ± 2 to 3.9 ± 1.2-p < 0.001, 10.2 ± 3.3 to 4.1 ± 1.6mg/dl-p < 0.001), K (5.2 ± 0.7 to 3.7 ± 0.3-p < 0.001, 5.3 ± 0.6 to 3.9 ± 0.3mM-p < 0.001), sP (5.4 ± 1.7 to 2.9 ± 0.6-p < 0.001, 5.7 ± 1.6 to 2.6 ± 0.6mg/dl-p < 0.001); increased values in albumin (4.3 ± 0.4 to 4.7 ± 0.4-p = 0.001, 4.2 ± 0.3 to 4.7 ± 0.4g/dl-p < 0.001), sCa (9.1 ± 0.7 to 11.3 ± 0.9-p < 0.001, 8.7 ± 0.6 to 9.9 ± 0.7mg/dl-p < 0.001). After increasing DFR from 500 to 700ml/min we observed no reductions in pre-HD urea, creatinine or URR (68.6 ± 8.1 to 69.9 ± 7.9%-p = NS), Kt/V (1.41 ± 0.4 to 1.42 ± 0.3-p = NS). However, under DFR of 700ml/min post-HD sCa, sP and CaxP were lower compared with those under DFR of 500ml/min (9.9 ± 0.7 vs 10.8 ± 0.8mg/dl-p < 0.001, 2.6 ± 0.6 vs 2.9 ± 0.6mg/dl-p = 0.02, 25.6 ± 6.2 vs 30.9 ± 6.7mg2/dl2-p < 0.001).

Conclusions

DFR increase from 500 to 700ml/min did not lead to favorable effects on HD adequacy but resulted in post-HD amelioration of sCa and sP levels and may be useful in cases of hypercalcemia, hyperphosphatemia and calcifications. DFR increase utility needs further investigation in patients with disorders of calcium-phosphate metabolism.

doi: 10.1016/j.cca.2022.04.162

W032

The effects of conivaptan and boric acid in post-ischemic renal function impairment

B. Can [b], F. Kar [b], E. Kar [b], M. Özkoç [b], H. Şentürk [a], G. Kanbak [b], İ.Ö. Alataş [b]

[a] *Department of Biology, Eskisehir Osmangazi University, Eskisehir, Turkey*
[b] *Department of Medical Biochemistry, Eskisehir Osmangazi University, Eskisehir, Turkey*

Background-Aim

The hypersecretion of antidiuretic hormone (ADH) has previously reported to be associated with pathogenesis of acute/chronic renal failure and ischemia-related diseases. Therefore, it has proposed that ADH receptors may be preferred as an important therapeutic target. The aim of this study was to investigate the effects of conivaptan, an antidiuretic hormone antagonist, and boric acid, an antioxidant agent, on renal function impairment in experimental renal ischemia-reperfusion (I/R) injury in unilateral nephrectomized rats.

Methods

Fourty Sprague-Dawley male rats randomly divided into 5 groups: Control (Sham-operated), I/R, I/R + dimethyl sulfoxide (DMSO), I/R + conivaptan, and I/R + conivaptan + boric acid. The right kidney nephrectomy was performed in all groups and animals allowed to recover for 15 days. After recovery, renal ischemia was performed for 45 minutes by occlusion of the left renal artery. At the end of the ischemia, kidney was reperfused, and 5% DMSO (i.v.), 10 mg/mL conivaptan (i.v.; in 5% DMSO) and 50 mg/kg boric acid (i.p.) were performed to the related groups at the onset of the reperfusion. Blood samples were taken immediately after the surgical operation at 6th hours of reperfusion (at the same time in the control). Serum Na, K, Cl, blood urea nitrogen (BUN), creatinine, uric acid, Ca, Mg and P levels were measured using commercial kits in order to evaluate renal function changes.

Results

Statistical analyses revealed that ischemia-reperfusion injury significantly increased serum Na, BUN, creatinine, Mg and P levels compared to the control (p < 0,0001). Although conivaptan treatment alone reduced the serum Na levels (141 ± 1,82 mmol/L), combination of conivaptan and boric acid treatment enhanced the Na levels (146,2 ± 1,88 mmol/L) compared to the control (140,1 ± 1,46 mmol/L). BUN and creatinine levels were high in the I/R group (68,79 ± 6,53 and 1,09 ± 0,33 mg/dL, respectively) but were significantly increased in the conivaptan and boric acid treatment group (78,46 ± 3,88 and 1,56 ± 0,10 mg/dL). Mg and P levels were high in all I/R operated rats but the highest value observed in the conivaptan and boric acid treatment group. There were no significant differences in Cl, uric acid and Ca levels among the groups.

Conclusions

Ischemia-reperfusion injury in unilateral nephrectomized rats caused important changes in serum electrolytes and renal function parameters. Conivaptan treatment alone caused no additional negative effect on renal function under single-kidney conditions. However, with data from all study groups, we determined that conivaptan showed an interaction with boric acid and influence kidney function unfavorably at the early injury period. This study may show the unfavorable effects of combined drug treatment in renal ischemia-reperfusion rat model.

doi: 10.1016/j.cca.2022.04.163

W033

Leukocyte esterase and leukocyte as a screening test for bacterial infection in preoperational patients with renal stone

N. Sae-Ung [c], L. Kateyam [c], R. Tavichakorntrakool [b], W. Chumpol [b], P. Tippayawat [b], Ckdnet [a]

[a] *Chronic Kidney Disease Prevention in the Northeast of Thailand, Medical Faculty, Khon Kaen University, 40002, Thailand*
[b] *Department of Clinical Microbiology, Associated Medical Sciences Faculty, Khon Kaen University, 40002, Thailand*
[c] *Department of Clinical Microscopy, Associated Medical Sciences Faculty, Khon Kaen University, 40002, Thailand*

Background-Aim

A urine sample with greater than 5 to 10 leukocytes/HPF under microscopy is abnormal and highly suggestive of urinary tract infection in symptomatic patients. We aimed to screen bacterial infection in patients with renal stone before a surgical operation by determination of positive-leukocyte esterase on urine dipstick with/without equal or greater than 5 leukocytes/HPF detected by an automated microscopic urine analyzer.

Methods

Sixty-one urine specimens of the preoperative patients with renal stone were collected for bacterial culture. These specimens were also performed urinalysis using dipstick and UriSed 3 Pro urine microscopic analyzer.

Results

Twenty-one urine cultures were positive for bacterial uropathogens while 40 cultures were negative. Thirty-nine specimens were positive for leukocyte esterase with/without equal or greater than 5 leukocytes/HPF while 22 specimens were negative. Sensitivity, specificity, PPV, NPV, accuracy, positive LR and negative LR for screening bacterial infection with positive-leukocyte esterase with/without equal or greater than 5 leukocytes/HPF were 90%, 50%, 49%, 91%, 64%, 1.81 and 0.19, respectively.

Conclusions

Positive-leukocyte esterase with/without equal or greater than 5 leukocytes/HPF shows high sensitivity and high negative predictive value thus it may be useful for negative screening of bacterial infection in the preoperative patients with renal stone.

doi: 10.1016/j.cca.2022.04.164

W034

Analysis of matrix effect of urine quality control materials for urine chemistry tests

C. Jeon, S. Mun, S. Kim

Department of Laboratory Medicine, School of Medicine, Daegu Catholic University, Daegu, South Korea

Background-Aim

National external quality assessment service on urinalysis in Korea have used control materials manufactured by the Yeongdong (YD Diagnostics, Yongin, Korea). The materials were composed of the phosphate buffered saline mixed with real material or chemical reagents. Therefore matrix effects would influence the results of participants.

Methods

We obtained 5 kinds of control materials from YD, Aution check plus (Arkray Inc., Kyoto, Japan), Liquichek urinalysis control (Bio-Rad Inc, Hercules, CA), UroColor control (Standard Diagnostics(SD), Seoul, Korea) and UC-control (Symex Co., Kobe, Japan). We also made normal and abnormal human urine. As test devices, we selected 6 instruments; Super+ (YD), Aution Max AX-4030 (Arkray), UroMeter 720 Pro (SD), Clinitek Adventus (Siemens Healthcare, Erlangen, Germany), URISYS 2400 (Roche) and UC1000 (Sysmex) based on the database of Korean Association of External Quality Assessment Service. With the 5 control materials and 2 human urines, and the six instruments, we performed urinalysis twice. The first was repeated 20 times and the second 40 times.

Results

In all control materials, matrix effects were found as follows: Aution Check (pH in URISYS, SG in Aution Max), Liquichek (UBG in Aution Max, SG in UC1000), UroColor (Bilirubin and SG in Aution Max, pH and SG in UC1000) and UC control (Bilirubin and SG in UroMeter, Bilirubin in UC1000, Ketone in Clinitek). YD controls also disclosed matrix effects: blood and UBG in Aution Max, glucose in Super+, SG in UC1000 and pH in URISYS.

Conclusions

As all control material showed matrix effects in the urinalysis, external quality assessment would be evaluated by results of peer group rather than by whole results.

doi: 10.1016/j.cca.2022.04.165

W035

Atypical cells detection in urine samples: Evaluation of analytical performance on UF-5000

G. Previtali, M. Seghezzi, V. Moioli, S. Buoro, M.G. Alessio

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-Aim

The automated fluorescence flow cytometer analyser UF-5000 (Sysmex, Japan) comprises a new interesting research parameter for Atypical cells (Atyp.C) detection. According to the new European "Regulation on in vitro diagnostic medical devices" (EU 2017/746 IVDR) we evaluate Limit of Blank (LoB), Limit of Detection (LoD), Limit of quantification (LoQ), Linearity, Repeatability and Carry over (CO) for the Atyp.C parameter adopting the Clinical & Laboratory Standards Institute (CLSI) protocols. The aim of this study is to demonstrate that the analytical performance of Atyp.C are suitable for clinical practice and could allow the early detection of patients that should be investigated for the presence of bladder cancer.

Methods

Normal reference range for Atyp. C previously calculated in our laboratory was 0.68 cells/μL.

For determining LoB, LoD, LoQ, 4 samples with known concentration of Atyp.C were analysed 15 times with two series of fixed reagents lots. Carry Over was evaluated on 6 samples (3 high target concentration and 3 low target concentration samples), a high concentration sample was used to asses the linearity of the Atyp.C parameter according to CLSI-EP6-A. 6 samples (3 low concentration, 3 clinical decision value concentration) were analysed 20 times for the repeatability evaluation as stated by CLSI H26-A2.

Results

Since the urine matrix is less stable than sera over time, it was necessary to adapt the CLSI protocols in order to guarantee an adequate number of evaluations and repetitions of fresh urine samples. The results obtained are summarized as follow: LoB = 0.3 cell/μL, LoD = 0.5 cell/μL, LoQ = 0.9 cell/μL; Linearity: linear range from 1.0 cell/μL till 40.0 cells/μL with R^2 = 0.995, Intercept = 0.6481, Slope = 1.004; Repeatability: CV = 30% or less and 50% or less for target samples of concentration 2.0 cells/μL and 1.0 cells/μL respectively; Carry Over: 0.0 cells/μl.

Conclusions

This type of evaluation is very useful in order to have a deeper known about limits and makings of everyday used analysers. The new research parameter Atyp.c showed very good analytical performances thus allowing us to plan for future clinical studies to investigate the pos-

sibility to use this parameter for the early detection of patients with bladder cancer.

doi: 10.1016/j.cca.2022.04.166

W036

Proteinuria reflex test: The importance of proteinuria characterization in patients with no history of renal or haematological diseases

V. Moioli, L. Michetti, R. Ravasio, M. Seghezzi, G. Previtali, G. Guerra, M.G. Alessio

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-Aim

As suggested by KDIGO guidelines, since April 2016 in our laboratory we had measured albumin to creatinine ratio (ACR) and protein to creatinine ratio (PCR) for all the patients with medical prescription of urinalysis. It is common to find patient with abnormal PCR values without albuminuria. In these cases, the presence of proteinuria could be the sign of renal or haematological diseases that can cause tubular or renal overload proteinuria respectively. The purpose of this study is to characterize the origin of proteinuria.

Methods

Between May and June 2018, we identified 48 patients (33M, 15F; age range 4-87, mean age 54, median age 61) with no history of proteinuria, PCR >200 mg/g, ACR <30 mg/g and no other alteration of urinalysis parameters). Urinary albumin (μALB_2, Siemens), total protein (UPRO_2, Siemens) and creatinine (ECRE_2 Enzymathic, Siemens) were measured on ADVIA 1800 (Siemens). Proteinuria characterization was conducted with gel agarose electrophoresis and immunofixation using Hydragel Urine Profil(e) (Sebia).

Results

13/48 (27%) patients had tubular proteinuria with ACR <10 mg/g 23/48 (48%) patients had mixed proteinuria with ACR between 10 and 30 mg/g; 6/48 (13%) had physiological proteinuria. Surprisingly, in 6/48 (13%) patients, with no history of haematological disease, Bence Jones proteinuria was found.

Conclusions

Our data shows the importance of proteinuria characterization in patients with no history of renal or haematological diseases to make early diagnosis and refer the patients to clinicians in order to start the correct therapies. If our data will be confirmed on a large population, it demonstrates the importance of the creation of a reflex test for proteinuria characterization.

doi: 10.1016/j.cca.2022.04.167

W037

Is it time to abandon timed collection urine for random urine samples?

M. Seghezzi, G. Previtali, V. Moioli, R. Marozzi, R. Ravasio, G. Guerra, M.G. Alessio

Clinical Chemistry Laboratory, ASST Papa Giovanni XXIII, Bergamo, Italy

Background-Aim

Albuminuria is the main marker of glomerular damage. Timed collected urine (especially nocturne) is widely used in general practice to detect albumin excretion, especially in diabetic patients, although it is limited by many preanalytical errors. A previous study has evaluated whether protein to creatinine ratio is a good indicator of 24h urinary protein excretion, but information about the reliability of albumin to creatinine ratio is lacking in a wide cohort to the best of our knowledge. Therefore, the aim of our study is to demonstrate that there is a correlation between random and timed collected urine for albuminuria.

Methods

5868 patients that simultaneously performed albumin to creatinine ratio (ACR) on first voided morning sample and nocturne (12h) albuminuria were enrolled from our routine samples. All subjects received written instructions for proper samples collection. U-albumin and creatinine concentration were determined using the immunoturbidimetric method (Sclavo Diagnostics International Srl, Siena, Italy) and the enzymatic method (Siemens Healthcare GmbH, Erlangen, Germany) respectively, on ADVIA 1850 (Siemens). ACR was calculated and expressed in mg/g of u-creatinine.

Statistical analysis was conducted on MedCalc software 19.1.3 To evaluate the relations between ACR and nocturne albuminuria, a single slope linear model was used. A logarithmic transformation of data was done before the analysis to correct the non-constant variability of the observations. Univariate correlation analysis was carried out with Pearson correlation coefficient (r).

Results

The correlations between ACR and nocturne albuminuria was high significant ($p<0.0001$) with r of 0.926 (95% Confident Interval [95% CI] 0.922 to 0.924); the correlation showed a linear regression almost identical to the line of unity with a slope of 0.96 (95%CI 0.95 to 0.97), an intercept of 0.04 (95%CI -0.001 to 0.07) and a determination coefficient (r^2) of 0.89.

Conclusions

Timed and first morning sample collections are both useful specimen to detect albuminuria. Random samples have less pre-analytical problems, are easier to collect and are cleaner than timed specimens; stated the high correlation demonstrated, it is high suggested to use random samples for determining albuminuria in clinical practice.

doi: 10.1016/j.cca.2022.04.168

W038

Performance evaluation of the Atellica 1500 automated urinalysis system

L. Chang Kyu, K. Dae Won, C. Jungim, Y. Seung Gyu, K. Sun-Young, C. Yunjung Cho

Laboratory Medicine, Korea University College of Medicine, Seoul, Republic of Korea

Background-Aim

The use of an automated urine analyzer enables large-scale sample processing by reducing the variation between inter-examiner/intra-examiner and saving labor and time. The recently introduced Atellica 1500 Automated Urinalysis System (Siemens, New York, NY, USA) combines the CLINITEK Novus Analyzer and the Atellica UAS 800 Analyzer into one fully automated unit. This study is to evaluate the performance of Atellica 1500 system (Siemens).

Methods

For the physical and chemical components of urinalysis, we collected 181 patient urine samples and compared the results from Atellica 1500 system (Siemens) to those of iRICELL2000 System (Beckman Coulter, Brea, CA, USA). In particular, the albumin-creatinine ratio (ACR) and protein-creatinine ratio (PCR) resulted by Atellica 1500 system were compared with the quantitative results measured by AU5800 Analyzers (Beckman Coulter). For Microscopic components, the agreement between the two urine instruments was first evaluated, and the results were compared with the manual microscopic review.

Results

The urine chemistry results from the Atellica 1500 system demonstrated ε89.0% within 1 level agreements with those of the iRICELL2000. The proportions of exact agreement for ACR and PCR between Atellica 1500 and AU5800 Analyzers were 83.1% and 66.4%, respectively. The proportions of agreement within one level were 99.6% and 97.5%, respectively. Except for urinary cast, the agreement rate within between the two items was above 91%.

Conclusions

The Atellica 1500 system showed good performance in routine Urinalysis samples obtained from clinic patients with or without kidney disease.

doi: 10.1016/j.cca.2022.04.169

W039

Opposite relationship of parathyroid hormone to age and reproductive hormones in male and female in maintenance hemodialysis pateints

A. Hazra [c], S. Mandal [a], J. Chakraborty [b]

[a] *AIIMS, Jodhpur, India*
[b] *ESI PGIMSR, Manicktala, Kolkata, India*
[c] *Government Medical College, Pali, Rajasthan, India*

Background-Aim

Parathormone is often deranged in maintenance hemodialysis pateints and regularly measured as the follow up tests. Many treatment decisions depend on parathormone blood level in chronic hemodialysis patients. It is important to know the preanalytical factors affecting the tests including biological variation like age.

Methods

PTH was measured in maintenance hemodialysis pateints. Relationship between PTH & age was analyzed in 330 observations. Inter-relatinoship of PTH with reproductive hormones e.g.Testosterone Prolactin, FSH, LH were noted by futher investigation in 30 male and 30 female subsets after IRB approval.

Results

PTH had a highly significant overall negative correlation with Age ($r = -0.24$, $p < 0.0001$) when ungrouped by gender. However when gender information were accessed in 30 male & female patients, there was significant difference in PTH ($p = 0.018$) in male vs female, and in females PTH tended to go up with age. Similarly for sex hormones there were gender difference in relation to change with age. The most significant was seen in prolactin male prolactin stronly went up with age ($r = 0.52$, $p = 0.0027$) whereas the female prolactin had a downward trend but not so significant.

Conclusions

PTH had a very highly significant negative correlation with Age in hemodialysis (oppsite to normal) the downward trend was contributed in 30 males, however in 30 females the PTH had an upright relation with age. In most sex hormones tested male and female had different age trend with male prolactin showing the most significant positive correlation and contrasted with negative but insignificant correlation for its female counterpart.

doi: 10.1016/j.cca.2022.04.170

W040

Relationship of neutrophil-lymphocyte-ratio and platelet counts with parathormone, and how they are affected by gender, age and sugar levels in maintenance hemodialysis patients in India

A. Hazra [c], S. Mandal [a], J. Chakraborty [b]

[a] *AIIMS, Jodhpur, India*
[b] *ESI PGIMSR, Manicktala, Kolkata, India*
[c] *Government Medical College, Pali, Rajasthan, India*

Background-Aim

Inflammation is associated with bad outcomes for hemodialysis patients. Inexpensive Blood Counts (CBC) and simple ratios e.g. Neutrophil-Lymphocyte-Ratio (NLR) and Neutrophil-Platelets-Ratio (NPR) have been shown to be meaningfully associated with disease subspectra with inflammation. Dialysis patients undergo secondary hyperparathyroidism and bone problems. Thus Parathyroid (PTH) testing is part of standard of care in hemodialysis. Scant data exist on relationship of NLR or NPR with PTH in hemodialysis patients.

Methods

Data of routine CBC and PTH (Access 2 iPTH) testing from 150 patients (M:F~2:1) under maintenance hemodialysis were analysed after IRB permission. R and RCmdr were used for Pearson correlation, t-test, regression etc.

Results

In our study Pearson correlation showed strong association of PTH with NLR and Platelet (Plt). PTH overall showed a significant negative correlation with NLR ($r = -0.224$, $p = 0.0018$) contributed mainly by lymphocytes which had a mild +ve correlation ($r = 0.186$, $p = 0.01$) augmented by ratio(NLR) with neutrophil which itself had insignificant but -ve relation. However ratio (NPR) with Neutrophils weakened the significant association Plt independently had with PTH ($r = -0.1696$, $p = 0.0187$) because both were in -ve. The Plt or NLR distribution in M & F groups were not statistically different.

When split into Male (M) & Female (F) groups, the PTH-Plt & PTH-NLR relations showed very different behaviour than the overall trend. The slope of least square lines for M vs F had entirely opposite slope for PLT-PTH & NLR-PTH scattergrams.

PTH also correlated independently with Age ($r = 0.24$, $p = < 0.001$) & Sugar ($r = -0.13$, $p = 0.015$).

Conclusions

We found significant negative correlations of NLR & Plt with PTH which is qualitatively opposite to a published study which had a female predominant population unlike ours. The significant negative correlation of platelet count with PTH hints at potential mechanistic link that needs to be more systematically explored.

Unbalanced distribution of Age and Sugar level in M vs F groups in our population were some of the confounding factors for the apparent gender based direction of change (increase vs decrease) in correlation between several factors including because they had independent association with PTH and potentially explains some disparity in the literature. Local prevalence of diabetic nephropathy or biological difference between the genders could be incidental factors behind some of these confounding variables.

doi: 10.1016/j.cca.2022.04.171

W041

Evaluation of inflammatory markers in hemodialysis

N. Trakas [a], E. Karakou [a], T. Chiras [c], A.P. Kakavelakis [e], J. Skarakis [d], P. Sklapani [b], A. Barbatsi [c], M. Sonikian [c]

[a] *Biochemistry Department, Sismanoglio General Hospital, Athens, Greece*
[b] *Cytopathology Department, Hygeia-Mitera Hospital, Athens, Greece*
[c] *Nephrology Department, Sismanoglio General Hospital, Athens, Greece*
[d] *Research and Development Department, DEMO S.A. Pharmaceutical Industry, Athens, Greece*
[e] *Rural General Practitioner, Phthiotis Region, Greece*

Background-Aim

C-reactive protein (CRP) and procalcitonin (PCT) are used as markers of inflammation and infection in general population and in chronic hemodialysis (HD). However, in dialysis (D) patients, CRP and PCT levels may be elevated even in the absence of inflammatory or infectious disease and diagnostic process is a challenge in such cases. We studied HD patients' laboratory profile concerning CRP and PCT.

Methods

We studied 25 stable HD patients, M/F = 22/3, aged 68(44-89) years, dialyzed thrice weekly for 55(6-274) months with a dialysate flow rate of 700 ml/min, with a residual daily diuresis less than 200 ml, Kt/V values of 1.44 ± 0.3 and no signs of infection. Patients were classified in 2 groups. Group A included 10 patients on pre-dilution online hemodiafiltration (HDF). Group B consisted of 15 patients on conventional HD with low-flux polysulfone membrane. 20 healthy subjects formed a group C. Serum CRP and PCT levels were measured in duplicate in A and B groups before and at the end of mid-week D sessions, also in C group.

Results

PreD CRP values in the total of patients were higher than those in controls (10.89 ± 19.29 vs 2.54 ± 1.28 mg/L-$p = 0.004$). Compared with group C, preD CRP values were higher only in B (15.98 ± 24.54-$p = 0.001$) but not in A group (4.09 ± 3.33-p = NS). There was a significant difference in preD CRP between A and B ($p = 0.028$). At the end of D session CRP showed a tendency to increase in both groups A (5.16 ± 4.81), B (17.00 ± 27.00) but differences were not significant. PreD PCT values in the total of patients were higher than those in controls (0.82 ± 0.9 vs 0.29 ± 0.05 ng/ml-$p < 0.001$). Compared with group C, preD PCT values were higher in both A (0.52 ± 0.15-$p < 0.001$), B (1.01 ± 1.13-$p = 0.006$). There was no difference in preD PCT between A and B ($p = 0.261$). At the end of D session PCT decreased in A (0.32 ± 0.11-$p < 0.001$) and increased in B (1.12 ± 1.21-$p = 0.014$).

Conclusions

In patients on both conventional low-flux HD and online HDF preD CRP and PCT levels were higher than those in healthy subjects. D modality and membrane flux did not affect postD CRP, but postD PCT decreased in online HDF. PCT usefulness might be limited in D with high-flux membranes. Cut-off values have to be established for both markers to eliminate confusion in diagnosis of inflammatory and infectious diseases in HD patients.

doi: 10.1016/j.cca.2022.04.172

W042

Serum uromodulin – A marker for diagnosis of chronic kidney diseases

I. Ivanova [b], D. Genov [a], A. Kundurdgiev [a], V. Pencheva [c], M. Nikolova [a], A. Kostadinova [a], N. Koleva [a], M. Hristova [a]

[a] *Clinic of Nephrology, Medical University – Sofia, UMHAT "St. Ivan Rilski", Sofia, Bulgaria*
[b] *Clinical Laboratory Department, Medical University – Sofia, UMHAT "St. Ivan Rilski", Sofia, Bulgaria*
[c] *Department of Propedeutics of Internal Medicine, Medical University – Sofia, UMHAT "Alexandrovska", Sofia, Bulgaria*

Background-Aim

Chronic damage in various renal pathologies leads to a change in urinary and serum uromoduline. This indicates that uromodulin plays an important role in the development of chronic kidney disease (CKD),

and its study makes possible early detection of renal impairment in CKD. The aim of this research is to assess the role of serum uromodulin as a marker of the renal impairment in patients with CKD.

Methods

A total of 70 patients were enrolled in this prospective observational study in the Clinic of Nephrology of the University Hospital "St. Ivan Rilski", Sofia, Bulgaria for a period of nine months (April – December 2019). The mean age of the patients was 56.53 ± 11.8 years with the male/female ratio 28/42 (40.0% / 60.0%). Laboratory blood and urine tests, abdominal ultrasound with resistive index measurement and serum uromodulin investigations were performed in all patients. Serum uromodulin levels were determined by ELISA kit - Uromodulin ELISA, EUROIMMUN, LOT E190627BD.

Results

Serum uromodulin levels were significantly negatively correlated with serum creatinine ($r = -0.467$, $p < 0.0001$), urea ($r = -0.495$, $p < 0.0001$), uric acid ($r = -0.296$, $p = 0.017$) and cystatin C ($r = -0.430$, $p < 0.0001$). Correspondingly, a positive relationship with estimated glomerular filtration rate ($r = 0.628$, $p < 0.0001$) was found.

Conclusions

Serum uromodulin levels significantly correlate with all already established laboratory parameters used for evaluation of renal impairment. It can be used as a potential marker for diagnosis and early assessment of CKD progression.

doi: 10.1016/j.cca.2022.04.173

W043

Sensitivity and specificity of plasma and urine NGAL for detection of acute kidney injury in critically ill children at Kenyatta National Hospital, Kenya

K. Kiptim [a], A. Amayo [b], F. Ndiangui [a], Z. Thaimuta [b], C. Gichuhi [b]

[a] *Kenyatta National Hospital, Kenya*
[b] *University of Nairobi, Kenya*

Background-Aim

Acute kidney injury (AKI) is a significant cause of morbidity and mortality in critically ill children. Early detection of AKI has a positive impact on outcome in affected children. Serum creatinine, which is the most frequently used biomarker for kidney dysfunction, is usually not sufficiently sensitive for diagnosis of early AKI. Neutrophil Gelatinase Associated Lipocalin (NGAL) has been studied as an early biomarker for AKI. Levels of NGAL in serum and urine have been found to be useful markers. There is little documentation of the performance characteristics of these assays in AKI detection in seriously ill children in our local set-up.

Objective

To describe the sensitivity and specificity of plasma and urine NGAL, in detection of AKI in critically ill children at Kenyatta National Hospital (KNH).

Methods

This was a prospective cross-sectional study carried out in critically ill children aged 1 -12 years admitted at Kenyatta National Hospital, Paediatric Unit. Forty study participants were enrolled, urine and blood samples were obtained from study subjects on admission. An additional blood sample was collected 48 hours post admission. Admission and 48 hour post admission plasma creatinine were estimated. Admission plasma and urine NGAL were analyzed using an NGAL kit (Bio Porto Diagnostics A/S, Denmark). KDIGO criteria were used for creatinine based diagnosis of AKI. The cut-off values recommended by the reagents manufacturer were used for interpretation of plasma and urine NGAL. Sensitivity and specificity of plasma and urine NGAL were determined using creatinine based criteria for diagnosis of AKI as the reference method. Ethical approval was obtained for the study.

Results

A total of 40 children were studied. Most were between 1 and 3 years of age and females (56%) were more than males. Nine children (28%) met the KDIGO creatinine based criteria for AKI diagnosis. For the prevalence of AKI based on the three individual markers, urine NGAL had the highest (90.0%) followed by plasma NGAL (85.4%) the lowest was creatinine with 29.5%. The sensitivity and specificity of urine NGAL for AKI detection were 100% and 16% respectively. For plasma NGAL, sensitivity and specificity were 85.7% and 13.3% respectively.

Conclusions

Plasma NGAL and Urine NGAL show high sensitivity for early detection of AKI in critically ill children. The specificity of NGAL is however low when measured against plasma creatinine. The perfomance of NGAL indicates it can be used as a screening test to identify critically ill children at high risk of AKI.

doi: 10.1016/j.cca.2022.04.174

W044

Renal stones chemical composition with age and gender distribution in Punjab Pakistan: A retrospective study

M. Zubair

Shalamar Medical and Dental College, Lahore, Pakistan

Background-Aim

The purpose of the present study is to find the chemical composition with age and gender related prevalence of the renal stones disease in Punjab Pakistan

Methods

It was a retrospective study over a period of one year from July 2018 to July 2019. In this study, the data was retrieved from Nexus Pro Laboratory Information Management System of Chughtai Lab Lahore of last one year. A total of 2900 stone chemical analysis was performed during this period. All analysis was done on Perkin Elmer instrument FT-IR spectrometer spectrum two.

Results

Out of 2900 stone analysis results, there were 1723(59.4)% males and 1177 (40.6)% females. The mean age of study population was 36.83 ± 15.51. The frequency of different types of stones were as follows: pure calcium oxalate stones were 1419, pure uric acid stones 239 and 1242 were mixed stones who had different percentages of (calcium oxalate, ammonium urate,carbonate apatite,cysteine,uric acid). In1723 males,851 had pure calcium oxalate stones,148 had uric acid stones and 724 had mixed stones. Similarly in 1177 females, 568 had calcium oxalate stones, 91 had uric acid stones and 518 had mixed stones. Highest prevalence of the renal stone disease was in the age group 31-40 year in which 788 patients had renal stones out of 2900.

Conclusions

The renal stones were more prevalent in males as compared to females in studied subjects.The most common renal stones were calcium oxalate and age group having highest number of nephrolithiasis was 31-40 year of age.

doi: 10.1016/j.cca.2022.04.175

W045

Epidemiological characteristics of acute kidney injury in neonates

S. Timovska Naunova [b], O. Jordanova [b], A. Sofijanova [b], M. Kimovska [b], H. Mangjukovska [b], S. Neskova [b], T. Voinovska [b], V. Timovski [a]

[a] *General Practise, Macedonia*
[b] *University Children Hospital-Skopje, Macedonia*

Background-Aim

Acute kidney injury (AKI) is defined as a rapid decrease in glomerular filtration, leading to an increase of urea and other nitrogenous waste products and most often to a decrease in urine output. It is common in neonates in intensive care unit. The incidence of neonatal AKI is the highest incidence followed by adults and children, depending of different factors such as gestational age, birth weight, predisposing factors and the facilities of the NICU. The aim of the study was to present the epidemiological characteristics of AKI in neonates.

Methods

The study evaluated neonates suffering kidney injury who at the period of three years were treated at the University Children's Hospital. It analysed medical records of 50 critically ill neonates with acute kidney injury. The severity of the disease was determined by RIFLE classification the material was statistically processed using methods of descriptive statistics.

Results

The calculated prevalence of kidney injury in neonates was 6.4%. Most of the involved neonates were born at term (68%) with predomination of male (64%). RIFLE classification was applied in the study. We reported "risk" in 36%, "injury" in 50% and "failure" in 14% of neonates with AKI. Perinatal asphyxia was the most common predisposing factors for kidney injury and was evaluated in 30% cases. The prevalence of prerenal, renal and postrenal AKI was 78.5%, 19.5% and 2.0%. The mortality rate was 32% and was significantly higher in neonates with congenital heard diseases.

Conclusions

Acute kidney injury is a serious condition which damages the kidney as a central mediator of the homeostasis of bodily fluids and electrolytes. Early identification of kidney injury in neonates represents the first step of prevention of this condition. Appropriate treatment of of predisposing factors could improve the outcome and prognosis of the disease.

doi: 10.1016/j.cca.2022.04.176

W046

Vitamin D deficiency in chronic kidney disease and dialysis patients of Kathmandu valley

R.K. Gupta

Department of Biochemistry, National Public Health Laboratory, Ministry of Health and Population, Nepal Government, Kathmandu, Nepal

Background-Aim

Vitamin D is a fat soluble vitamin as well as a hormone synthesized from precursors in the skin when exposed to sun light. : Vitamin D deficiency are common among patients with chronic kidney disease (CKD) or undergoing dialysis. In addition to nutritional and sunlight exposure deficits, factors that affect vitamin D deficiency include race, sex, age, obesity and impaired vitamin D synthesis and metabolism. Serum 1,25 (OH)2D levels also decrease progressively because of 25(OH)D deficiency, together with impaired availability of 25(OH)D by renal proximal tubular cells. Vitamin D status is assessed by measuring the concentration of serum 25-hydroxyvitamin D [25(OH) D].

Methods

This study was performed in 182 CKD and patients under dialysis (106 male and 76 female) who were visiting National Public Health Laboratory (NPHL) Hospital, Kathmandu, Nepal from December 2018 to December 2019. The people with normal blood sugar and normal renal function tests were considered as healthy population and taken as control (182 people). We excluded the patients with preexisting history of bone disorder, thyroid dysfunction, dyslipidemia, diabetes and patients who have previously taken vitamin D supplementation. Serum total 25-hydroxyvitamin D (25(OH) D) level was measured by chemiluminescence immunoassay method.

Results

The mean serum 25(OH)D concentration of CKD patients was 16.30 ±8.92 ng/mL, with 70.4% of the subjects having 25(OH)D deficiency (<20 ng/mL), 22.4 % having insufficiency (20-29 ng/mL), and 7.2% of the subjects having sufficient 25(OH) D (ε30 ng/mL) levels.

Conclusions

Vitamin D deficiency was highly prevalent in CKD patients living in Kathmandu valley of Nepal.

doi: 10.1016/j.cca.2022.04.177

W047

Determination of renal stones composition and epidemiology of the disease at NHLS-DGM Academic Laboratory, South Africa

S.M. Pheeha, D.M. Tanyanyiwa

Chemical Pathology Department, Sefako Makgatho Health Sciences University, Dr. George Mukhari Hospital (DGM)-National Health Laboratory Services (NHLS), Pretoria, South Africa

Background-Aim

Renal calculi are solid masses that are formed as a result of a pathological accumulation of minerals and organic substances in the urinary system. The prevalence of renal stone disease is increasing worldwide, with increasing recurrence rates. This is also currently witnessed in South Africa. However, a lower incidence was reported previously which implicated ethnic differences in the development of Kidney stones. With the exception of ethnicity, other factors such as environmental conditions, diet and fluid intake, metabolic syndrome, genetic disorders, urinary tract infections, age and gender are also involved in renal stone formation. Therefore, the aim of this study was to determine kidney stone composition using Fourier-Transform-Infrared-Spectroscopy (FTIR) and to analyze the trends/epidemiology of disease at NHLS-DGM Academic Laboratory.

Methods

This is a retrospective study involving qualitative and quantitative analysis of laboratory data. Patient renal stone samples sent to NHLS-DGM academic laboratory for analysis were selected (N = 513). The present study is of a retrospective design and it took place within a period of 3.5 years (January 2016 – June 2019). An FTIR spectrometer instrument was used to determine kidney stone composition. Renal stone composition results and patient information (age, gender, type of stone and other factors such as environment, as well as date of stone collection), were used for compilation of an epidemiological report.

Results

The highest prevalence of renal calculus disease was observed more in males (N= 345) than in females (N= 155). There were eight different stone components detected in the present study, with calcium oxalate (58.6%), carbonate apatite (18.7), uric acid/ammonium urate (15.9%), protein (3%) and struvite (2.8%) stones being among the top 5 most common. The age was ranging between 8 months & 12 days to 103 years.

Conclusions

Apart from knowing risk factors influencing stone formation, it is of great importance to determine the kidney stones constituents. This will aid in identifying the type of stone, and hence allow appropriate, individualized patient treatment. Moreover, clinicians will be able to guide patients on how to modify their lifestyle habits such as diet & fluid consumption, thus helping them to prevent recurrence of stones. Successively, the prevalence of kidney stone disease will decrease, and costs involved with detection and treatment of the condition will be reduced.

doi: 10.1016/j.cca.2022.04.178

W048

A comparative study of serum cystatin C, serum electrolytes, urea and creatinine in early detection of kidney injuries in morticians exposed to formaldehyde

C.M. Olisah [c], S.C. Meludu [b], C.E. Dioka [a]

[a] *Department of Chemical Pathology, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria*
[b] *Department of Human Biochemistry, Nnamdi Azikiwe University, Awka, Nnewi Campus, Nigeria*
[c] *Department of Medical Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*

Background-Aim

Background: Human exposure to formaldehyde is associated with multiple adverse effects. Chronic exposures may cause oxidative stress and may result in some vital organ damage. Chronic renal diseases may result due late detection of kidney toxicities from chronic formaldehyde exposures. Chronic renal failure (CRF) is characterized by gradual decline in glomerular filtration rate (GFR). GFR is presently being monitored by serum creatinine concentration and calculated creatinine clearance using Cockcroft and Gault equation. However, serum creatinine does not increase until the GFR has moderately decreased (about 40 ml/min/1.73 m2). This insensitivity for small to moderate decreases in GFR in creatinine gives a false sense of alert and leads to late detection of kidney damage.

Aim: To ascertain early detection of possible kidney injuries in Morticians occupationally exposed to formaldehyde by assessing cystatin C, serum electrolytes, urea and creatinine.

Methods

Materials/Methods: The exposed group (n = 45) comprised of male embalmers (morticians) who have had occupational exposure for a minimum of five years, while apparently healthy age-matched male subjects (n = 45) without considerable exposure to formaldehyde served as control subjects. Informed consent was obtained from all enrolled subjects. A structured questionnaire was utilized to capture the bio-data and other pertinent information on work place exposure. Eight milliliters of blood samples obtained were used for assessment of cystatin C using Eliza. Serum electrolytes were determined using Ion selective electrode, urea and creatinine were determined using spectrophotometric methods. Data obtained was analyzed using SPSS.

Results

Results: From the results obtained, mean serum electrolytes of the exposed group in mmol/l were (Na-142.89 ±2.023 ;K = 3.45 ± 0.233; Cl = 101.17 ±3.67 and HCO3 = 21.73 ±1.60), these were not statistically significant when compared with non-exposed subjects (Na-143.22 ± 2.48; K = 3.47 ±0.30; Cl = 101.37 ± 3.58; HCO3 = 23.28 ±1.82) mmol/l ($p > 0.05$). The mean levels of serum urea of exposed (3.03 ± 0.54) mmol/l, non-exposed (3.03 ± 0.59) mmol/l and serum crea-

tinine (72.10 ± 9.20) mol/L and non-exposed (74.50 ± 12.04) mol/L were also found to be statistically insignificant ($p > 0.05$). However, the mean levels of cystatin C of the exposed group (177.96 ± 30.98) ng/ml were statistically significant when compared with the non-exposed (191.85 ± 13.14) ($p < 0.05$).

Conclusions

Conclusion: Formaldehyde exposures may induce a gradual deterioration of renal functions in chronically exposed morticians. Serum electrolytes, urea and creatinine may not be sufficient to indicate an early signs of kidney damage. From the study, serum cystatin C may be a better marker of renal impairment in early stages.

doi: 10.1016/j.cca.2022.04.179

W049

The P21-mediated and senescence-associated hyperglycemic memory in diabetic nephropathy is therapeutically amendable

M.M. Al-Dabet [a b], K. Shahzad [c], A. Elwakiel [c], S. Zimmermann [c], B. Isermann [c]

[a] *Department of Medical Laboratories, Faculty of Health Sciences, American University of Madaba (AUM), 11821 Amman, Jordan*
[b] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, 04103 Leipzig, Germany*
[c] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, 04103 Leipzig, Germany*

Background-Aim

Diabetic nephropathy (dNP) is a major chronic microvascular complication among diabetic patients and a leading cause of end stage renal disease (ESRD) worldwide. dNP is characterized by albuminuria. Moreover, In dNP, the tubular compartment undergoes a particular pattern of damage characterized by induction of CDKi (mainly p21) leading to proliferative arrest, tubular hypertrophy and senescence-like phenotype. Chronic senescence-state would hamper elimination of damaged cells. The progression of those complications, despite improvement of hyperglycemia can lead to long-lasting renal effect. This phenomenon, which is referred to as “hyperglycemic memory”, is still poorly understood. Evidence suggests a key regulatory role of epigenetic mechanisms (DNA methylation & histone modifications).

Methods

Two mouse models with established dNP (16 weeks after STZ-induced persistent hyperglycemia or 16 weeks old db/db mice) were used. Blood glucose was reduced for 6 weeks using an SGLT2-inhibitor, mimicking therapy in diabetic patients. Furthermore, we investigated the role of p21in human dNP and its relevance as a potential diagnostic marker for dNP.

Results

Despite a marked reduction of blood glucose using SGLT2 inhibition, albuminuria, histomorphological changes and glucose, the expression of p21, a senescence-associated cyclindependent kinase inhibitor, remained elevated. Sustained p21 expression was linked with demethylation of its promoter and reduced DNA methyl transferase (DNMT) activity and expression. A role of miR-148a, identified in-silico as a potential regulator of DNMT1, was confirmed in tubular cells. Reducing miR-148a in addition to normalizing blood glucose reversed sustained tubular p21 expression, senescence and renal damage in mice with dNP. In human renal biopsies, tubular p21 expression and senescence marker were observed in patients with dNP compared to those without dNP. We analyzed the presence of p21 in urine of controls and diabetic patients with(out) dNP. dNP patients were recruited from a study in which a subgroup of patients was randomly assigned to a diet to lower blood glucose. Expression of p21 was elevated in the urine of dNP patients and remained high despite reduction of blood glucose levels upon intermittent diet. Conversely, no p21 expression was detected in the urine of healthy controls.

Conclusions

Epigenetically sustained p21 expression and associated senescence contribute to the hyperglycemic memory in dNP. In human, renal induction of p21 is a specific hallmark of dNP, indicating that urinary p21 identify patients with dNP and remains high despite improving of blood glucose. This pathogenic mechanism can be targeted by inhibiting miR-148a.

doi: 10.1016/j.cca.2022.04.180

W050

The effect of orange water kefir on malondialdehyde (MDA) level and superoxide dismutase (SOD) activity in the kidney tissue of the hyperlipidemic rats (rattus norvegicus)

R. Prabowo [a], S. Mariana [b], M.D. Pramaningtyas [c]

[a] *Clinical Program of Medicine, Faculty of Medicine, Universitas Islam, Indonesia*
[b] *Department of Chemical Engineering, Faculty of Industrial Technology, Universitas Islam, Indonesia*
[c] *Department of Physiology, Faculty of Medicine, Universitas Islam, Indonesia*

Background-Aim

Hyperlipidemia can cause kidney tissue injury due to high oxidative stress. One of oxidative stress signs is the increase of malondialdehyde level (MDA) and the decrease of superoxide dismutase activity (SOD). Previous research has shown that probiotics (water kefir) may contribute to improving lipid profile and oxidative stress in the kidney tissue. This research aims to know the effect of orange water kefir on MDA level and SOD activity in the kidney tissue of hyperlipidemic rats.

Methods

This research design is quasi-experimental. Three groups (K+, K-, P) each consist of 5 rats. All intervention was given by the sonde method. All groups were given fed ad libitum until the end of this research. The first 4 weeks, K+ and P groups were induced by quail egg yolk with the dosage is 5 ml/200 gram body weight (grBW). In the second 4 weeks, group of P was given orange water kefir with the dosage is 5 ml/ 200 grBW. Orange water kefir was made in accordance with good manufacturing product (GMP) standards procedure to making probiotic beverage. All rats are terminated by anesthesia and decapitation to take the kidney. Then, the kidney tissue will be examined for MDA level and SOD activity.

Results

Mean of MDA (mg/dl) levels were 10.17 + 0.11 (K +), 0.79 + 0.09 (K-), 2.78 + 0.12 (P). Mean of SOD activity (%) were 43.21 ± 3,45 (K +), 83,57 ± 2,48 (K-), 73,92 ± 1,65 (P). The results showed significant differences in MDA level and SOD activity in kidney tissue between all groups after the intervention of orange water kefir ($p < 0.001$). There is a significant difference between all groups ($p < 0.0001$).

Conclusions

The intervention of orange water kefir has an effect to improve the MDA level and SOD activity in the kidney tissue of the hyperlipidemic rats with a significant difference $p < 0.0001$.

doi: 10.1016/j.cca.2022.04.181

W051

Study of kidney disease in a Spanish cohort of HIV patients after five years of follow up

R. Fernández [a], J. Miró [a], F. Cofàn [a], C. Ruiz [a], J. Laguna [a], A. Moreno [a], J. Trullas [b], L. Macias-Muñoz [a], J. Bedini [a], N. Rico [a]

[a] *Hospital Clinic, Barcelona, Spain*
[b] *Hospital de Olot i comarcal de la Garrotxa, Spain*

Background-Aim

Currently 37 million people are living with HIV infection, the antiretroviral therapy and longevity in these patients has increased the risk of chronic kidney disease (CKD). The Kidney Disease: Improving Global Outcomes (KDIGO) recommend classified the prognosis of CKD by glomerular filtration rate (GFR) and a marker of kidney damage, stands out the proteinuria. The aim of this study is to know the evolution of kidney disease in a Spanish cohort of HIV patients after five years of follow-up.

Methods

Retrospective observational study in a cohort of 3324 HIV patients followed from 2014 to 2019. We classified this cohort according to the GFR and consecutively we estimated, for each stage, the percentage of patients who evolved to other stages in 2019. Ultimately, we classified the prognosis of CKD, according KDIGO guidelines.

Results

According to the GFR, in 2014, 72% of patients were classified in stage 1 (normal kidney function (NKI);GFR > 90mL/min), 25% in stage 2 (mild kidney impairment (MKI); GFR 60-89mL/min) and 3% in stage 3 or higher (CKD; GFR < 60mL/min). In 2019, 42% were classified in stage 1, 50% in stage 2 and 8% in stage 3 or higher.

After five years, a total of 43% (1043) of patients classified as NKI in 2014 progressed to MKI in 2019; and 19% (156) classified as MKI in 2014 progressed to a CKD in 2019. After evaluate the proteinuria, we observed a significant change in the prognosis of CKD, especially in those patients who evolved into a CKD. Of these, a 60% were classified as moderately risk, 21% as high risk and 19% as very high risk in 2019; any patient was classified as low risk. This implied a 45% more of patients in the moderately increased risk category, a 7% more in the high risk category and a 19% more in the very high risk category respect to 2014.

Conclusions

In our cohort we observed that after 5 years of follow up, a 43 % of patients evolved into a MKI and 19% to a CKD. After evaluate the proteinuria we observed a worst prognosis risk of CKD in 2019. The results confirm the importance of identifying, in early stages, those patients with a predisposition to develop kidney disease. Recognizing this population could help the clinicians to implement renoprotection strategies.

doi: 10.1016/j.cca.2022.04.182

W052

Morpho-constitutional study of uric acid lithiasis in southern Tunisia

M. Boudaya, F. Ayadi, S. Fendri, M. Turki, F. Gargouri, N. Aissaoui, R. Marrekchi, F. Ayedi

Laboratory of biochemistry, Sfax University Hospital, Tunisia

Background-Aim

Uric acid lithiasis is a frequent disorder that affects especially young men;therefore, it presents a serious health and socio-economic problem. It is often related to metabolic factors.

The spectrophotometric analysis of stones provides information that can contribute to the better understanding of the mechanisms involved in their formation, allowing a secondary prevention.

Our purpose was to determine the morphology and composition of urinary uric acid lithiasis in southern Tunisia by morpho-constitutional analysis and to study the epidemiological particularities of these stones.

Methods

This is a mono-centric retrospective and descriptive study of urinary lithiasis cases. The lithiasis were identified by the morpho-constitutional study,they were collected in the Laboratory of biochemistry, Sfax University Hospital of TUNISIA during the period from January 2011 to December 2019.

Results

Our study involved 93 uric acid stones which represents 10.4% of all urinary stones analyzed during the study period (n = 897).Our patients were aged from 1 to 80 years. sex ratio = 1.6.

Nephritic colic was the most common discovery circumstance (50%). Renal localization was the mostcommon (47.2%).

The global constitutional analysis of the calculi showed that 42.55% of the calculi had a pure composition mostly type AU0, the rest had a mixed composition and Whewellite (c1) was the major component.

The morphological study of the surfaces showed that 58.06% of the calculi were formed type IIIa .

An association Ia + IIIa was noted in 11.82%. 32% of the intermediate layers was formed by types IIIa and 21% IIIb. The nucleus was formed in 35.71% of type Ia and in 28.57% of type IIIa.

The constitutional study of the calculi showed that AU0 was the majority in the surface (37.63%). The association of AU0 with C1 was the most frequent (24.73%).

45.89% of the intermediate layers were constituted by anhydrous uric acid AU0 and 12.95% by C1.The association of AU0 with C1 was found in 11.77%. The nucleus was formed in 37.5% cases by AU0.

Conclusions

The evaluation of lithogenic risk factors is essential to allow an appropriate etiological approach to prevent recurrence.

doi: 10.1016/j.cca.2022.04.183

W053

To refit or not to refit? Impact of the CKD-EPI 2021 creatinine refit equation for a south-east Asian laboratory

J.G. Tan [a], Y. Lee [a], S.Y. Wong [b], P.H.M. Er [a], G.E. De La Pena [a], M.S. Wong [a]

[a] *Lab Medicine, Khoo Teck Puat Hospital, Singapore*
[b] *Renal Service, Khoo Teck Puat Hospital, Singapore*

Background-Aim

Estimated glomerular filtration rate (eGFR) provides an important tool for clinicians to assess the degree of renal impairment. It affects access to healthcare including eligibility for medical subsidies for certain drugs, nephrology referrals and initiation of dialysis. Currently, eGFR is calculated from serum creatinine, age, gender using the 2009 CKD-EPI creatinine equation, with the black race having a multiplier of 1.159. Recognizing the use of race factor may perpetuate healthcare disparities, a new equation without race factor has been developed (CKD-EPI 2021 Refit). We, therefore, seek to assess the impact of CKD-EPI 2021 Refit equation on our laboratory.

Methods

We data-mined serum creatinine requests received by our laboratory from July to September 2021 from the laboratory information system. The corresponding patients' age and sex were used for the calculation of eGFR. We studied the absolute and percentage difference in eGFR between the CKD-EPI 2009 creatinine and CKD-EPI creatinine 2021 Refit equations. The changes in the classification of CKD Stages with the new equation were examined. Creatinine was assayed Roche Cobas c702 using an IDMS-Traceable enzymatic method.

Results

A total of 59,430 serum creatinine results over 3 months period were retrieved. The CKD-EPI creatinine 2021 Refit equation reported a higher eGFR than the 2009 CKD-EPI creatinine equation. The median absolute difference was 3.3 ml/min/1.73m^2 [Wilcoxon Signed Rank Test, p-value <0.001]. The eGFR derived from the new equation is 5.9% (median) higher than the current equation. The 2.5th and 97.5th percentile differences are -1.0% and 9.6% respectively. 7,606 out of 59,430 results (12.8%) were reclassified to a better CKD Stage (higher eGFR) using the new equation. The percentage of CKD Stage II, IIIA, IIIB, IV, V that were reclassified when using the new equation were 22.1%, 27.5%, 23.5%, 14.5% and 6.5% respectively.

Conclusions

There exist significant differences in eGFR calculated using 2009 CKD-EPI creatinine vs 2021 CKD-EPI creatinine Refit equation, with the latter reporting higher eGFR (median 5.9%). 12.8% of the results were reclassified to a better CKD Stage (higher eGFR) using the new equation. We therefore advocate discussion with clinicians before implementing the new equation. The US National Kidney Foundation and the American Society of Nephrology now recommend all US laboratories adopt the new equation. As such, we may expect future drugs approved by FDA to use renal dose adjustment based on eGFR calculated using the 2021 refit equation. If local clinicians use these drugs, it may become necessary for the laboratories to provide the new equation.

doi: 10.1016/j.cca.2022.04.184

W054

Suggestion of cystatin C indication using muscle mass-based parameter for the desirable prediction of glomerular filtration rate

J. Yim [e f], N. Son [h], K.M. Kim [b], D. Yoon [a], Y. Cho [c], S. Lee [d], Y.J. Park [d], K. Kim [d], J.E. Lee [g], J. Kim [d]

[a] *Department of Biomedical Systems Informatics, Yonsei University College of Medicine, Seoul, South Korea*
[b] *Department of Endocrinology, Yonsei University College of Medicine, Seoul, South Korea*
[c] *Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Anyang, South Korea*
[d] *Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, South Korea*
[e] *Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, South Korea*
[f] *Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, South Korea*
[g] *Department of Nephrology, Yonsei University College of Medicine, Seoul, South Korea*
[h] *Divisions of Biostatistics, Yonsei University College of Medicine, Seoul, South Korea*

Background-Aim

The aim of this study is to evade the creatinine-based estimated glomerular filtration rate (eGFRcr), which may overestimate eGFR in the case of low muscle mass, and to suggest cystatin C indication using muscle mass-based parameters for the estimation of desirable glomerular filtration rate.

Methods

Using a cross-sectional analysis from this study of 138 (male, 53; female, 79) Koreans aged 40-95 years, including inpatients and health-check subjects, eGFR was compared between the eGFRcr (derived from CKD-EPI_2021 excluding race parameter) and eGFRcys (derived from CKD-EPI_2012 based on Cystatin C measurement). We determined eGFRcys_CKD-EPI_2012 as the reference, excluding the subjects without inflammation, insulin resistance, and obesity, although we did not measure GFR directly. We determined calf circumference (CC), skeletal muscle mass (SMM; appendicular lean mass/heigt^2) measured by

bioelectrical impedance analysis (BIA). We calculated eGFR % difference of eGFRcr against eGFRcys We defined the cases of detection of hidden renal impairment (DHRI) as eGFRcr ε 60 mL/min/1.73 m^2 and eGFRcys < 60 mL/min/1.73 m^2. We analyzed the results using Pearson's correlation correlation coefficient (r) and the association of age, sex, and SMM with the assigned eGFR category was determined via logistic regression. We also derived cutoff values to determine which subjects would test cystatin C test, based on muscle mass.

Results

We confirmed significant correlation between SMM and serum creatinine levels on both sexes (r, 0.344 for male, 0.348 for female). We confirmed significant negative correlation between SMM and eGFR % difference (r, -0.603 for male, -0.478 for female). We also SMM can be a significant parameter for DHRI as the cut-off values of 7.5 kg/m^2 for male ($p < 0.0001$) and 5.7 kg/m^2 and for female ($p < 0.0001$) by logistic regression analysis, which are similar to those of Asian Working Group for Sarcopenia 2019 to suggest to undergo cystatin C testing rather than creatinine testing for renal function evaluation. We suggest 31 cm or below for male ($p < 0.02$) and 29 cm or below for female ($p < 0.001$) as cutoff of CC for DHRI by logistic regression test to indicate Cystatin C test.

Conclusions

We suggested the criteria to test Cystatin C based on muscle mass-based parameters.

doi: 10.1016/j.cca.2022.04.185

W055

Serum erythroferrone in chronic kidney disease patients

V. Manolov [b], V. Pencheva-Georgieva [g], V. Vasilev [a], R. Emilova [c], O. Georgiev [g], M. Dayova [a], I. Petrova [f], B. Bogov [d], S. Hadjidekova [e], K. Tzatchev [b], L. Traykov [f]

[a] *Clinical Laboratory Ramus, Sofia, Bulgaria*
[b] *Dept. of Clinical Laboratory, Medical University, Sofia, Bulgaria*
[c] *Dept. of Immunology, NCIPD, Sofia, Bulgaria*
[d] *Dept. of Internal Diseases, Medical University, Sofia, Bulgaria*
[e] *Dept. of Medical Genetics, Medical University, Sofia, Bulgaria*
[f] *Dept. of Neurology, Medical University, Sofia, Bulgaria*
[g] *Dept. of Propaedeutics of Internal Diseases, Medical University, Sofia, Bulgaria*

Background-Aim

Erythroferrone' main function is to suppress hepcidin synthesis during inflammation, thereby regulates iron homeostasis. Chronic kidney disease (CKD) involves high number of populations worldwide, which on its way increases brain-vascular diseases risk. Among the main reasons for increased brain disorders evidence in patients with CKD is iron homeostasis disregulation.

Methods

62 patients with chronic kidney disease (stages II to V) were included; age 46.6 ± 9.9. The established results were compared to sex and age matched healthy controls. Routine blood analyses plus CBC, serum iron, ferritin, hsCRP and specific erythroferrone were measured in the included groups. IMT, MMSE, CERAD tests were used for atherosclerotic changes evaluation.

Results

We found decreased serum erythroferrone levels in CKD patients with IMT, MMSE, CERAD changes (3.2 ± 1.9 ng/mL) compared to healthy controls (10.7 ± 2.9 ng/mL); $P < 0.001$. A negative correlation was found in CKD patients with brain disorders between IMT and serum erythroferrone levels ($r = -0.809$, $P < 0.01$). Serum erythroferrone correlates negatively to atherosclerotic evidence changes in patients with impaired kidney function ($r = -0.799$, $P < 0.005$).

Conclusions

Brain-vascular disease risk factors are connected to chronic kidney function impairment. Disregulation of iron homeostasis is one of the main risk atherogenesis factors. Early hepcidin quantification might predict cognitive disturbances as atherosclerosis symptoms in chronic kidney disease patients, which might be very important for better clinical diagnosis and practice.

doi: 10.1016/j.cca.2022.04.186

Clinica Chimica Acta 530 (2022) S241–S244

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Laboratory InformationTechnology

M091

Impact of sample storage and retrieval in the Nairobi hospital laboratory (Kenya) using labware LIMS

W. Ochieng [a], F. Ndungu [a], A. Amayo [b]

[a] *The Nairobi Hospital, Kenya*
[b] *The University of Nairobi, Kenya*

Background-Aim

More than 2/3 of laboratory errors occur in the pre-analytical phase. Laboratories are therefore required to retain specimens to allow for repeat testing if needed. ISO 15189 requires the laboratory to have documented procedures for retention, storage and tracking clinical samples to safeguard sample integrity and ensure ease of retrieval. Most laboratories store blood samples randomly in racks. For busy laboratories, retrieval of a sample would require going through each rack to identify the required sample. This exercise is time consuming and inefficient. If the required sample is not easily found repeat specimen collection may be required with attendant patient dissatisfaction. Most bio-repositories use Laboratory Information Systems (LIMS) for efficient management of sample storage and retrieval. Since clinical laboratories have LIMS, they also have the opportunity to improve on their specimen management by applying this functionality. The Nairobi Hospital Laboratory stores an average of 200 blood samples daily for each section. Risks associated with conventional ways of sample retrieval including sample integrity, quality verification challenges, and time wastage necessitated the Laboratory to roll out the LIMS (LabWare Inc) sample storage function. This study was undertaken to evaluate the effectiveness of the LIMS function in reducing time for specimen retrieval.

Methods

The sample size for the evaluation was determined as 5% of samples stored daily (10 samples). The samples were randomly selected and four technical staffs were asked to retrieve the samples; two staff using the LIMS system to retrieve and the other two using the conventional method. Time taken to retrieve the samples was documented.

Results

The average times for sample retrieval for the first two technologists were 38.1 and 3.2 minutes for one using conventional and one using LIMS respectively. For the second pair average sample retrieval times were 36.4 Vs 2.4 minutes using conventional and LIMS respectively. The average times saved by use of LIMS were 34.9 and 34 minutes respectively for first and second teams.

Conclusions

Using the LIMS for managing specimen storage significantly reduces time wasted in specimen retrieval and increases staff productivity. The objective evidence of time saved in this study helped in managing the change from conventional to a LIMS based storage by enhancing staff buy-in. Sample storage was included in the daily tasks and samples are stored in a timely manner and their integrity is guaranteed.

doi: 10.1016/j.cca.2022.04.188

M092

Customer satisfaction level of android application laboratory results

P. Liana [b], V. Patricia [a], M. Shofiarani [a]

[a] *Central Laboratory Instalation, Dr. Mohammad Hoesin Hospital, Palembang, Indonesia*
[b] *Clinical Pathology Department, Sriwijaya University/Dr. Mohammad Hoesin Hospital, Palembang. Indonesia*

Background-Aim

Clinical laboratories are challenged to provide quality services, meet the standards and expectations of their customers. Turn around time laboratory results are expected to be short because the delay in delivering results will have an impact on patient safety, and cost inefficiency. But in practice, there are still often obstacles such as the absence of laboratory results in the form of pdf files on Hospital information system computer due to a disrupted network, power outages, etc. therefore the clinical laboratory is making a breakthrough for the delivery of laboratory results with an online application via android.

Methods

This research was a quantitative study using primary data from questionnaires. The sample of the study was the doctors who used the android application laboratory results. Customer satisfaction is analyzed by the SERVQUAL (Service Quality) method with a five-point Likert scale. To develop a service improvement strategy, Cartesian Diagram was

used the validity and reliability test of the questionnaire was done. The data were processed by SpSS software.

Results

The highest customer satisfaction was the empathy dimension with an average of 95.3%, while the lowest one was the assurance dimension with an average of 85%. The Cartesian diagram showed that as many as five variables were in quadrant B and quadrant C. There was each one variable in the assurance and responsiveness dimension in quadrant A. All dimensions of service quality had a negative gap.

Conclusions

Most variables of the empathy dimension are in quadrant B which means that importance and high satisfaction level which is an achievement that must be maintained. The responsiveness dimension, especially in the time of handling customer complaints variable is the highest priority dimension for improvement.

doi: 10.1016/j.cca.2022.04.189

M093

Efficient operation of point of care testing (POCT) through the network system in Asan Medical Center

J. Wi [a], O. Kwon [a], H. Hong [a], J. Hong [b], H. Kim [b], W. Lee [b], S. Chun [b], M. Won-Ki [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-Aim

Point of care testing (POCT) is clinical diagnostic testing at the site of point of care, that is, outside the clinical laboratories. Commonly performed POCT includes blood sugar test, arterial blood gas analysis (ABGA), and blood clotting test etc. As POCT is performed by personnel who do not specialize in the clinical laboratory testing, quality management activities including internal quality control monitoring and device maintenance should be regularly performed by experts in central laboratory to ensure the reliability of test results.

In addition, POCT users should be periodically trained and their training record should be documented to allow for user authentication based on the training history.

Methods

Asan Medical Center has implemented point of care (POC) glucometer and arterial blood gas analyzers. For POC glucose testing, 261 Roche ACCU-CHEK Information II glucometers are used and for POC blood gas analysis, 20 Instrumentation Laboratory GEM PREMIERs, 16 Siemens Rapid500s, and 7 Radiometer ABL90 FLEX PLUS' are used. Each POC device is operated in wards, emergency rooms, intensive care units and operating rooms and the test results are delivered to the server system controlling each device located at the central laboratory and then transferred to the hospital information system (HIS) using the same protocol used for the clinical tests performed in the central laboratory.

Results

As internal quality control (IQC) and external quality assessment (EQA) activities are mandatory for all the clinical laboratory testing, IQCs are performed using at least two quality control materials with different concentration daily by the user as guided by the College of American Pathologists and the Laboratory Medicine Foundation of Korea. If a quality control result fails, QC should be repeated. And it fails again, the POC device becomes locked and further testing is not possible and the event is automatically reported to the server system and allows for person in charge to resolve the problem. Also the quality control test results are delivered real-time to the quality control analysis program, Unity Real Time 2.0 (Bio-Rad Laboratories, Inc. Hercules, CA, USA) to allow for easier detection of issues in POCT sites.

Our institution has been regularly participating in the EQA programs of the CAP survey and Korean Association of External Quality Assessment Service (KEQAS).

Furthermore, our institution established a web-based training program for POCT on our own and all the personnel can be trained by just visiting the website of cyber education program. And user authorization is provided through the POCT Server provided by each vendor.

Conclusions

Asan Medical Center maintains quality control of POCT with systematic and standardized internal and external quality management programs, and web-based periodic user training and user authentication are maintained efficiently.

doi: 10.1016/j.cca.2022.04.190

M094

Evaluation of moving average as continuous analytical quality control for routine chemistry and hematology parameters

L. Henricks [a], E. Van Andel [a], W. Den Elzen [a], C. Cobbaert [a], H. Van Rossum [b], J. Gillis [a], P. Schenk [a]

[a] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands*
[b] *Department of Clinical Chemistry, the Netherlands Cancer Institute, Amsterdam, the Netherlands/Huvaros, the Netherlands*

Background-Aim

Moving average quality control is a patient data-based real-time quality control monitoring system with many potential advantages, such as absence of commutability problems, sensitivity to pre-analytical errors and, above all, continuous monitoring of performance. We evaluated the use of moving average quality control for three chemistry parameters (bicarbonate, calcium and free thyroxine (FT4)) and four hematology parameters (MCV, MCHC, hemoglobin and erythrocyte sedimentation rate (ESR)) in our university hospital.

Methods

For each parameter, patient data from one year were extracted from our laboratory information system (LIS). Next, moving average optimization was performed using bias detection curves with the MA Generator tool. Optimal moving average settings for each parameter were derived from the program and incorporated into the LIS. The performance of

moving average as quality control was then evaluated over six months, by the number of alarms generated and proportion of false alarms.

Results

For ESR and hemoglobin, MA Generator showed that only very large biases (>40%) could be identified, even when excluding outliers. Therefore these parameters were not chosen for further evaluation. For all other parameters, incorporation into our LIS was shown to be useful. For example, for MCV and MCHC substantial shifts (>~5%) after periodic maintenance and differences between analyzers could be identified, which are not seen with regular internal quality control (IQC). The moving average for bicarbonate detected a drift that was not detected via regular IQC, and erroneous results could be prevented. Optimal settings for moving average of FT4 and calcium were more difficult to obtain due to series of extremely high or low results from selected departments (e.g., Intensive Care Unit). Therefore, data extractions were adapted and repeated; evaluation will take place during three months.

Conclusions

In our laboratory, the use of moving average quality control appears to be useful for chemistry and hematology parameters, especially for long-term drift monitoring of parameters without external quality assessment such as bicarbonate, and has added value to existing IQC for the hematology parameters MCV and MCHC.

doi: 10.1016/j.cca.2022.04.191

M095

AI infrastructure for the digitization and automation of clinical laboratories

S. Cope

Intel Corporation, United States

Background-Aim

Laboratory Clinicians agree that AI is elemental in driving the digitization and automation of Laboratory 4.0. As workflow efficiency and personalized medicine advance, the ability to move, store and analyze vast amounts of data will place new demands on laboratory instrumentation and infrastructure. In order to glean insights on these data, one needs to deploy advanced AI models while maintaining required bandwidth, latency and privacy requirements specific to the laboratory environment and ecosystem. The ability of AI-specific toolkits to provide workflow speedup on Intel® architecture is presented through relevant health and life science use cases.

Methods

All performance measurements were conducted on systems powered by Intel® architecture, including Intel® Xeon® Scalable Processors and Intel® AI accelerators; deep learning models were optimized with OpenVino® toolkit.

Results

AI techniques for image recognition provide laboratory scientists the ability to rapidly decrease time-to-result and improve sample throughput, despite the exponential growth of data. Through the development and deployment of AI software development kits and interfaces, we are able to achieve order of magnitude speedups across multiple Intel® Silicon implementations and diverse workloads. Results highlighted in this presentation include 20X speedup on training and 10-180X speedup on inferencing for image classification, image segmentation and anomaly detection use cases. Use cases also exploit large amounts of system memory, an increasingly important requirement in next generation applications.

Conclusions

To address the unique challenges of Laboratory 4.0, we apply deep neural network acceleration techniques to process large datasets in significantly less time while extracting greater insight. These best known methods include the use of Intel® architecture and AI-optimized toolkits. Intel is collaborating with leading health innovators to create new data-driven solutions that will drive transformation in the field of laboratory science enabled by the breadth of the Intel AI portfolio from edge to cloud. Intel envisions a future where insights from all available healthcare data across instrumentation, pathology, genomics, and other sources are combined to extract insights and open up new possibilities to predict and prevent disease.

doi: 10.1016/j.cca.2022.04.192

M096

Automated, integrated and connected: Creating an interoperable laboratory medicine network in the UK

S. Box, R. Crees, M. Barrow

X-Lab, UK

Background-Aim

In the UK and Ireland, diagnostics laboratories exchange work with other laboratories within the NHS, Public Health organisations, and private laboratories across Europe. This is because a laboratory might not have the capacity or means to test in-house, or that they need to access a specialist assay offered by an external laboratory. However, this presents an industry-wide informatics challenge as laboratories operate on a variety of information systems. Without interoperability, the exchange of work has historically relied on paper-based systems and manual processing. This, then, opens exchanged samples to the risks of human error, slow turnaround times, lack of visibility and tracking, and forfeited patient safety. The presence of this testing in every laboratory discipline, too, means that labs are, essentially, referring tests across a network of laboratories; therefore, site-to-site interfacing is not a sustainable solution, in terms of cost or maintenance, across an international network of laboratories. This presentation will discuss a globally unique solution to this challenge which exists in most healthcare systems and organisations across the world.

Methods

Labgnostic, a diagnostic exchange powered by X-Lab, is a unique, global technology which enables any diagnostic laboratory to refer tests or return results to any diagnostic laboratory, anywhere in the world. It is a technological hub which is versatile, interoperable, and offers laboratories access to a network of laboratories through a single connection. By installing an interface into a laboratory's information system, the laboratory can connect to any laboratory on the network to send or receive any test data almost instantly. Labgnostic digitises and automates the lab-to-lab referral and reporting process and removes the need for inef-

ficient manual processes that are time-consuming, incur unnecessary costs, and result in errors.

As a cost-effective, universal, and systems-agnostic solution, Labgnostic has successfully been delivering a fully interoperable laboratory medicine network at scale to the UK, Ireland and Europe since 2007. Labgnostic, which operates under the National Pathology Exchange (NPEx) in the UK, is currently used in 90% of UK NHS laboratories, which includes 100% of English and Scottish NHS laboratories, Public Health organisations, and private laboratory organisations.

Results

When COVID-19 hit the UK, the UK NHS mandated that all NHS trusts were to transfer all externally referred SARS-COV-2 tests requests and results through X-Lab's system for the faster turnaround times, safer data exchanges, and network access it offered. All UK NHS labs, through the solution, have the opportunity for capacity uplifts by transferring or receiving COVID-19 test requests to any other laboratory on the network to ensure all tests are performed in timescale required. In the UK, most laboratories work to a rapid turnaround time of under 24 hours for COVID-19 antigen tests. The project was commissioned by the NHS in mid-March and in a matter of weeks, the X-Lab team were able to deliver and implement their solution in all remaining unconnected NHS sites. Additionally, NPEx (or Labgnostic) has been the key data infrastructure for delivering COVID-19 data back to Public Health organisations, primary care facilities, and NHS bodies who deliver results to subjects and monitor data. The X-Lab team are currently working to deliver COVID-19 antibody tests at scale in the UK through their system.

Conclusions

With a hub connecting diagnostic systems across the UK, Ireland and into Europe, X-Lab are now exploring use cases for this technology across other continents with laboratory business, proficiency testing providers and public sector service organisations in areas such as surveillance. This presentation will put forward the need for an integrated and interoperable laboratory medicine network throughout the world with a case study on its success in the UK and the similarities it shares with the other testing arenas.

doi: 10.1016/j.cca.2022.04.193

M097

Parametric and non-parametric estimation of reference intervals for routine laboratory tests: a health check-up data analysis of 260,889 young Korean soldiers

T. Kim [a], H. Choi [b], S. Lee [b]

[a] *Department of Internal Medicine/The Armed Forces Goyang Hospital, Goyang, South Korea*

[b] *Department of Laboratory Medicine/Pusan National University Yangsan Hospital, Yangsan, South Korea*

Background-Aim

Determination of reference intervals using a big data has several obstacles due to heterogeneity in analyzers, time span, and ethnicity. The present study aims to establish reference intervals (RIs) for routine common blood count (CBC) and chemistry laboratory tests in homogeneous 20's healthy Korean male soldiers using a large-sized health check-up data.

Methods

A total of 609,649 men underwent health examination when promote to the corporal between January 2015 and September 2021. Of them, eligible 260,889 individuals aged 20-25 were included in the analysis. The RIs were established by both parametric and non-parametric method. In parametric approach, the maximum likelihood estimation was applied to measure the Box-Cox transformation parameter, and the values at 2.5th and 97.5th percentiles were recalculated. Non-parametric approach adopted the Tukey's exclusion test, and then the values at 2.5th and 97.5th percentiles were obtained. Partitioning by body mass index (BMI) was additionally performed.

Results

The obtained RIs of hematology parameters were comparable between devices. In case the values followed Gaussian's distribution, parametric and non-parametric methods were well-matched in both hematology and chemistry markers. When the values were right skewed, upper limits were higher in parametric than in non-parametric methods. Obese individuals showed higher RIs of CBC, some liver function tests, and some lipid profiles than non-obese 20's soldiers.

Conclusions

Using big data in healthy 20's Korean male soldiers of single race, we proposed RIs of CBC and chemistry parameters. As approach to the massive, stored data gets easier and prevalent, further studies are needed to exquisitely discriminate eligible individuals and determinate RIs in an extrapolated sample.

doi: 10.1016/j.cca.2022.04.194

Clinica Chimica Acta 530 (2022) S245–S259

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Laboratory Management

M098

utility of the icteric index in the management of total bilirrubine in the biochemistry emergency laboratory

R. Rubio Sánchez, E. Lepe Balsalobre, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville, Spain

Background-aim

The heme group from hemoglobin and other hemoproteins is metabolized to bilirubin and transported to the liver. There, it is conjugated with glucuronic acid and it is eliminated by the digestive tract.

The determination of total bilirubin (BT) is frequently requested in the Biochemistry Emergency Laboratory, due to its usefulness in the assessment of hepatobiliary function and hemolytic alterations. The measurement of serum indices, among which is the ichteric index (II), is performed automatically in all samples that are requested a biochemical analysis to detect possible interference.

The objective of this study was to analyze the requests for total bilirubin and implement a protocol of adequacy of the demand, according to the icteric index, for a better use of the available resources.

Methods

Descriptive and retrospective study of 55,590 BT applications received at the Biochemical Emergency Laboratory during 2018. A contingency table was made, classifying the samples according to whether BT levels were normal (δ1.2 mg/dL) or pathological (>1.2 mg/dL).

Results

Of the 55,590 samples analyzed, 10.6% had a pathological II while in 89.4% the values were within normal range. Of the normal II, only in 48 samples (0.1%) bilirubin was pathological; due to this, the probability of finding a pathological BT when the II is normal is 1/1000. Taking into account the cut-off point for II of 2, the sensitivity is 70%, the specificity is 99.9%, the positive predictive value is 98.8% and the negative predictive value is 96.6%.

Conclusions

The application in clinical practice of the cut-off point proposed for II could prevent the performance of a considerable number of BT determinations. In 2018, 89.4% of the BT determinations would not have been necessary because the II was normal.

Only the determination of BT in 48 patients in whom it would have been indicated would have been stopped, since it ended up being pathological, although in no case the value exceeded 1.7 mg/dL.

Despite the low cost of each BT determination, the high number of determinations requested makes the annual cost of conducting this test very high.

doi: 10.1016/j.cca.2022.04.196

M099

Comparison of four matrixes for diluting insulin in routine clinical measurements

H. Li, D. Wang, X. Cheng

Peking Union Medical College Hospital, China

Background-aim

Clinician relies on the specific measurement of insulin to know the peak time of insulin in the insulin release test and the multiple of insulin growth compared with fasting. In our laboratory, 2.36% (6626/280765) serum insulin concentrations were higher than 300 mU/L which was higher than linearity. Accordingly, it is important to determine the accurate insulin concentration by dilution test. To evaluate effect of original diluent, pure water, 0.9% NaCl, and low insulin concentration serum to dilute high insulin concentration serum in routine clinical measurements.

Methods

According to Clinical Laboratory and Standard Institution (CLSI) EP15-A2, the precision of method was validated. Residual serums of high insulin concentrations ranged from 200 to 300 mU/L were collected in Peking Union Medical College Hospital from August to November in 2017. Four different matrixes including Siemens original diluent, pure water, 0.9% NaCl and low insulin serum (labeled as A to D) were used to dilute the obtained serums by 1:2, 1:5, and 1:10.

Results

The repeatability ranged from 1.3 % to 1.9 %, and the within-laboratory CV (%) ranged from 1.9 % to 3.2 %. The linear correlation coefficient of A to D were higher than 0.9. The linear regression R2 were 0.871-0.913,0.893-0.924,0.879-0.953,0.910-0.965 from A to D, respectively. The recovery rates are 86.4%-104% (original diluent),73.2%-

99.3% (pure water),76.4%-101.3% (0.9% NaCl), and 84.2%-99.7% (low insulin serum).

Conclusions

Using 0.9% NaCl, pure water and low insulin serum to dilute high serum insulin (>300 mU/L) with dilution factor 1:5 on the SIEMENS ADVIA Centaur XP® instrument, and the clinical reported range is 0.5-1500 mU/L.

doi: 10.1016/j.cca.2022.04.197

M100

Laboratory medicine committee its fingerprints on the profession and practice of laboratory medicine (2010–2019)

S. Al Rashdi [a], W. Mulaabed [b]

[a] *Diagnostic Laboratories Services, Ministry of Health, Oman*
[b] *National Genetic Center, Ministry of Health, Oman*

Background-aim

As part of the Ministry of Health (MOH) vision towards having high professional input in the management and leadership of the different health specialties, a Technical Committee for Improving the Diagnostic Laboratory Services was established in November 2010 that was renewed in December 2018 (Ministerial Decrees 143/2010 & 249/2018). The Committee included ten members who represent the leaders and experts in the different specialties of the profession.

The aims and job profiles of the Committee were to: 1. Review laboratory services in diagnostic labs, specialized labs and public health labs, 2. Recommend the policies, strategies and plans in lab management including reviewing the specifications, selection, and development of equipment, consumables and other requirements to provide consultation view towards ensuring the most effective selection and high quality service, 3. Improve the guidelines in the implementation of laboratory service, both technically and medically in all lab specialties as per international the profession, 4. Review the manpower status including the need, skill and competency necessary in the profession, and 5. Report the overall lab performance and ensure proper inter-laboratory communication.

Methods

- MOH Laboratories in ALL regional hosp & majority (Wilayat & Local Hosp & Polyclinics) were visited for assessment and professional evaluation. The laboratory Services review included equipments, manpower, infrastructure, consumables, safety, quality, strengths/weaknesses).
- Discussed with Lab HOD, pathologists, technicians, clinicians & hospital directors.
- Cumulative report data base for Fx planning strategies for overcoming

weaknesses & implementing recommend in clinical/cost effective manner.

Results

The committee has significant input in reviewing and improving laboratory service in the different health institutions ensuring effective utilization of service at technical, medical and cost manner as well as quality improvement. The Committee is regularly discussing and making recommendations on different professional issues, as appropriate. The Committee also implemented in the majority of health institutions the use of external quality assurance programs, and prepared and distributed many professional guidance documents in the relevant area in addition to their input in providing consultation input for the inter-laboratory IT connectivity, creating POCT committees in regional hospitals, organizing professional educational activities in certain areas of interest and critically reviewing equipments' specification with advisory input in selection procedures and many other activities and enrollments.

Conclusions

The Committee through its input in the Laboratory profession has contributed obviously in implementing the necessary policies and guidance in the service that resulted in evidenced improvement in the effectiveness of the service.

doi: 10.1016/j.cca.2022.04.198

M101

Assessing the performance of Abbott M2000 for HIV-1VIRAL load testing on a sigma scale; Malawi pilot study

E. Chipofya [b], R. Khunga [a], B. Mpote [b]

[a] *Jhpiego, Lilongwe, Malawi*
[b] *Thyolo District Hospital, Malawi*

Background-aim

In laboratory medicine, Sigma metrics may be used to improve assay quality by identifying biased and/or imprecise assays. The aim of the study was to calculate Sigma metrics of HIV-viral load testing (VL) to determine the appropriate quality control strategy.

Methods

A retrospective study was conducted with ethical approval at Thyolo District Hospital. Internal quality control (IQC) and external quality assessment (EQA) data were extracted from Malawian laboratories using the Abbott M2000 system for the months of May 2018, September 2018 and June 2019. IQC data were used to calculate the coefficient of variation percent (CV %) and EQA data were used to calculate the bias percent. Sigma metrics were calculated with the following formula: Sigma = (TEa % − Bias %) /CV%. TEa is the total error allowable and was calculated using the Tonks rule with the following formula: TEa= ([Reference interval ÷ 4] ÷ mean of the reference range) ×100. Internationally, HIV-1 viral load within the range of 40 - 200 cps/ml is clinically significant; in Malawi 839 to 1000 cps/ml is currently in use.

Results

TEa calculated using the range 40-200cps/ml was 33.3%. The equivalent TEa using the range 839-1000cps/ml was 4.38%. Based on untransformed data the CV results for May 2018 (n=21) were 26.6% (low control) and 18.2% (high control). Equivalent CV results for September 2018 (n=23) were 22.9% (low) and 25.5% (high). In June 2019 (n=26) corresponding results were 16.4% (low) and 19.6% (high). The Sigma results (TEa=33.3%) for the high control were 4.81; -2.41; and -1.46 for the three study windows. Corresponding Sigma results for the low control were 3.29; -2.69; -1.76. Using TEa = 4.38% the Sigma results for the high control were 3.22; -3.54; -2.93

for the three study windows. Corresponding Sigma results for the low control were 2.20; -3.95; -3.52.

Conclusions

The data reveals that Sigma metrics for VL testing are not as good in Malawi as in developed countries. No evidence of systematic improvement was found across the three study windows. We recommend that regular EQA participation should occur in Malawi, backed up by educational support in quality management.

doi: 10.1016/j.cca.2022.04.199

M102

Laboratory errors in clinical biochemistry: The quality of laboratory testing in B.P. Koirala institute of health sciences, Nepal

A. Niraula, M. Lamsal, B.K. Lal Das, O. Sherchand, B. Mishra

B.P. Koirala Institute of Health Sciences, Nepal

Background-aim

Errors associated with medical care is taken as one of the major priority be it in the field of health care provider or laboratory medicine with no exception in clinical chemistry laboratories. The quality laboratory reports contributes for about 70% to medical diagnosis and treatment. Substantial development of automation in the field of clinical chemistry has aided for the significant improvement in medicine and laboratory science, but the errors in the pre-analytical phase pertains to be maximum in the whole laboratory cycle. This study aims to evaluate the common pre-analytical errors occurring in routine biochemistry laboratory and subsequently imply strategies applicable in our laboratory setting to minimize their occurrence.

Methods

This is a hospital based cross-sectional study conducted in routine biochemistry laboratory in B.P. Koirala Institute of Health Sciences, Dharan, Nepal. All the samples in routine biochemistry laboratory were screened for a period of 1 year from November, 2018 to October, 2019 respectively. All the types of pre-analytical errors were assessed and recorded.

Results

A total of 34,540 samples were screened during the study period. Out of the total samples 1015 samples were subjected to rejection which accounted for total of 2.94%. Among the rejected samples, maximum were due to hemolyzed samples (1.5%), wrong identification (0.6%), samples misplaced (0.4%), improper sample collection (0.2%), inappropriate sample collection time (0.12%), missing samples(0.1%) and lipemic samples (0.02%) respectively.

CONCLUSIONS

The study revealed a significant number of pre-analytical error existed in our laboratory which has a direct impact in quality laboratory results and patient service. In addition, the study indicated that there is an extensive need of awareness for laboratory personnel regarding the errors commonly occurring in the biochemistry laboratory.

doi: 10.1016/j.cca.2022.04.200

M103

Low proficiency of microscopists with poor laboratory is a challenge for malaria diagnosis in Ethiopia

D. Nega, A. Abebe, A. Abera, B. Gidey, A. Gibretsadik, G. Tasew

Ethiopian Public Health Institute, Ethiopia

Background-aim

Successful malaria elimination calls for rapid and accurate tracking of cases so that the personnel can be promptly treated before the occurrence of transmission. This study assessed the competency of malaria microscopists working in health facilities.

Methods

A cross-sectional study was conducted from February to June 2018 in 20 malaria elimination targeted districts in Ethiopia. A maximum of 5 malaria-microscopists per facility were conveniently included. Structured questionnaires and 10 WHO certified malaria slide panels from EPHI slide malaria bank were administered to each study participant.

Results

In this assessment, 17 district hospitals, 71 health centers (HCs) and 18 private clinics (PCs) were included. Of 1896 malaria-positive & 474 negative slides administered to 237 participants, 318 slides (16.8%) reported falsely negative & 47 slides (9.9%) reported falsely positive. The participants achieved “good” grade [Agreement (A): 84.6%, Kappa (K): 0.6] on parasite detection and “Poor” agreement (A: 43.8%; K: 0.11) on species Identification. The agreement is lower in PCs (Detection A: 77.8%; Identification A: 37.2%), followed by HCs (Detection A: 84.14%; Identification A: 41.64%) and hospitals (Detection A: 86.7%; Identification A: 48.1%). No or slight agreement seen on differentiation of P. falciparum from other species (A: 28.41%; K: 0.29). Above 95% of participants, (201/237), did not count or used only plus system of parasite count. Of the 18 PCs, only 10(55.6%) had registration certificate. Of all facilities, 91.5% (97/106) use microscopy, 2.83% (3/106) use RDTs and 2.9% (3/106) use both. In 84.6% (11) hospitals, 35.7% (25) health centers & 26.7% (4) PCs, the laboratories comply fully with the national quality assurance guidelines.

Conclusions

The low competency of malaria microscopists particularly in species identification & poor to moderate capacity laboratories in the current study may place challenges to malaria elimination path. Therefore, comprehensive In-service training of professionals with fulfillment of the laboratory equipments and supplies is required.

doi: 10.1016/j.cca.2022.04.201

M104

Application and modification of reference change values for delta checks in clinical laboratory

J. Hong[a], E. Cho[b], H. Kim[a], W. Lee[a], S. Chun[a], W. Min[a]

[a] *Department of Laboratory Medicine, Asan Medical Center and University of Ulsan College of Medicine, Seoul, South Korea*

[b] *Department of Laboratory Medicine, Hallym University Dongtan Sacred*

Heart Hospital and Hallym University College of Medicine, Hwaseong, South Korea

Background-aim

Delta check is a patient-based quality control tool for detecting laboratory errors by comparing current and previous test results of each patient. Reference change value (RCV) has been adopted in the guideline as a method for delta check in clinical laboratories, but the performance is not verified. In this study, RCV-based delta check method was applied to actual patients' data, and modified for application.

Methods

RCV with 95% confidence interval (CI) and with 99% CI were calculated using results of internal quality control materials and publication-based biological variation data. Test results of 17 analyte in inpatients, outpatients, and health examination recipients were collected for analysis. The detection rates of the current delta check method of our laboratory and those of the RCV-based delta check method were compared. Several modifications were tried to lower the excessive detection rate.

Results

RCV-based delta check method had higher detection rates compared to conventional method. Applied modifications reduced detection rates. Removing the pairs of results within reference interval reduced detection rates (0.42%~10.92%). When RCV was divided by time interval, the detection rates were similar to prior rates in outpatients (0.19%~1.34%). Using RCV multiplied by twice the upper limit of reference value as cutoff reduced the detection rate (0.07%~1.58%).

Conclusions

RCV is a robust criterion for delta check and already included in clinical laboratory practice guideline. However, RCV-based delta check method generates high detection rate which can increase the laboratory workload, so it seems to be subject to modification for actual application in clinical laboratories.

doi: 10.1016/j.cca.2022.04.202

M105

The journey of the implementation of total laboratory automation – A large Indian medical centre in focus

A. Gupta [a], A. Raizada [b]

[a] *Aster Labs, Bangalore, India*
[b] *POCT Services, India*

Background-aim

The laboratory is arguably the core of diagnostic based medicine and has come to play an invaluable role in the making of decisions by medical personnel. Clinical pathology has placed high demands on the laboratory causing decision makers to rapidly adapt to methods in order to keep pace. These challenges have been tackled via various means with the most effective one being the implementation of the total laboratory automation system (TLAS).

This study was a journey of the implementation of TLAS including challenges that are facet during the comprehensive overview of the process involved in pre-implementation, implementation and post-implementation decision making, advantages of this system, architecture and the technicality of its operation process, at one of the India's largest multi-superspeciality institute, Medanta-The Medicity,Gurgaon.

Methods

This study involves the analysis and the comparison of the pre-automation situation with the post-automation situation from cost perspective, turn around time (TAT), equipments, sample rejections, timely report availability, complaiance for urgent samples. The method initially involved interviewing key leadership personnel, studying the documented specimen flow, operator flow and process flow. Challenges of the Pre-implementation included space- constraint, designing of the automated statement. During implementation phase, the major challenge was barcode labelling.

Results

The implementation of TLAS has led to reduced urgent TAT, decrease in number of sample rejections, reduced rate of post release modification of reports, increase in number of morning round reports availability, thus overall improved lab productivity that was helping in improving the brand-value of the hospital, increase revenue and savings by reducing the average length of the stay for patients and improving patient care standards.

Conclusions

The importance of TLA is to give timely and accurate results, however, the best of automation alone is not enough. If the processes around automation mainly information technology are not streamlined most of the advantages will fail. Automation is well suited to a laboratory when coupled with a process like LEAN that can bring path-breaking improvements in the overall efficiency of the laboratory.

doi: 10.1016/j.cca.2022.04.203

M106

Proactive health care service, experience from Thailand

T. Intorn, K. Tuntitavatchaikul, A. Worachartcheewan, P. Dansethakul, P. Pidetcha

Faculty of Medical Technology, Mahidol University, Thailand

Background-aim

Annual health-checkup is a government subsidized item for Thai government officer and most are setting inside hospital. Mobile health-checkup model (MHM) designed as proactive health care service by managed outside hospital and set the value as "your health can be touch". The aim of study is to evaluate the model satisfaction and perception of customers.

Methods

MHM established by the faculty of Medical Technology, Mahidol University, Thailand, which service for more than 30,000 persons each year. The service processes were designed into five parts, follow the medical laboratory service phases (pre-pre analytical, pre analytical,

analytical, post analytical, and post-post analytical, respectively), and met the ISO 15189: 2012 and ISO 15190: 2003 requirement. Since 2017 to 2019, questionnaire performed as a tool for the service processes quality evaluation and customer perception for "your health can be touch". The satisfaction scores were allocated from 1 to 5 (strongly dissatisfied to strongly satisfied), and the corrective action stand up when the average score in each process less than 4.5 or have the customer comment or complaint.

Results

Most of the service processes in four parts using 3 years evaluation have the average score more than 4.5, except in 2017, pre-pre analytical part including 1) public announcement and information documents, 2) registration steps, and 3) total time in these part were less than 4.5. The corrective action was generated by changed the operation steps from fixed 5 to 4 steps composed of fixed 1 step and flexible 3 steps. The score raised to 4.5 after implemented in next year. The service processes respond to "your health can be touch". The score was exhibited from 4.4 in 2017, and changed to 4.5 in 2019 after added the two year backwards results in each parameter and health status interpretation in the individual's book report.

Conclusions

MHM act as the effective proactive health care service model not only stimulate individual health-awareness and self-management but also incidental impact for reduce over-crowding problem in hospital.

doi: 10.1016/j.cca.2022.04.204

M107

Improving troponin T turnaround time in the emergency department

S. Sobki, M. Alseeni, A. Naitah, A. Aljohi

Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Background-aim

Troponin T is a protein which is released from Cardiac Muscle following myocardial damage and is therefore an indicator of Cardiac events such as myocardial infection. The availability of Troponin T results allows clinicians to triage patients effectively within an appropriate time period, the time taken to finish triaging, consultation and referral of each patient can affect the clinical management and patients outcome. Therefore, the prolonged Turnaround Time (TAT) for reporting laboratory results may have negative impact on; time to receive treatment, patients outcome, customer satisfaction, bed utilization time and cost. The National Academy of Clinical Biochemistry (NACB) turnaround time benchmark for STAT samples is (1) Hour. The aim of this study was to identify overall TAT (order to complete) for Troponin in Emergency Department (ED) patients and determine the length of time for components of overall TAT to ascertain opportunities for improvement.

Methods

Data were collected on all patients seen in ED with Troponin Laboratory Testing. A multi-disciplinary team was formulated from ED Laboratory, Nursing, Physicians, Portering and Quality Specialists. The team used FOCUS (Find, Organize, Clarify, Understand) PDCA (Plan, Do, Check, Act) tool as a problem solving technique to review the current situation. Process mapping was done which included infrastructure, manpower system, equipment and materials. Improvement opportunities were identified and the proper resolutions such as Training and Education, Separation of Tests, autoverification and increasing the numbers of STAT Centrifuges, using pneumatic tests system was implemented.

Results

Target of 90% of all reported Troponin results within (60) minutes was in place since January 2019. Causes for prolonged TAT were identified including inefficient sample delivery, lack of awareness of the importance of such results, improper tests group and not fully utilizing available technology. Actions coming from process mapping and FOCUS-PDCA tool were implemented. The results of reported Troponin T within (60) minutes following introduction of those solutions were 96% of all reported results.

Conclusions

Continuous monitoring of TAT of Troponin T within ED and ED Laboratory by implementing changes, adopting solutions from Quality tools with collaboration between ED Physicians, Nurses and ED Laboratory improved TAT of Troponin T which had positive impact on ED patients flow and safety.

doi: 10.1016/j.cca.2022.04.205

M108

Developing approaches to reagent lot-to-lot validation for an integrated health network of ortho-clinical vitros chemistry analyzers in suburban centers

M. To, B. Goudreau, J. Raizman, T. Higgins, A. Tsui

Alberta Precision Laboratories, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada

Background-aim

Reagent lot-to-lot comparisons are recommended by accreditation bodies to ensure that performance of each reagent lot meets acceptable standards, and patient results have not significantly shifted to impact clinical decisions. There have been guidelines to perform such comparisons, but practices are found to be highly variable between sites, ranging from a simple (quality control (QC) only) to a comprehensive (QC + patient sample) to a reflexed approach. This study aimed to determine an approach to standardize reagent lot-to-lot comparison practices between suburban laboratories within an integrated health network of Ortho-Clinical Diagnostic VITROS chemistry analyzers.

Methods

This study consists of retrospective and prospective analysis of reagent lots on VITROS 350 or 4600 analyzers for 20 chemistry analytes. Two years of retrospective reagent lot comparison data with QC and patient samples were obtained from one site. In the prospective study, QC and 10 patient pools were sent to 9 sites. One-way ANOVA and total allowable error were used to identify any statistically or clinically significant differences between reagent lots ($p < 0.05$).

Results

For sodium, retrospective analysis showed that 43% (3 out of 7) of QC comparisons between lots correlated with patient comparisons to correctly predict reagent lot pass or fail. Sodium also demonstrated the greatest reagent lot variation between reagent lots, with 25% (9 out of 36) of reagent lots failing patient sample comparisons in the prospective analysis. Other analytes such as glucose and creatinine were stable and did not demonstrate reagent lot variability.

Conclusions

We demonstrate that certain analytes (e.g. sodium) with a history of reagent lot differences would benefit from a comprehensive lot-to lot evaluation with QC and patient samples, whereas other analytes (e.g. glucose) with stable long-term performance would use a simple QC approach. A reflex algorithm using patient sample comparison can be applied when the simple QC approach exceeds tolerance limits. These approaches allow small laboratories to optimize limited resources for reagent lot validation without sacrificing quality of results.

doi: 10.1016/j.cca.2022.04.206

M109

Monitoring of the proportion of unacceptable specimen for laboratory quality improvement

S. Kim [a], H. Hong [a], J. Hong [b], H. Kim [b], W. Lee [b], S. Chun [b], W. Min [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

Unacceptable specimen refers to the specimen collected in incorrect container, insufficient specimen, clotted specimen in anticoagulant containers, grossly hemolysed sample, inadequately labeled sample, and so on. They cause delay of testing and increase of work load, requiring resampling, which causes inconvenience to patients. As a quality improvement activity, we tried to reduce the proportion of unacceptable specimen.

Methods

A specialized data analysis module implemented in the laboratory information system was used to collect data including corresponding unacceptability criteria and department where the tests were ordered. Using the result from the data, intern education program was revised, and targeted education including skill practice was conducted. Necessity of reducing unacceptable specimen and guide for blood collection were promoted using printed poster and intranet message board, especially for the department with high proportion of unacceptable specimen. Unacceptable specimen proportion was calculated monthly, the goal was set as below 0.55%, following the instruction from the performance improvement department.

Results

Annual average of unacceptable specimen proportion reduced consistently: 0.62% at 2014, 0.56% at 2015, 0.38% at 2016, 0.33% at 2017, and 0.29% at 2018. Between 2015 and 2016, performer of ward blood collection was changed from intern to nurse. In the corresponding period, unacceptable specimen proportion sharply decreased from 0.56% to 0.38%. Gradual decrease seems to be an outcome from the quality improvement activity.

Conclusions

Unacceptable specimen proportion decreased following the monitoring and targeted promotion and education. This could contribute to the reduced patient inconvenience and waste of laboratory resources including consumables and labor. Laboratory test result could be also delivered without delay caused by resampling.

doi: 10.1016/j.cca.2022.04.207

M110

Efficiency improvement and cost impact with auto verification in hematology laboratory in Thailand

P. Pilakongdechakorn, T. Thanomsin, S. Theangdung, L. Surapat, C. Sancharernsook, C. Santaweesuk, N. Teerawatanasuk

Laboratory Department, Sunpasitiprasong Hospital, Ubonrachathanee, Thailand

Background-aim

The Hematology laboratory testing is fundamental blood testing in laboratory that are under pressure to provide accurate and fast result reporting to physicians and patients. This pressure forced laboratories to ensure the accurate result by perform 100% slide review but the problem that followed are high cost from slide usage and not achieve guarantee Turn Around Time especially when the workload is increasing. The Auto verification via middleware was chosen to be a tool to solve the problems with expect outcome to reduce cost of slide and achieve Turn Around Time goal.

Methods

One-month data of Turn Around Time and percentage of slide review comparing between before and after implement Auto verification. The verification rules were designed in the middleware AlinIQ AMS (Analyzer Management System) followed the guidelines of the International Consensus Group for Hematology Review of International Society for laboratory hematology (ISLH) and additional validation criteria as specified by the Laboratory Professional.

Results

The comparison results are as follows,

- Average Total TAT reduces from 100 minutes to be 55 minutes or 45% reduction
- No change of the time spent in Pre-Analytic processes
- Average Analytic time reduces from 15 minutes to be 9 minutes or 40% reduction
- Average Post Analytic time reduces from 63 minutes to be 24 minutes or 62% reduction
- %Achieve TAT goal at 90 minutes increase from 57% to be 100% or 43% increasing
- Slide review reduces from100% to be 63% or 37% reduction

Conclusions

The auto verification abled to use to be a tool to solve the problem of high cost from slide usage and not achieve TAT goal that Hematology Laboratory facing, the results shown the significant reduction of % slide review and increasing of % Achieved TAT goal by 37 % and 43 % respectively.

doi: 10.1016/j.cca.2022.04.208

M111

Impact of unnecessary spare tubes received in a clinical chemistry laboratory

J. Cole, E. Samsodien, H. Vreede

National Health Laboratory Service, Cape Town, South Africa

Background-aim

Waste reduction is one of the core goals of lean management, which entails an all-encompassing quality improvement philosophy that is commonly applied in clinical laboratories. Waste of any kind results in higher costs, poorer quality and reduced value for customers. Disposal of hazardous and plastic waste bears higher costs than other waste materials. Medical laboratories are among the top contributors to hazardous waste, and hospitals that have an on-site laboratory generate hazardous waste at much higher rates than other hospitals. In our laboratory, which is attached to a teaching hospital in Cape Town, it was noted that many test requests are accompanied by additional filled serum separator tubes (SSTs). These spare SSTs are not used, but are refrigerated for 7 days, before being discarded and destroyed according to national hazardous waste policy. The lifecycle of these tubes produces waste of many types, including hazardous and plastic waste. Most of the costs are not quantifiable. We aimed to determine the direct financial impact of spare SSTs received as a proxy for other costs.

Methods

We performed a retrospective data extract from our laboratory information system to determine the number of spare SSTs received in our laboratory in one month. Through calculating the sample volume requirements for each request, we were able to estimate how many of these samples were never used. From that number, we determined the amount of unnecessary hazardous and plastic waste produced, and the direct financial cost of this.

Results

We found that 99% of spare tubes received were immediately refrigerated and never used. This equates to > EUR 200 per month wasted on buying and disposing of SSTs. It does not include labour costs, electricity costs, nor costs to the patient or the environment. The only time a spare tube was sometimes necessary was when a test would be referred to a different laboratory. Requested minimum volumes for referral are 500μL to 1mL of serum, which could likely be revised.

Conclusions

We shall send a memo to the heads of each clinical department listing the chemistry, virology and immunology tests offered in our laboratory and explaining that only one full SST is required if tests from this list are requested. We shall also provide a simple calculation to approximate the blood volume required in case of paediatric tube use. Re-audit will be performed to determine the impact of the notification and further intervention will be implemented as required. With continued improvements in automation, implications for other laboratory processes should be considered. Sample volume requirements may change, and with them sample collection systems and procedures could be revised to reduce waste.

doi: 10.1016/j.cca.2022.04.209

M112

Analytical performance specification for thyrotropin (TSH) by critical difference according to clinician survey

T.P. Loh

Department of Laboratory Medicine, National University Hospital, Singapore

Background-aim

Thyroid hormone disorder is one of the most common endocrine disorders affecting the general population. Central to the assessment of thyroid status is the thyroid function tests. They usually involve the laboratory measurement of thyrotropin (thyroid stimulating hormone, TSH). TSH is also used in monitoring of therapeutic effect and disease progression. It is important to set the analytical performance specification of TSH that supports the clinical utility.

Methods

In this study, we administered a survey questionnaire to clinician participants of an endocrine course conducted in Singapore to determine the minimum change (critical difference) in TSH under three different clinical scenarios. The results of the survey were then used to determine the analytical performance specification for these measurands. The analytical performance specification for precision (CVa) is calculated by CVa = [(CDˆ2/2zˆ2) - CViˆ2]ˆ0.5, where CD = critical difference, Z-value was set as 0.84 (for 80% probability) and 1.64 (for 90% probability) and CVi = within-person biological variation, 15.9% (published within-person biological variation as curated by European Federation of Clinical Chemistry and Laboratory Medicine).

Results

In total 66 clinician participants attempted the survey, who were made up of 39 (59%) specialists, 19 (29%) trainees and 5 (8%) general practitioners. The median expected change (critical difference) for the three clinical scenarios ranged 40%-93%. The analytical performance specification for precision is calculated as 29%-75% (at 80% probability) and 6.2%-37% (at 95% probability).

Conclusions

This survey provided insights on how analytical performance specification may according to the clinical scenarios where TSH is in use. At lower probability (Z-value), the analytical performance specification for precision is much wider than the desirable CVa of 7.8% calculated from within-person biological variation, and is comparable when a more stringent Z-value is used.

doi: 10.1016/j.cca.2022.04.210

M113

Hemolysis masked hypocalemia by albuterol sulfate (salbutamol)

L. Barbato [e], S. Pigozzo [c], M. Danielle Campelo [a], R. Barbazza [e], C. Bovo [d], A. Gandini [e], P. Marini [e], G. Lima-Oliveira [b]

[a] *Clinical Laboratory Bioanalise, Teresina, Piaui, Brazil*
[b] *Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy*
[c] *Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*
[d] *Medical Direction, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy*
[e] *Pharmacy, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy*

Background-aim

The aim of this report is to show that hemolysis due to inadequate phlebotomy procedure masked hypocalemia by albuterol sulfate (salbutamol).

Methods

Case report

A 10-year-old male with asthma attack was admitted in the emergency room. The patient was properly medicated with intravenous infusion of albuterol sulfate – 4 mcg/Kg/min –. Briefly, this drug increases cyclic AMP levels resulting in bronchial smooth muscle relaxation (bronchial dilatation), and inhibition of release of immediate hypersensitivity mediators from mast cells. Technical nurse was oriented to perform blood collection – complete blood count, electrolytes (sodium, potassium, cloro, calcium, and magnesium), glucose, and blood gas analysis – within half an hour after albuterol sulfate infusion. Samples where delivered to stat laboratory by pneumatic tube system with a note in the test-order "I had difficult to locate an appropriate venous access to perform the blood collection".

Results

Complete Blood Count: red blood cells 4.86x1012/L; haemoglobin 14.1g/L; hematocrit 40.5%; mean corpuscular volume 83.3fL; red blood cell distribution width 12.6%; white blood cells 6.12x109/L; neutrophils 3.24x109/L; lymphocytes 2.32x109/L; monocytes 0.43x109/L; eosinophils 0.13x109/L; platelets 18x109/L. Platelet clumps were observed in peripheral blood smear from blood sample collected with EDTA.

Blood gas analysis: results were not reported, laboratory inform that had possible analytical problem on their blood gas analyser.

Glucose: 4.77mmol/L (86mg/dL)

Electrolytes: laboratory did not report the results; sample hemolyzed +++.

The angry physician called the laboratory informing that the patient present sign and symptoms of hypocalemia. Therefore, the physician need the potassium laboratory result. However, the laboratory professional verbally (by phone) inform that analyses and report of result are not allowed, because sample was hemolyzed. 3.9 mmol/L of potassium was the unreported result on the hemolyzed sample. The new blood collection had shown a potassium of 2.1mmol/L.

Conclusions

The laboratory need to understand the physician needs, the patient situation, and the preanalytical variability before to decide to report or not the results. Therefore, it is time for the laboratory professional start a personalized service for both patients and physicians to guarantee the best service in the health institution.

doi: 10.1016/j.cca.2022.04.211

M114

Optimization of the thyroid panel for screening and diagnostic purposes

J.A. Delgado RodrÍguez, J.M. BauÇa, D. Morell GarcÍa, M.I. Pastor GarcÍa, D. Ramos Chavarino, M.A. Ballesteros Vizoso, P. Argente Del Castillo, A. Barcelo Bennasar

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

Measurement of serum thyrotropin (TSH) is currently the recommended test for the screening of thyroid dysfunction, especially in the outpatient setting, while serum free thyroxine (FT4) is kept as a reflex test (two-step strategy). In our laboratory, this strategy is followed in adult individuals (>15 years) from Primary Care (PC). Likewise, it includes a 'safety margin' for requests with a thyrotropin δ1.0 or ε4.0mIU/L (normal: 0.35–4.95mIU/L).

Our aim was to optimize the TSH cut-off values for the addition of FT4 as a reflex test, allowing us to detect ε95% of pathological FT4 results. Moreover, we retrospectively applied the new cut-off values from this study in order to analyze avoidable FT4 measurements and possible adverse clinical consequences.

Methods

Retrospective observational study performed in a tertiary care hospital between 01/01/2013-31/12/2018. All laboratory requests for adult PC patients that contained thyroid function tests for diagnostic purposes (TD protocol) were considered. All requests from patients with a previous diagnosis of thyroid disease or pregnant women were excluded.

Both TSH and FT4 are quantified on the Architect i2000 (Abbott Diagnostics, USA).

Different receiver operating characteristic (ROC) curves were performed for the TSH cut-off values and the percentages of FT4 tests avoided were compared.

Clinical consequences were assessed by reviewing the medical records.

Results

During the period assessed, a total of 554,529 TD protocols were included. 119,504 requests had automatically an FT4 test added, which accounted for 2.4% abnormal results for this test.

From the ROC curve that enables ε95% of abnormal FT4 results to be detected, the TSH values obtained were ε4.82 mIU/L and δ0.90 mIU/L. This TSH cut-off values would lead to a saving of 32.0% of annual FT4 measurements (27,696 euro/year) without adverse clinical consequences.

Conclusions

Setting optimized TSH cutoffs for reflex testing of FT4 would reduce the need for FT4 testing. Clinical laboratories need to offer not only true

results, but also become the cornerstone in the optimization of resources.

doi: 10.1016/j.cca.2022.04.212

M115

Request adequacy of cobalamin and folate in primary care

J.A. Delgado RodrÍguez, J.M. BauÇa, M.I. Pastor GarcÍa, M.A. Ballesteros Vizoso, P. Argente Del Castillo, A.M. GarcÍa Raja, A. Barcelo Bennasar

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

The prevalence of cobalamin and folate deficit ranges around 2.9%, reaching up to 35% among elderly people. This fact leads to an increase in the demand for both tests. However, the NICE does not recommend the repetition of cobalamin and folate in the follow-up of patients with replacement treatment, and suggests the complete blood count (CBC) instead. Others scientific societies recommend checking cobalamin levels every six months for patients treated with metformin or patients whose cobalamin deficiencies have been corrected.

In accordance with international guidelines, our laboratory established a measure of demand adequacy for primary care (PC). The laboratory information system (LIS) retains the determinations of cobalamin and/or folate with normal previous results within a six-month period. The decision on its processing is based on: diagnostic suspicion, CBC results, iron status panel. For the repetition of folate, previous cobalamin values are also considered.

Our aim was to evaluate the adequacy of the rule implemented, through the number of tests avoided.

Methods

Retrospective observational study performed in a tertiary care hospital between 12/04/2019-31/07/2019. Requests from PC including cobalamin and/or folate were considered. Requests with normal previous results for at least one of the two tests (B12: 138-652pmol/L; folate: 7.05-46.59nmol/L) within a six-monthperiod, were retained by the LIS.

The percentages of B12 and folate determinations retained and not performed were evaluated.

Results

This study included 2033 requests from PC containing only cobalamin, 1368 only folate and 12779 with both tests. The adequacy rule (retention) was activated for 1141 requests (7.1%). 791 requests showed normal prior results in less than six months for both tests (69.3%). Cobalamin was responsible for the retention of 199 requests (17.4%) and in 151 the cause was folate (13.3%).

Among all requests retained by the LIS, 480 cobalamin tests were rejected and not performed (48.5%), along with 453 folate tests (48.1%), in accordance with the abovementioned items.

Conclusions

Given the increasing demand of cobalamin and folate from PC, clinical laboratories need to offer not only true results, but also become the cornerstone in the optimization of resources.

doi: 10.1016/j.cca.2022.04.213

M116

Performance evaluation of Aptio automation system for calibration and quality control process

H. Hwang, H. Choi

Laboratory medicine, Kosin University Gospel Hospital, Busan, South Korea

Background-aim

The calibration and quality control process are the first step before we start testing patient samples in clinical laboratory. This calibration and quality control process occupies some portion of total consumed time for testing. If such manual process for the calibration and quality control can be automatically completed before technicians arrive for their work, and the testing process could be shortened. This not only makes test results reported faster but also improves the efficiency of the laboratory routine. As the Aptio automation system (Siemens, Germany), laboratory automation system can perform capping, recapping, storing, and testing the reagent and sample tubes, we designed the Aptio automation system to conduct the calibration and quality control processes before working time. We evaluated the performance of Aptio automation system when it performed the calibration and quality control process by comparing those processes conducted by conventional manual system.

Methods

We performed the calibration and quality control process in two ways. One was a conventional manual system and the other was automation system where all processes were conducted by the Aptio automation system. All calibrators and quality control materials needed for 5 days were prepared on the day before the first automated process began. Capping, recapping, and storing processes were done by automation system. When the calibration and quality control processes were completed, we tested patient samples for 5 consecutive days. We divided patient samples into two groups. Each group was composed of equally spaced 5 levels of tested items (ALT, cholesterol, creatinine, direct bilirubin and lipase). The first group (group 1) covered the analytical measurement range (AMR) and the second group (group 2) ranged around cutoff value of each tested item. Each sample was tested in triplicate. We compared the precision in each group and analyzed the statistical difference of test results in two groups between the manual and automation systems. We also analyzed whether the test results were significantly different as the day proceed from the first day to the fifth day. Statistical analysis were performed by analyze-it (Analyze-it Software Ltd., UK) and SPSS v.25 (IBM Corp, USA).

Results

As a whole, there were no significant differences between the two systems in group 1. The mean CVs of repeatability in group 1 were 2.1% and 2.0% in automation and manual systems, respectively. The within laboratory CVs in group 1 were 2.8% and 2.6% in automation and manual systems, respectively. In group 2, automated system show relatively low CVs in both repeatability and within laboratory CVs. The mean CVs of repeatability were 1.8% and 3.8% in automation and manual systems, respectively, and the within laboratory CVs were 3.4% and 4.2% in automation and manual systems. When we analyzed

the differences of test results between the two systems with paired t-test, there was statistically significant difference in group 1 ($p = 0.008$), while no significant difference was noticed in group 2 ($p = 0.413$). When we analyzed the differences of test results on each day, there was no remarkable tendency of increase or decrease between days.

Conclusions

We selected 5 test items of our concerns in the laboratory, which may make the conclusion of this study difficult to be generalized. However, the difference of performances between automation and manual systems was negligible. The Aptio automation system is thought to be a good tool for automated calibration and quality control processes in clinical laboratory.

doi: 10.1016/j.cca.2022.04.214

M117

Persistently increased cobalamin concentration due to macrocomplexes-B12 without supplementation: A case report

J.A. Delgado Rodríguez, J.M. BauÇa, M.I. Pastor García, M.A. Ballesteros Vizoso, P. Argente Del Castillo, A. Barcelo Bennasar

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

A 73-year-old woman showed persistently increased cobalamin concentrations during the last 2 years, without toxic habits. Physical and neurological examinations were normal. The last fasting biochemical control showed a normal complete blood count, and a normal general metabolic panel, including iron profile, liver panel, folate and glomerular filtration rate. Serum cobalamin was quantified using a chemiluminiscent microparticle immunoassay on the Architect i2000 platform (Abbott Diagnostics), yielding a result of >1476pmol/L (normal: 134–651pmol/L). No supplementation was reported.

Given the persistent elevation of serum cobalamin, tumor biomarkers, serologies and autoimmunity panels were requested. Moreover, the laboratory manager also decided to perform an interference study in order to rule out a spurious cobalamin result.

Results

Different pathologies have been associated with high levels of cobalamin, such as myeloproliferative syndromes, autoimmune lymphoproliferative syndrome and fibrolaminar hepatocellular carcinoma. Other pathologies with a likely association are cancer and metastasis of unknown origin, liver or kidney disease.

Tumor biomarkers, the tests for autoimmune diseases and the serologies were all negative, and her medical records did not reflect any alteration in liver tests or kidney function.

Conclusions

In order to rule out analytical errors, the test was repeated and quantified using an alternative methodology (enzimoimmunoanalysis, Elecsys, Roche), yielding the same result.

The blockage and removal of possible heterophilic antibodies led to the same cobalamin result. In addition, the negative result for the rheumatoid factor in our patient allowed ruling out such interference. Finally, all possible interfering antibodies or macrocomplexes were removed by precipitation with polyethylene glycol. 8 samples of patients with cobalamin supplementation were selected as a negative control. The mean recovery obtained in these controls was 80.8%. In our case, cobalamin recovery was <5%.

The cause of the hypervitaminemia was concluded to be the existence of macrocomplexes-B12, which may interfere in some commercially available cobalamin assays, resulting in falsely elevated cobalamin levels.

doi: 10.1016/j.cca.2022.04.215

M118

Contingency plan for potential vibration impact on a large-scale chemical pathology laboratory during hospital redevelopment construction period

H.K. Wong [b], C.Y. Law [b], C.W. Lam [a]

[a] *Department of Pathology, University of Hong Kong, Hong Kong*
[b] *Division of Chemical Pathology, Queen Mary Hospital, Hong Kong*

Background-aim

Established in 1937, Queen Mary Hospital (QMH) is a major acute hospital in Hong Kong Island. It provides a full range of acute and tertiary services, and specialist services to the public. It also serves as a tertiary and quaternary referral center for many complex and advanced services such as organ transplant, burns and reconstructive surgery for the entire territory. It is also the only designated liver transplant center in Hong Kong providing world-class liver transplant service. QMH Chemical Pathology Laboratory was established in 1982 to provide high quality and timely laboratory services through competent laboratory professionals and the application of advanced technologies in analyzing clinical samples. The laboratory needs to handle over 5000 patient samples daily. The demand for laboratory has increased substantially in recent years and the Division strives to cope with the surging workload through continuous re-engineering of the testing platforms and wider application of state-of-the-art technologies. However, the services development and technological advancement of QMH have been limited by the outdated design of its facilities, insufficient clinical space and unsatisfactory service zoning. Hence, the hospital is undergoing a board-scale redevelopment project to cope with the growing clinical service demands. Three buildings, the Clinical Pathology Building (CPB), University Pathology Building (UPB) and Housemen Quarters (HQ) besides Block K were planning to be demolished for the construction of new block to enhance hospital services. Chemical Pathology Laboratory was in fact located in lower ground floor of Block K, where in close proximity to the piling construction site. As evaluated by Vibration Consultant from Hong Kong Polytechnic University, the very short distance will substantially brings more severe vibrational impacts to the laboratory equipment. In addition, the construction-induced vibration are distributing to the equipment continuously therefore brings more uncertainty to the impact of the laboratory instruments.

Methods

With the anticipation of potential vibration impact on Chemical Pathology Laboratory, a Task Force on Mitigation Measures for Construction-induced Vibration was formed in Dec 2017 to stipulate new policy on protecting vibrational-sensitive laboratory equipment and develop the Contingency Plan to provide emergency response for any laboratory service interruption. After 1.5 years of planning and actual

execution, the final contingency plan was established by the task force and submitted to Hospital Authority Head Office (HAHO).

Results

The first tier of the plan was the protection of vibration-sensitive laboratory equipment proactively. All laboratory equipment were evaluated by Vibration Consultant to analyze the actual impact to the equipment, via the calculation of the estimated vibration levels from piling construction site to the actual equipment position, with reference to each equipment's vibration criterion curve. Table top and small size sensitive equipment were protected by installation of anti-vibrational plate/table. After installation and evaluation, it was confirmed that more than 90% of the vibration from the floor could be isolated by the plate/table. For those floor-type large equipment that could not use anti-vibrational plate/table, a 24-hour vibration monitoring system was installed in the laboratory to monitor for any abnormal vibration levels. The contingency plan outlined the structure of the management and implementation of response operations when abnormal levels were detected. For the second tier planning, a rapid response satellite laboratory was established in another location for contingency use. When the major analyzer for short turn-around-time testing services in Chemical Pathology Laboratory breakdown due to the continuous vibration impact, it will serves as a back-up lab and will cover the most timely and critical specimens (around 30%) of the laboratory's urgent services. For the rest of the 70% urgent specimens, we also gain kind and professional technical supports from two local Chemical Pathology Laboratories: Department of Pathology in Kwong Wah Hospital and Hong Kong Children Hospital to participate in the contingency plan too. With detailed design in operational logistics and wider application of laboratory informatics system, these urgent specimens will send to their laboratory directly for test analysis during the contingency plan activation. Comprehensive stress tests for the logistics and sample processing has been performed and passed too.

Conclusions

The main piling construction works for new block were commenced in October 2019 and will last for 18 months. The contingency plan will be activated anytime during these 18 months when major analyzers in Chemical Pathology breakdown.

doi: 10.1016/j.cca.2022.04.216

M119

Impact of Kaizen implementation on TAT in the Nairobi hospital laboratory (Kenya)

W. Ochieng, F. Ndungu, L. Kamau

The Nairobi Hospital, Kenya

Background-aim

Laboratory tests are very critical in patient management and a large contributor to clinical decisions by clinicians/doctors according to American Society for Clinical Laboratory Science (ASCLS) document on value of clinical laboratory services in healthcare. If the results are not delivered in a timely manner then consequences such as delayed treatment, irritated clientele, loss of customers and even loss of lives can occur. Shorter turnaround time for delivery of quality Laboratory results is thus a goal for all laboratories as they are key contributors to patient management. Complaints from clients on long TAT necessitated The Nairobi Hospital Laboratory to roll out kaizen achieve this noble target. Kaizen is a concept that means change for the better and aims at continuous improvement of processes to achieve desired goals. Biswajit Dey et al in their paper on Laboratory Turnaround Time state that Improving TAT is a continuous process and a wholesome approach is needed for reducing the obstacles for optimum TAT. The need to improve on the turn-around time necessitated the laboratory management to train staff and roll-out Kaizen in the laboratory.

Methods

Staff were initially trained on the Kaizen process and value stream mapping processes. The staff identified the current processes, mapped out the value adding and non-value adding components in the Laboratory process from the time a client arrives in the lab to the time they get results. Kaizen was rolled out with the sole objective of reduction of TAT for CRP, Tbc and Urinalysis as indicators for other laboratory tests. The TAT was tracked from patient arrival to issue of results. This was recorded and tracked using Labware LIMS (Laboratory Information Management System). Target TAT was set for each test post roll-out. Data was then collected on the current TAT before Kaizen for three selected tests which are routinely requested by practitioners in the three major sections of the lab i.e. Heamatology, Chemistry and Microbiology. This was data for one month. 2 months after roll-out, data was then collected again for the three tests for the 3rd month and analyzed. The data was for samples for out-patients only.

Results

The results obtained were as follows:
Test TAT before Kaizen Target
CRP 34% within 60 minutes 70% within 60 minutes
Tbc 48% within 45 minutes 90% within 45 minutes
Urinalysis 52% within 60 minutes 80% within 60 minutes
TAT after Kaizen
CRP 56% within 60 minutes
Tbc 60% within 45 minutes
Urinalysis 79% within 60 minutes.

Conclusions

The training before roll-out helped in change management and buy-in by staff. It is evident that Kaizen helped in reducing the TAT for patients. Customer complaints (both patients and practitioners) on waiting for too long for laboratory results also reduced significantly. This eventually leads to effective management of patients by practitioners. Success in this kaizen project is illustrated by the percentage improvement in TAT towards the Target TAT.

doi: 10.1016/j.cca.2022.04.217

M120

AN Unusual case of haemolysis in the clinical biochemistry laboratory: A case report and literature review

C.H. Yong, S.Y. Tan, Z.X. Yang, S. Saw, S. Sethi

National University Health System, Singapore

Background-aim

Haemolysis is a commonly encountered phenomenon in the laboratory, usually due to poor phlebotomy techniques or inadvertent trauma to the red blood cells during transport.

The result is a visible red tinged serum following centrifugation, a result of lysis of the red blood cell membranes. Clinical chemistry laboratories commonly utilize haemolysis index on automated analyzers to detect this, and rejection of the sample may be required depending on the test in order to reduce possible interference. Herewith, we present an unusual case of persistent haemolysis despite repeated blood draws.

Methods

A 74 year old Chinese gentleman with a history of diabetes, hyperlipidemia and haemodialysis presented with acute abdominal pain. Haemoglobin was 11.6g/dL, platelets were 226 x 10^9/L and total white cell count was 21.04 x 10^9/L.

His physician had ordered multiple serum samples for liver panel, renal panel and amylase but these were persistently haemolysed (x 4) with a rusty color being noted after centrifugation of the sample.

Results

Interestingly, his potassium taken on whole blood and measured on the blood gas analyser was 4.2 mmol/L. This led us to suspect potential in-vivo haemolysis contributing to the persistent haemolysis.

True enough, his lactate dehydrogenase was several times the upper reference limit at 7170 U/L and haptoglobin was reduced as well. Peripheral blood film was otherwise unremarkable. The results were released to his physician upon request with an interpretive comment.

Conclusions

This case illustrates the unusual cause of in-vivo haemolysis in the laboratory. Laboratory personnel must be cognizant to this phenomenon and withholding chemistry results in such scenarios is not advised.

doi: 10.1016/j.cca.2022.04.218

M121

The impact assessment of valued based laboratory medicine and recent practices in Nigeria

A.S. Mahmud

Lagos University Teaching Hospital (LUTH), Nigeria

Background-aim

Recently, the advent of contagious and infectious diseases are predominant in West Africa such as the consistence prevalence of Lassa fever which is known to take its victim during laboratory diagnosis and laboratory testing. Public administrators, clinicians and policy makers fails to look at what possible outcome a specific action could have and what methods could be used to minimize, prevent or manage any negative outcomes of such actions in the prevention and treatment of infectious diseases that could affect the population. The introduction of modern clinical methods, combination of procedures, and tools used to promote and evaluate effective health care delivery are being compromised resulting to low quality services and clinical hazards. Value Based Laboratory Medicine (VBLM) implementation is a serious challenge for most public and private hospitals and healthcare providers in West Africa making it difficult for their organization to adapt into a patient-centered clinical pathway based on both classical outcomes and innovative patient-evaluation.

Methods

A cross-sectional survey was administered followed by an interview and discussion with laboratory stakeholders about a proposed impact assessment that included all the six procedural elements of laboratory assessment. An online questionnaire was sent via email to different public hospitals and health care centres in Nigeria.

Results

A total of 98 out of the 180 (54.4%) laboratory health workers responded to the emailed survey administered. Among the survey respondents, 24 out of the 98 (24.5%) participated in the structured interview and the discussion and formed the community of practice group that provided insights about the laboratory practices and procedures used in their centres. Among stakeholders, 76.2% practiced in accredited laboratories. Results of interviews and discussions revealed suggestions about continuous ongoing assessment, such as the inclusion of laboratory quality management and safety as separate items to be unified for all sections.

Conclusions

It could be concluded that policy makers and health care providers must understand the need to provide accurate diagnosis which is essential to the prevention and treatment of disease in Nigeria, and, although the paradigm applied to this region must of necessity be different, it cannot, however, embrace a practice of medicine that routinely involves presumptive diagnosis based on clinical syndrome.

doi: 10.1016/j.cca.2022.04.219

M122

Sample rejection of mixed body fluids for CSF identification – The utility of fluid protein concentration

J. Rusch, R. Dalmacio

University of Cape Town, South Africa

Background-aim

In patients with potential cerebrospinal fluid (CSF) leaks, traditional chemical analyses such as glucose, protein and specific gravity are unreliable. The gold standard investigation is fluid electrophoresis to determine the presence of beta-2 transferrin. However, there is a paucity of information in the literature with regards to the analysis and diagnostic performance of undetermined mixed body fluid samples. Mixed samples with total protein concentrations that are much higher than CSF values (0.15 to 0.45 g/L) require large dilutions to decrease the protein concentration to approximately 0.5 g/L stipulated by the assay (Sebia Hydragel 3 CSF). The risk is that beta-2 transferrin may be diluted below the limit of detection of the assay and lead to a false negative result with significant clinical implications as patients with CSF leaks are treated medically with antibiotic prophylaxis, while some require surgery. Moreover, rejecting all fluid samples with evidence of contamination with other body fluids is likely to put patients and clinicians at risk, when beta-2 transferrin could have been confidently identified. The aim of this study was to assess the utility of a cut-off value for fluid total protein concentration in determining whether a mixed fluid sample should be rejected for beta-2 transferrin analysis.

Methods

Retrospective data were reviewed for fluid beta-2 transferrin analyses performed over a three-year period, together with the corresponding total protein result. Diagnostic performance was assessed and receiver operating characteristic (ROC) curve analysis was completed using STATA (StataCorp, USA). A set of dilution experiments was performed to determine the dilution value at which beta-2 transferrin was no longer visible.

Results

194 test results were assessed of which 101 were positive and 93 were negative for beta-2 transferrin. The median (interquartile range) of the total protein concentration was 3.53 g/L (0.928-35.9). Using a TP cut-off of 16.8 g/L and the beta-2 transferrin result as the comparator, ROC curve analysis demonstrated a specificity of 100% and a sensitivity of 73% (AUC 0.88; 95%CI 0.82-0.93). Dilution experiments illustrated that at a 1 in 20 dilution beta-2 transferrin could no longer be identified (approximate concentration 0.25 mg/L). This dilution factor would be required for a sample with an initial total protein concentration of approximately 10 g/L.

Conclusions

There are significant challenges and risks to identifying CSF in mixed body fluid samples with high total protein concentrations requiring big dilutions. This study demonstrated that rejecting all samples with evidence of contamination with other body fluids is unnecessary as a significant number of patients can be confidently identified with total protein values below the cut-off value for total protein of 10 g/L. Above the conservative cut-off of 16.8 g/L, our data show that the test will be negative, irrespective of the presence of beta-2 in the original sample. Values between 10 and 16.8 g/L may be positive, negative or equivocal, with an increased risk of false negative results. As a result of this study our laboratory now uses a cut-off of 16.8 g/L for rejecting samples for beta-2 analysis. Samples with concentrations between 10 and 16.8 g/L are analysed and negative results discussed with clinicians.

doi: 10.1016/j.cca.2022.04.220

M123

Minimum consensus specifications for vitamin B12: From theory to practice

M. Ballesteros Vizoso, P. Argente Del Castillo Rodríguez, B. García García, J. Delgado Rodríguez, B. Badal Cogul, J.M. Bauça, M. Pastor García, A. Barceló Bennassar, G. Perez Esteban

Department of laboratry medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

It is recommended for laboratories to establish quality specifications for biomarkers. In 2017,different laboratory medicine societies in our country(AEBM,AEFA,SEQCML,SEHH) published minimum consensus quality specification(EMC) recommendations that laboratories should comply with.The aim is to obtain a new control procedure for vitaminB12 based on the new EMC of 14%.

Methods

The determination of B12 was performed by chemiluminescence on an Architect i2000(Abbott Diagnostics,US)autoanalyzer.Level1 of internal quality control material Bio-Rad's Lyphochek Immunoassay Plus lot 40350 was used.The tool used to obtain the control procedure was the Westgard Advisor of the Unity-Real-Time.

The desirable quality specification based on the biological variability for total error(ETa) established so far had been 30%.The new quality control procedure based on the new EMC recommendation(14%)was calculated in two different ways:According to the cumulative data of Immunoassay Plus from April-2019 to May-2020,13 different reagent lots have been used during this time,and using only the reagent lot that had a higher sigma during this period.

Results

Between April/2019-May/2020 in our laboratory we implemented a procedure control where we used the 1-3s|2-2s control rules and 2 control per series(N).This procedure was based on:

Eta of 30.00%
Systematic error = 3.00%
coefficient of variation = 5.44%
Sigma = 4.96
probability of detecting the error(Ped) = 94.30%
probability of false rejection(Pfr) = 0.60%

By setting the ETa at 14% the recommended control procedure would be 1-3s|2/3-2s|R-4s|3-1s|8-X(N = 6) 13lots with Ped = 14.8%, Pfr = 9.5% and 1-3s|2/3-2s|R-4s|4-1s(N = 2) with Ped = 95.50%, Pfr = 1.20% using the best sigma lot.

Conclusions

The establishment of this new procedure based on the new recommendations would require a multi-rule procedure, 6 control materials per series,which requires a great effort(numerous calibrations, control repetitions, technical working time, reagent lot maintenance) to obtain a totally insufficient Ped and a high Pfr,while no clinical impact of the use of the current quality specifications.

Our results show that not only is it necessary for the total error to be lower than the fixed specification,but that it must be in such a magnitude that the error of the results is detectable with a high probability.

doi: 10.1016/j.cca.2022.04.221

M124

Clinical thresholds for pseudohyperkalemia and pseudonormokalemia in patients with thrombocytosis

J.A. Delgado RodrÍguez [b], D. Morell GarcÍa [b], B. LÓpez Andrade [a], M.A. Ballesteros Vizoso [b], J.M. BauÇa [b]

[a] *Department of Haematology and Hemotherapy, Hospital Universitari Son Espases, Palma de Mallorca, Spain*
[b] *Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain*

Background-aim

Lysis of platelets during in vitro coagulation leads to a false increase in potassium concentration. We aimed to establish the cut-off value for

platelet count interfering potassium and to estimate the percentage of cases of pseudohyperkalemia and pseudonormokalemia in our hospital.

Methods

This was a retrospective observational study. Subjects were divided into two groups: on a branch, individuals with essential thrombocytosis who were appointed for a control blood examination [complete blood count and basic metabolic panel (potassium concentration in serum)]; and in the other branch, individuals with thrombocytosis and high C-reactive protein (reactive thrombocytosis) for which essential thrombocytosis had been excluded (potassium concentration in lithium heparin plasma). The cut-off value for the interference of platelet count on potassium results was calculated using the reference change value. Sensitivity and specificity were calculated using a ROC-curve, and the size of the effect by the Cohen's d. The clinical impact of both phenomena was assessed by reviewing the medical records of individuals reclassified as such, and also looking for potential cases in 2019 on the laboratory information system.

Results

Fifty-four individuals with essential thrombocytosis were included along with 156 individuals with reactive thrombocytosis. Potassium concentration correlated with platelet count (P-value <0.001; Spearman's $>=0.394$) in serum but not in plasma. The cut-off value of platelet count interfering potassium was $578 \cdot 103/|L$ [CI 95%: 513 – 642 · 103/|L], with an associated sensitivity and specificity of 0.67 [CI 95%: 0.52 – 0.80] and 0.58 [CI 95%: 0.42 – 0.72] respectively. The medical records of patients reclassified as pseudohyperkalemia or pseudonormokalemia did not include any medical action for the modification of potassium levels. In 2019, up to 0.14% of the total serum potassium determinations were susceptible to be pseudohyperkalemia or pseudonormokalemia.

Conclusions

This study provides an optimized cut-off value for platelet count, and brings to light not only pseudohyperkalemia-related issues, but also the pseudonormokalemia phenomenon, which usually go unnoticed.

doi: 10.1016/j.cca.2022.04.222

M125

Simulation models to predict biochemistry test capacity in Covid-19 pandemic surge capacity planning in tertiary care centres

B. Kyle [b], A. Bajkov [a], D. Haugrud [b], F. Wu [b], A. Lyon [b], M. Lyon [b]

[a] *Roche Diagnostics, Canada*
[b] *Saskatchewan Health Authority, Canada*

Background-aim

The COVID-19 pandemic has re-emphasized the need for the timely delivery of clinical laboratory results to support optimal patient care. The objective of this study was to determine if current instrumentation in Saskatoon hospital chemistry laboratories could accommodate the anticipated COVID workload in addition to non-COVID testing for the existing acute care hospitals and proposed field hospitals.

Methods

A simulation model was utilized to assess workload and turn-around-time (TAT) capacity for pre-analytic, total analytic, chemistry, ion-selective-electrode and immunoassay testing to accommodate an expanded COVID workload. Anticipated COVID patient numbers and a COVID specific test menu were incrementally introduced into a 24 hour pre-COVID testing workload. The impact of field hospital location, courier schedule and daily instrument maintenance schedule were also considered when calculating a TAT from specimen collection to result reporting.

Results

Instrumentation throughput, scheduled times for instrument daily maintenance and the time of day when the specimen surge is received in the laboratory were found to be significant predictors of laboratory's ability to accommodate anticipated COVID workload. Courier schedule and proximity of the field hospital to the laboratory significantly influenced the TAT for field hospital testing.

Conclusions

A simulation model is a helpful tool to provide useful information for optimal delivery of multi-site clinical laboratory services during the COVID-19 pandemic.

doi: 10.1016/j.cca.2022.04.223

M126

Effective utilization management strategies to limit inappropriate referred-out test requests

A. Lou [a], A. Thoni [a], B. Nassar [a], J. Craig [b], S. Hynes [b], E. Santilli [b], M. Elnenaei [a]

[a] *Dalhousie University, Canada*
[b] *Nova Scotia Health, Canada*

Background-aim

Specialized testing performed in reference laboratories is relatively low in volume, but costly. Sizable efforts have been made in our institution since Oct. 2016 to ensure their appropriateness. This study investigates the impact of standardized approval criteria for each of these tests, and sending automated messages requesting clinical details from ordering physicians before sample processing.

Methods

Prior to October 2016, the approval process involved sending an automated message indicating the need for a written request with clinical information within 30 days; but was limited in both scope and number of tests. Commencing October 2016, 84 tests were targeted for the same process using more stringent approval criteria. Responses were then reviewed by assigned medical lab staff. Based on the clinical relevance, some tests were preapproved for certain specialists. Numbers of each test requested and completed during 2015 to 2018 were collected to calculate annual cost savings.

Results

In 2015, prior to the new process, 973/5028 (19.4%) RO tests were canceled due to no response within 30 days. Total test cancelation rates increased to 25.1%, 34.4% and 38.5% despite the annual total request numbers increasing to 5067, 5143 and 5778 in 2016, 2017 and 2018 respectively. The total annual cost saving increased from $61,454 in 2015 to $132,120 in 2018. In 2018, the test in greatest demand was fecal calprotectin (FC) which increased to 553 from only 17 in 2015; 23% of those requests were canceled, accounting for the highest number of a canceled test. Request numbers for some tests also decreased in 2018 compared to pre-vetting in 2015; mostly zinc from 375 to 195, urine 5HIAA from 259 to 130 and IgG subclasses from 428 to 306. Moreover, cancelation rates increased in 2018 compared to 2015 from 14.7% to 47.2%, 13.1% to 16.2% and 13.1% to 30.1% for Zinc, 5HIAA and IgG subclasses, respectively.

Conclusions

Automated messaging with LIS assisted rules is an effective utilization strategy for costly tests suspected of being redundant or unjustified. Establishment of further communication with ordering physicians by providing evidence- based approval criteria for each case is necessary for better patient care and cost saving.

doi: 10.1016/j.cca.2022.04.224

Clinica Chimica Acta 530 (2022) S260–S264

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Laboratory Statistics

M127

Internal quality control procedure for urine test strip analyzer: Multi-rule based on sigma metric

H. Park, Y. Ko

Department of Laboratory Medicine, Bucheon St. Mary's Hospital, College of Medecine, The Catholic University of Korea, Seoul, Republic of Korea

Background-aim

Usually, for quality control of urine test strip analyzer, control materials were measured once in a run and it was checked if the test results were not more than one grade away from the expected value. A quantitative result, delta-E (⊗E), from the controls could be used for estimation of precision and for designing multi-rule based on the sigma metric. The aim of this study was to determine whether the current method of a single measurement of controls was appropriate and if not, to find an appropriate way.

Methods

Between Nov 2018 and Oct 2019. URiSCAN Super Plus (YD Diagnostics, Yongin-si, Korea) was used as an automated urine analyzer with URiSCAN strip (YD Diagnostics) and URiTROL™ Liquid Urinalysis Control (YD Diagnostics). Range table of ⊗E for each grade of urine test strip reading was obtained from the manufacturer. Difference of the midpoint ⊗E between two neighboring grades was regarded as total allowable error (TEa) for the grade. Mean, SD, and CV were calculated for each lot of control. Sigma-metric was calculated as TEa/CV assuming that bias was zero. The sigma-metric based QC rule was determined using Westgard EZ Rules 3 software (Westgard QC, Madison, WI, USA).

Results

Four lots of level 2 control for blood, ketone, glucose, leukocyte, and pH, and 4 lots of level 3 control for bilirubin and protein were used for data analysis. Sigma-metrics were as follows: 3.59–15.30 for blood; 3.91–6.30 for ketone; 4.46–9.83 for glucose; 3.02–6.53 for leukocyte; 1.79–2.15 for pH from level 2; 3.62–7.63 for bilirubin; 5.15–6.42 for protein from level 3. Corresponding QC rules (number of control measurements) were as follows: $1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_x$ (4)–$1_{3.5s}$ (2) for blood; $1_{3s}/2_{2s}/R_{4s}/4_{1s}$ (4)–$1_{3.5s}$ (2) for ketone; $1_{2.5s}$ (4)–$1_{3.5s}$ (2) for glucose; $1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_x$ (4)–$1_{3.5s}$ (2) for leukocyte; $1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_x$ (4)–$1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_x$ (4) for pH from level 2; $1_{3s}/2of3_{2s}/R_{4s}/3_{1s}$ (6)–$1_{3.5s}$ (3) for bilirubin; 1_{3s} (3)–$1_{3.5s}$ (3) for protein from level 3.

Conclusions

For blood, ketone, glucose, leukocyte, bilirubin, and protein, a single measurement of each level of control was appropriate in the lots showing the best precision among the four. Corresponding QC rules for the 6 test items were $1_{2.5s}$ (2)–$1_{3.5s}$ (2 or 3). Meanwhile, for pH, $1_{3s}/2_{2s}/R_{4s}/4_{1s}/8_x$ (4) with duplicate measurement was necessary.

doi: 10.1016/j.cca.2022.04.226

M128

Use of six-sigma for quality control for biochemistry parameters

A. Mishra, B.K. Yadav, U. Budhathoki, R. Pokhrel, V.K. Sharma, E.T. Tuladhar, A. Bhattarai, M. Raut

Department of Biochemistry, Tribhuvan University Teaching Hospital, Nepal

Background-aim

Background: The clinical laboratory must provide the accurate test results to patient on which decisions of the physicians rely. Hence, the clinical laboratory must undergo performance testing regularly. Six-sigma is currently the method of choice for performance testing of laboratories.

Aim: To evaluate the Six-sigma value for different biochemistry Parameters.

Methods

Materials and methods: Using BT 1500 and BT 3500 biochemistry analyzers 12 clinical analytes were measured for a period of three months. Internal Quality Control (IQC) performed routinely for both levels were noted from both analyzers and used for calculation of coefficient of variation (CV%). Bias was estimated based on the difference of the average obtained for each analyte from the target values provided. Values for Total Allowable Errors (TEa), were taken from Clinical Laboratories Improvement Act guidelines for various clinical analytes. Sigma values were calculated using- CV%, percentage bias and TEa.

Results

Results: Sigma value >6 were found for AST and ALT which shows less strict QC rules are needed to be followed for high error detection

doi:10.1016/j.cca.2022.04.226

and low false rejection. Sigma-values between 3 and 6 were found for uric acid for both levels of control and AST for L1 in both analyzers. Less than 3 sigma values were obtained for parameters- Urea, Creatinine, Albumin, Triglyceride, Total Cholesterol, Alkaline Phosphatase, Magnesium for both level of controls in both the analyzers indicating the need towards the improvement in these methods.

Conclusions

Conclusion: Incorporation of Six-sigma rule would be useful for evaluation of performance testing of clinical laboratories.

doi: 10.1016/j.cca.2022.04.227

M129

Trains and veins: Research of the data of fasting glucose level in the annual medical examination of the October railway employees in 2019, Saint-Petersburg

O. Zakharenkova , A. Shkouro

Private Health Institution "Clinical hospital "Russian Railways - Medicine" Saint-Petersburg, Russia

Background-aim

The structure of the organization's preventive care process in our company leads by a strong algorithm of immediately received laboratory reports and subsequent high speed of therapists' decision-making. Basically, the laboratory part of the medical examination of railway employees includes measure levels of glucose, cholesterol, hematology analysis, routine urine tests, and urine drug testing.

We made a decision to review statistic data in our biochemistry laboratory department based on the fasting glucose level.

Methods

We made a decision to review statistic data in our biochemistry laboratory department based on the fasting glucose level.

On the basis of the private health institution "Clinical hospital" Russian Railways - Medicine " in St. Petersburg, in 2019 a routine preventive examination of fasting glucose 24,961 employees of the October Railway was conducted. We've used analyzer Beckman Coulter AU-680 and measured serum fasting glucose level.

Results

The following data were collected. The percentage of employees going through a routine examination in the category of under 25 years old was 15.7%, 25-40 years old - 37.2%, 40-60 years old - 44.3%, and more than 60 years old - 6.6%.

As it is had expected the percentage of men, who works in the Russian Railways, higher, than woman's, and maintain the level 70% and 30% respectively.

The proportion of patients with an increased glucose value among all going through a routine examination in 2019 was 6.2%.

Data review helps us to immersed in the diagnostic process more precisely.

Glucose level between 5.9-7.0 mmol/l was found at 4.6% of all employee's numbers, and the percentage of glucose level higher, than 11 mmol/ l included 0.2 % of railway staff.

In case of deviations from the reference interval for glucose, the patient must perform an additional examination, which includes endocrinologist examination and performing laboratory tests such as HbA1C, TSH and glucose tolerance test.

Additionally, we found a few cases (0.5 %) collecting samples for laboratory tests from non-fasting patients.

In addition, the age group from 40 to 60 years turned out to be the largest in the number of cases of increased blood glucose levels. This group makes 72% of all patients with elevated fasting glucose levels. Primary, this group includes 80% of patients with decompensated diabetes, 19 % with impaired glucose tolerance and only 1% with firstly diagnosed diabetes.

Over the 2019 year we find 14 cases (0,06 % of all examined samples) of dangerous glycemic level, higher than 15 mmol/l. These patients were immediately transferred to the hospital after receiving the result from the laboratory department.

Conclusions

In conclusion of our research, intense supervision not only guarantees early diagnostic, following treatment of diabetes and the prevention of complications but also leads to the advanced quality assistance and to an outstanding level of healthcare for employees and safety passengers' of the company.

doi: 10.1016/j.cca.2022.04.228

M130

Validation of an approach using only big data from clinical laboratories to establish reference intervals for common biochemical analytes

L. Hou, C. Ma , L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, China

Background-aim

To evaluate an approach using only big data from clinical laboratories to establish reference intervals (RIs) for common biochemical analytes.

Methods

Reference individuals were screened through variables such as blood pressure, Body Mass Index (BMI), and subjects' electronic health records, and RIs1 were established. Based on statistical distribution judgment and outlier recognition method, RIs2 were established using only big data from clinical laboratory. The consistency of RIs1 and RIs2 was compared synthetically by the method of comparative confidence interval (CI) and the method of calculating decision consistency.

Results

RIs1 and RIs2 of common biochemical analytes have a high degree of range coincidence. Among them, Alb, Cr for females, Na and Cl obtained exactly the same RI based on the two data sets. The width of CI for the upper and lower limits of RIs1 and RIs2 was less than 0.2 times of the corresponding RIs width. For all common biochemical analytes, the decisions consistency rate of RIs1 and RIs2 was greater than 95%.

Conclusions

It was possible to establish RIs for most common biochemical analytes using only patient data from clinical laboratories after adopting appropriate statistical methods.

doi: 10.1016/j.cca.2022.04.229

M131

Assessing workflow efficiency with the implementation of a total laboratory automation system

G. Ramaloo [b], S. Sivaneson [b], N.A. Mokhsin [b], K.M. Choo [b], M. Giddy [b], N. Nheu [b], T. Abdul Rahman [a]

[a] *Faculty of Medicine, Universiti Teknologi MARA & Integrative Pharmacogenomics Institute, Universiti Teknologi MARA, Malaysia*
[b] *Gribbles Pathology, Malaysia*

Background-aim

With increasing workload, introducing a lean system into a medical laboratory is key to ensuring efficient workflow. Our laboratory saw a significant incline in workload following the merge of several small labs into one. In order to improve workflow efficiency to accommodate the growing number of samples, the lab established a total laboratory automation (TLA) system that was more integrated than the previous system.

Methods

A new TLA system was implemented in December 2020 and the turnaround time (TAT), defined as the time from data entry of samples to result validation, was documented to assess the lab's key performance index (KPI) following the implementation of the new TLA system (Roche Diagnostics, Germany). A TAT greater than 95% was considered to achieve the KPI of routine biochemistry testing of <24 hours. In addition, the testing capacity of TLA, TLA space utilization, space capacity for reagent storage, and sample archiving as well as sample flow (defined as the number of manual steps involved) were captured as well.

Results

There was a significant improvement in TAT KPI from 83.6% to 97.2% (14% increment) with an average time taken to achieve a 95% TAT of 416 minutes on the previous TLA and 188 minutes with the newly established TLA (55% reduction in time). Furthermore, testing capacity on the new TLA showed a 16% increment compared to the previous TLA. The new TLA showed a 23% reduction in space utilization and had a 45% and 88% increment in reagent storage and sample archiving storage capacity respectively compared with the previous TLA. There was also a 44% reduction in sample flow (32 steps vs 18 steps).

Conclusions

Findings from this study highlights the relevance of selecting an appropriate TLA to achieve optimum lean lab. The newly implemented TLA system served to accommodate our laboratory with its increasing number of samples and wide range of testing menu.

doi: 10.1016/j.cca.2022.04.230

M132

Comparison between polynomial regression and weighted least squares regression analysis for verification of linearity of quantitative measurements

T. Jeong [c], S.K. Kim [a], S. Kim [d], C. Lim [a], J. Chung [b]

[a] *Department of Biostatistics, School of Medicine, Dongguk University, Goyang, Republic of Korea*
[b] *Department of Laboratory Medicine, Dongguk University Ilsan Hospital, Goyang, Republic of Korea*
[c] *Department of Laboratory Medicine, Ewha Womans University College of Medicine, Seoul, Republic of Korea*
[d] *Department of Laboratory Medicine, Ilsan Paik Hospital, Inje University College of Medicine, Goyang, Republic of Korea*

Background-aim

Periodic verification of the analytical measurement interval (AMI) of the quantitative test is very important in clinical laboratory. Recently, the linearity evaluation protocol by Clinical & Laboratory Standards Institute (CLSI) has been revised from EP6-A to EP6-ED2 and the statistical method of interpreting the linearity evaluation data has been changed from polynomial regression to weighted least squares linear regression (WLS). We compared and analyzed the AMI verification results according to the linearity evaluation guidelines.

Methods

The evaluation of AMI of clinical chemistry tests was performed by using five samples with two replicates in three different laboratories. After analyzing the same evaluation data in each laboratory by polynomial regression analysis and WLS methods, it was compared with each other whether linearity was verified across the five sample concentrations. The cases where the 90% confidence interval of deviation from linearity within the allowable deviation from linearity (ADL) in the WLS were additionally compared.

Results

The linearity of 42.3% to 56.8% of the chemistry items was verified by polynomial regression analysis in three laboratories. When the same data was analyzed by WLS, the linearity of 63.5% to 78.3% of the test items was verified where the deviation from linearity of all five samples was within ADL criteria, and the cases where the 90% confidence interval of all deviation from linearity overlaps the ADL was 78.8% to 91.3%.

Conclusions

Interpreting the AMI verification data by the WLS method according to the newly revised CLSI document EP6-ED2 will be helpful in laboratory practice.

doi: 10.1016/j.cca.2022.04.231

M133

An approach for determining allowable between reagent lot variation

M. Van Schrojenstein Lantman [m], H. Can Çubukçu [a], G. Boursier [i], M. Panteghini [e], F.A. Bernabeu-Andreu [o], N. Milinkovic [h], P. Mesko Brguljan [g], S. Linko [k], D. Brugnon [c], R. O'Kelly [b], C. Kroupis [f], M. Lohmander [l], L. Šprongl [d], F. Vanstapel [j], M. Thelen [n]

[a] *Ankara University Stem Cell Institute, Interdisciplinary Stem Cells and Regenerative Medicine, Ankara, Turkey*
[b] *Association of Clinical Biochemists in Ireland, Ireland*
[c] *Clinical Chemistry Laboratory, Spedali Civili, Brescia, Italy*
[d] *Clinical Laboratory, Hospital Kladno, Kladno, Czech Republic*
[e] *Department of Biomedical and Clinical Sciences "Luigi Sacco", and Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milano, Italy*
[f] *Department of Clinical Biochemistry, Attikon University General Hospital, Medical School, National and Kapodistrian University of Athens, Haidari, Greece*
[g] *Department of Clinical Chemistry, University Clinic for Respiratory and Allergic Diseases, Golnik, Slovenia*
[h] *Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia*
[i] *Dept of Genetics, Rare Diseases and Personalized Medicine Rare Diseases and Autoinflammatory Unit, CHU Montpellier, Univ Montpellier, Montpellier, France*
[j] *Laboratory Medicine, University Hospital Leuven, Belgium and Department of Public Health, Biomedical Sciences Group, KU Leuven, Leuven, Belgium*
[k] *Linko Q-Solutions, FI-00950 Helsinki, Finland*
[l] *Regional Laboratoriemedicin, Sahlgrenska Universitetssjukhuset, Trollhättan/Göteborg, Sweden*
[m] *Result Laboratory for Clinical Chemistry, Amphia Hospital Breda, the Netherlands*
[n] *Result Laboratory for Clinical Chemistry, Amphia Hospital Breda, the Netherlands,*
[o] *Servicio Bioquímica - Análisis Clínicos, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain*

Background-aim

Clinicians trust medical laboratories to provide reliable results on which they rely for clinical decisions. Laboratories fulfil their responsibility for accurate and consistent results by utilizing an arsenal of approaches, ranging from validation and verification experiments to daily quality control procedures. All these procedures verify, on different moments, that the results of a certain examination procedure have analytical performance characteristics (APC) that meet analytical performance specifications (APS) set for a particular intended use. The APC can in part be determined by estimating the measurement uncertainty component under conditions of within-laboratory precision (uRw), which comprises all components influencing the measurement uncertainty of random sources. To maintain the adequacy of their measurement procedures, laboratories need to distinguish aspects that are manageable versus those that are not.

Methods

One of the aspects that may influence uRw is the momentary significant bias caused by shifts in reagent and/or calibrator lots, which, when accepted or unnoticed, become a factor of the APC.

Results

In this paper, we postulate a model for allocating a part of allowable uRw to between-reagent lot variation, based on the need for long-term consistency of the measurement variability for that specific measurand.

Conclusions

The allocation manages the ratio between short-term and long-term variation and indicates laboratories when to reject or correct certain variations due to reagent lots.

doi: 10.1016/j.cca.2022.04.232

M134

Epidemiological profile of intestinal parasite carriage observed in the Medical Parasitology Mycology Laboratory of Mohammed VI University Hospital of Oujda

D. El Moujtahide

Parasitology mycology laboratory of Mohammed VI University Hospital of Oujda, Morocco

Background-aim

Intestinal parasitism is a frequent phenomenon and occupies an important place in infantile pathology, especially in countries of the third world.

The human digestive tract can be colonized by various species of parasites belonging to different classes. Although some of them are cosmopolitan, their prevalence varies from region to region. This variation is due to various factors including environmental, socio-economic and / or those related to eating habits. The study of the intestinal parasite carriage of a population reflects its level of food and fecal hygiene.

Methods

This is a retrospective study carried out at the medical parasitology-mycology laboratory of Mohammed VI University Hospital in Oujda over a period of 38 months between 12/01/2018 to 01/01/2022 We have included all the stool samples received at laboratory. For each coprological sample, we performed a parasitological examination of the stools in the fresh state and after staining with Lugol.

Results

Samples from 2,916 cases were studied during this period. The parasitological examination of the stool was positive in 27% of cases (n = 787), with a mean age of 35.3 years (01 - 96 years), sex ratio M / F = 0.79. The most frequently found germ was Blastocystis hominis in 60% of cases (n = 1778) followed by Endolimax nana in 17.69% of cases (n = 495), Entamoeba coli was found in 14.51% of cases (n = 408), Giardia intestinalis in 3.5% (n = 102), Trichomonas intestinalis in 2.1% (n = 58), Entamoeba histolytica / dispar in 1.91% of cases (n = 55), Entamoeba hartmani in 1.03% (n = 29), and Dientamoeba fragilis in 2.8% of cases (n = 81).

Conclusions

The intestinal parasite carriage in our laboratory was moderate and represented almost entirely by protozoa, mainly by Blastocystis hominis. It is necessary to conduct such studies, mainly dedicated to the population of rural areas. We must encourage faecal hygiene measures in our population, hence the need to strengthen the means of information, awareness and education.

doi: 10.1016/j.cca.2022.04.233

Clinica Chimica Acta 530 (2022) S265–S296

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Method Evaluation, Reference Interval, Decision Levels

M135

Source of variation evaluation of specific proteins in apparently healthy Tibetan Chinese adults: A multicenter cross-sectional study

L. Honglei, D. Wang, Y. Zou, L. Qiu

Peking union medical college hospital

Background-aim

The aim of this study was to evaluate the source of variations (SVs) and establish reference intervals (RIs) of component C3 (C3), component C4 (C4), immunoglobin A (IgA), immunoglobin M(IgM), and immunoglobin G (IgG) in Tibet, China.

Methods

Apparently healthy individuals (n = 1379) were enrolled from Ali, Shigatse, Lhasa, Linzhi in Tibet, China. All the tests were measured by Beckman Coulter automatic analyzer. Multi regression analysis (MRA) was performed to evaluate the (SVs. The 2.5th and 97.5th percentiles (P2.5, P97.5) were calculated as the upper and lower limit of RIs, respectively.

Results

The levels of IgG and IgM were significantly higher in female than that in male. However, sex was not a significant source of variation for C3, C4 and IgA. Altitude showed no significant association with any specific proteins except IgG. The serum IgG levels in Shigatse & Lhasa was significantly higher than that in other two altitudes. RIs for C3, C4, IgA, IgM, IgG were 0.85-1.67g/L, 0.10-0.35g/L, 1.03-4.80g/L, 0.46-3.10g/L, 9.0-21.5g/L, respectively.

Conclusions

In this cross-sectional study, RIs for C3, C4, IgA, IgM, Ig G were established for Tibetan population in China.

doi: 10.1016/j.cca.2022.04.017

M136

Reference interval of spot urinary oxalate (OX) in children less than six years of age

S.B. Hashmi, A.H. Khan, J. Talati, S. Qazi, L. Jafri

Aga Khan University Hospital Karachi

Background-aim

Hyperoxaluria is an important risk factor for stone formation. Diagnosis of hyperoxaluria in children can be made using 24-hour urine collection. Random collections normalized to urinary Creatinine may be of some clinical use in patients who cannot collect a 24-hour specimen, typically small children. Reference interval for oxalate to creatinine ratio (Ox:Cr) for Pakistani children are not available.

Methods

A cross sectional study was conducted at Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, in collaboration with section of Urology, Department of surgery AKU from June 2018 to August 2019. Apparently healthy children less than 6 years of age were included. Children on vitamins or with either past history of renal stones or family history of renal stones were excluded. Random urine samples were collected in sterile container with no preservative. Urine samples were stored at -30°C until analysis after adding 6M HCl. Urinary Oxalate was measured on Micro lab 300 using a kit based on oxalate oxidase principle by Trinity Biotech, while creatinine was measured on ADVIA 1800 by Siemens, US based on kinetic Jaffe reaction. Oxalate to creatinine ratio (Ox:Cr) was calculated. Data was analyzed by EP evaluator and SPSS 23. Subjects were categorized based on ages into following groups: group I comprised of children between 0-6 months; group II 6 months to 1 year; group III 1-2 years; group IV 2-4 years and group V 4-6 years.

Results

Mean age of study subjects (n = 120) was 29 months (±22.3) with M:F ratio 1:1. Subjects from various districts of Karachi were part of the study; majority were from District East (30.8%) followed by Central (28.3%), West (21.7%), Malir (15%) and (4.2%) from South. Majority of

doi:10.1016/j.cca.2022.04.016

the subjects were Urdu speaking (n = 45, 37.5%), followed by Pakhtoons (n = 27, 22.5%), Punjabi (n = 24, 20%), Sindhi (n = 19, 15.8%) and Baloch (n = 5, 4.2%). No significant difference was noted in median Ox:CR ratio between various ethnicities (p value > 0.05). Significant difference was noted median Ox:Cr in group I to V were 0.25 (0.06), 0.19 (0.11), 0.15(0.04), 0.11 (0.06) and 0.08(0.04) respectively (p-value < 0.001). The established RIs of Ox:Cr was 0.05-0.34 (90%CI).

Conclusions

Ox:Cr ratio showed a declining trend with age in children. In children < 6 years of age RI for Ox:Cr was calculated as 0.05-0.34. Interpretation of random urine Ox:Cr ratios is challenging in children and large scale reference interval studies are encouraged taking diet and age into consideration.

doi: 10.1016/j.cca.2022.04.018

M137

Comparison of two analyzers for the determination of fecal calprotectine

R. Rubio Sánchez, E. Lepe Balsalobre, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville

Background-aim

Fecal calprotectin (CF) is a calcium-fixing protein that is used to assess inflammation of the intestinal mucosa, allowing to distinguish between inflammatory bowel disease (IBD) and irritable bowel syndrome. In the determination of CF there is a great variability in the results obtained with different commercial kits.

The objective of this study was to evaluate the concordance of the results of CF obtained in the BÜHLMANN fCAL turbo analyzers (PETIA: turbidimetric particle-enhanced immunoassay) and LIAISON Calprotectin ASSAY from Diasorin (CLIA: chemiluminescent immunoassay).

Methods

44 stool samples were analyzed, obtaining the CF concentration through the two previous analyzers. Statistical analysis for the comparison of both methods was performed by Passing-Bablok regression and the analysis of differences by Bland-Altman.

Results

The Passing-Bablok test shows the following equation of the line: y = -9,126443 + 0.642356 x, showing systematic differences (95% CI of the ordinate at the origin: -17.3999 to -3.6111) and proportional differences (95% CI of the slope: 0.5556 to 0.7529).

The results showed a high correlation between both teams (r = 0.9596), with those obtained in the BÜHLMANN analyzer (mean: 196.4 g/g) higher than those obtained in the LIAISON analyzer (mean: 129.9 g/g).

Taking into account the established cut-off point of 50 g/g of CF for the study of IBD, there is a clinical concordance of 86.3% between the two techniques. This agreement is 100% in results > 50 g/g obtained by LIAISON. In contrast, there are 6 cases < 50 g/g according to LIAISON, but superior to cut-off according to BÜHLMANN.

Conclusions

A good correlation is observed between the CLIA (LIAISON) and the PETIA (BÜHLMANN). The reason why the results obtained in the BÜHLMANN analyzer are slightly higher than those of the LIAISON could be the contamination by detected sample drag and/or the use of different calibration standards.

As in most laboratory parameters, patient follow-up should be done with the same method and instrument and, in case the change is inevitable, it is necessary to indicate in the report this circumstance and the possible non-comparability with the results previous.

doi: 10.1016/j.cca.2022.04.019

M138

Study of the hemolithic index in the samples received in the biochemistry emergency laboratory

R. Rubio Sánchez, E. Lepe Balsalobre, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville

Background-aim

Hemolysis is the rupture of erythrocytes, causing the release of hemoglobin and the rest of its content to the plasma. It is the main cause of preanalytic rejection of blood samples (approximately 70%), since it interferes in the determination of lactate dehydrogenase (LDH) and bilirubin, among other tests. It is an avoidable preanalytic effect in most cases, since it is due to incorrect sampling and/or transport.

The objective of this study was to detail the hemolytic index of the samples received in the Biochemistry Emergency Laboratory, and to evaluate the results obtained according to the different clinical units of origin of the samples.

Methods

Observational and retrospective study carried out during the year 2018 in which 89,790 samples from the different hospital units were analyzed. The degree of hemolysis was divided into four categories according to the hemoglobin concentration: < 15 mg/dL (without hemolysis), 15-40 mg/dL (slight hemolysis), 41-100 mg/dL (moderate hemolysis) and > 100 mg/dL (intense hemolysis). The degree of hemolysis was measured by absorbance in the COBAS 8000 analyzer (Roche Diagnostics).

Results

The percentage of hemolysis varies greatly according to the hospital unit, observing that the most hemolyzed samples came from Emergency (29.5%) and Neonatology (27.1%). On the other hand, samples from Internal Medicine (4.4%) and Surgery (5.3%) have the lowest degree of hemolysis.

Conclusions

The high degree of hemolysis of samples from Emergency and Neonatology may be due to the difficulty in extracting the samples; in Emergency the extractor staff rotates too frequently and it has less practice, while in Neonatology the patients collaborate less, favoring venous collapse.

The control of the degree of hemolysis is essential in the determination of numerous tests since, otherwise, an erroneous diagnosis can be established and an inadequate treatment can be given to patients.

doi: 10.1016/j.cca.2022.04.020

M139

Comparison of the clinical diagnosis performance of a novel TSI immunoassay versus an automated TRAB immunoassay in Graves' disease: A Chinese multicenter study

M. Chaochao [a], C. Xinqi [a], C. Xiaofeng [a], J. Qiang [c], Z. Honggang [b], Q. Ling [a], L. Xiaolan [a]

[a] *Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science*
[b] *Nuclear medicine department, The Second Hospital of Tianjin Medical University, TianJin 300211, China*
[c] *Nuclear Medicine Department, Tianjin Medical University General Hospital, Tianjin*

Background-aim

Thyroid-stimulating hormone receptor antibodies are diagnostic hallmarks of Graves' disease (GD). At present, two different types of fully automated assays can detect the level of TRAb: thyroid-stimulating immunoglobulin (TSI) and TRAb immunoassays. The aim of this study was to evaluate the clinical diagnostic performance of these two assays and their agreement.

Methods

Sera were evaluated from 1000 subjects from three centers representing a variety of conditions: 100 subjects with untreated GD, 200 with treated GD, 62 with autoimmune thyroid disease, 216 with other thyroid diseases, 214 with non-thyroid autoimmune diseases, and 208 others (including 17 healthy controls and 191 subjects with other diseases). TSI and TRAb immunoassay were parallelly used on 1000 serum samples. The Thyretain™ TSI Reporter Bioassay was performed on 86 samples whose TSI results were inconsistent with the TRAb results. A human thyroid-stimulating blocking antibody (TSBAb) ELISA was used on TSI-negative and TRAb–positive samples in the control groups (n = 52).

Results

When comparing untreated GD patients with the control groups, the area under the curve for the TSI immunoassay was 0.991 (95% confidence interval (CI): 0.985–0.997), which was not inferior to that of the TRAb assay (0.987, 95% CI: 0.976–0.994) ($P = 0.4471$). Compared to the TRAb immunoassay, the TSI immunoassay showed higher specificity (96.86% vs. 88.71%, $P < 0.0001$), positive predictive value (PPV) (81.67% vs. 55.37%, $P < 0.0001$), and positive likelihood ratio (LR+) (31.18 vs. 8.68), respectively. The rest of the measures of accuracy were at least comparable to those of the TRAb immunoassay. A good agreement between TSI and TRAb assays was found in agreement study. The agreement rate for the TSI immunoassay with the bioassay was significantly higher than that of the TRAb immunoassay (87.21% vs. 12.90%).

Conclusions

The clinical diagnostic performance of the TSI immunoassay for Graves' disease is not inferior to that of the TRAb immunoassay. The TSI immunoassay shows good agreement with the TRAb immunoassay. The TSI immunoassay could be a more accurate diagnostic method for GD than the TRAb immunoassay, as we provide evidence that the former is only specific for TSI.

doi: 10.1016/j.cca.2022.04.021

M140

Compared the influence of four outlier recognition methods on the establishment of biochemical tests reference interval with big data of physical examination population

M. Chaochao, Y. Yicong, W. Jie, Z. Yutong, W. Danchen, Q. Ling

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science

Background-aim

The big data of physical examination population are cleaned, and the pre-processed data can be used to establish the reference intervals of tests after mining. The identification and processing of outliers is a very important step in the process of data cleaning. In this study, four different outlier identification methods were used to clean the data, compare and analyze the influence of different methods on the establishment of reference intervals. Moreover, a combination of outliers identification methods of reference interval based on big data of physical examination population was established.

Methods

In this study, all the physical examination data of Peking union medical college hospital from 2014 to 2015 were washed. The outliers were identified by four methods, Tukey (coefficient 1.5/3) and ESD (coefficient 3/4), and the 2.5 and 97.5 quantiles of the 33 biochemical test indexes after the outliers were eliminated by four methods were calculated. Wilcoxon rank sum was used to compare the reference intervals of each test index established by 5 methods to identify outliers and eliminate them. The results of the indicators of the physical examination population in 2016-2017 were judged by the reference interval used in clinical practice and the reference interval established by the four methods. The consistency of the results of the four methods and the reference range used in clinical practice was calculated and expressed by kappa value. The distribution level of each indicator Kappa of the four methods was compared to determine whether the four outlier recognition methods had an impact on the establishment of reference interval with big data.

Results

hsCRP, TBil, TG, UA, HCY, Glu, GGT, AST, ALT, LP(a), TBA and ALP were significantly different after the outliers were removed by four methods ($P < 0.05$). There was no significant difference in the distribution of K , LD. Na, P, TC, TP, Urea, HDL-C, DBil, Cysc, Cr, Cl, Ca, APol1,APoB, ChE, FFA,PA, CO2 and Alb ($P > 0.05$).There was no difference in the distribution of Kappa values between the four methods ($P = 0.974$).

Conclusions

Different identification methods of outliers may affect the establishment of reference interval of some biochemical indexes based on the big data of physical examination population. We established the data cleaning combination for establishing the reference interval through data

mining for different biochemical indexes, and suggested that different methods should be selected according to different indexes and characteristics of data when establishing the reference range with the physical examination population.

doi: 10.1016/j.cca.2022.04.022

M141

Is it possible to establish reference intervals for all common biochemical analytes and thyroid hormones by mining big data from clinical laboratories?

M. Chaochao, X. Liangyu, H. Lian, X. Tengda, G. Xiuzhi, Q. Ling

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science

Background-aim

Many studies have established a reference intervals based on big data, but none has evaluated whether the method is feasible. Therefore, based on big data mining, this study aims to establish the reference interval of common biochemical analytes and thyroid hormones, analyze the feasibility of establishing the reference interval using big data, and provide theoretical basis for the establishment of personalized reference interval.

Methods

The study randomly divided the data into derivation set and validation set through the method of data mining, established the reference interval of common biochemical analytes and thyroid hormones based on the derivation set, evaluated the precision through the iterative algorithm, and then compared the consistency of the two methods on the validation set.

Results

The reference intervals established by big data were close to the reference intervals being used in clinical laboratories for most indicators. The 90% CI of Reference intervals (RIs) for common biochemical analytes and thyroid hormones were very narrow. The consistency rate for most of common biochemical analytes and thyroid hormones was greater than 0.8.

Conclusions

The results of using big data to establish reference interval are stable and feasible. Personalized reference intervals can be established with big data.

doi: 10.1016/j.cca.2022.04.023

M142

Analytical and clinical performance of the vitros® immunodiagnostic products B·R·A·H·M·S PCT assay on the vitros immunodiagnostic systems

G. Ogbonna, S. Alvey, E. Hryhorenko, J. Parsells, S. Phonethepswath, B. Smith

Ortho Clinical Diagnostics, Rochester, NY, USA

Background-aim

The performance of the VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT (Procalcitonin) assay was validated on the VITROS® Immunodiagnostic Systems.

Methods

The assay is a two-step dual monoclonal immunometric assay that uses anti-PCT antibody immobilized on the well surface to capture PCT in the patient sample. The assay is calibrated against the B·R·A·H·M·S PCT™ sensitive KRYPTOR™ with time to first result of 24 minutes.

Results

The reportable range of the assay is 0.030 ng/mL to 100 ng/mL and patient samples can be diluted accurately up to 10-fold. A precision study over 20 days using ten precision pools with sample concentrations of 0.037 ng/mL to 76.0 ng/mL resulted in within-laboratory percent coefficient of variation (%CV) of 2.4% to 6.4%. Method comparison with B·R·A·H·M·S PCT sensitive KRYPTOR using 246 patient samples showed excellent correlation ($r = 0.994$; VITROS B·R·A·H·M·S PCT = 1.057* B·R·A·H·M·S PCT sensitive KRYPTOR - 0.010). Positive and negative percent agreements compared to the B·R·A·H·M·S sensitive KRYPTOR using 2168 patient samples at medical decision points of 0.100 ng/mL, 0.250 ng/mL, 0.500 ng/mL, 2.00 ng/mL and 10.0 ng/mL were excellent (overall agreement >97%). The assay was evaluated for prediction of cumulative 28-day all-cause mortality using samples obtained from the Multicenter Procalcitonin MOnitoring SEpsis (MOSES) Study collection. Blood samples were collected from subjects with severe sepsis or septic shock on Days 0, 1, 3 and 4 of ICU and hospital stay. Patient's vital status was verified at Day 28. The change in PCT values (⊗PCT) from Day 0 to Day 4 and Day 1 to Day 4 was calculated and evaluated against 28-day-all-cause mortality. ⊗PCT decline >80% (negative result) or δ80% (positive result) was significantly associated with 28-day-all-cause mortality ($p = 0.006$). Univariate Cox proportional hazards regression analysis showed a 1.93-fold increase in risk for mortality in subjects with a positive ⊗PCT result.

Conclusions

The VITROS B·R·A·H·M·S PCT assay demonstrated excellent analytical performance and agreement with B·R·A·H·M·S PCT sensitive KRYPTOR method. Clinical performance shows ⊗PCT information can be used to classify patients diagnosed with severe sepsis or septic shock into lower and higher risk for cumulative 28-day all-cause mortality.

doi: 10.1016/j.cca.2022.04.024

M143

Erythroferrone serum reference ranges for bulgarian population

V. Manolov[b], V. Vasilev[a], O. Georgiev[f], R. Emilova[c], I. Petrova[e], V. Pencheva-Genova[f], E. Hadjiev[d], T. Kunchev[e], K. Tzatchev[b]

[a] *Clinical laboratory, "Aleksandrovska" hospital, Sofia, Bulgaria*
[b] *Dept. of Clinical Laboratory, Medical University-Sofia, Bulgaria*
[c] *Dept. of Immunology, NCIPD, Sofia, Bulgaria*
[d] *Dept. of Internal Diseases, Medical University-Sofia, Bulgaria*
[e] *Dept. of Neurology, Medical University-Sofia, Bulgaria*
[f] *Dept. of Propaedeutics of Internal Diseases, Medical University-Sofia, Bulgaria*

Background-aim

Erythroferrone was discovered in year 2014 as regulator of hepcidin synthesis and is synthesized by erythroblasts. Erythroferrone main function is to suppress hepcidin synthesis during inflammation, thereby regulates iron homeostasis. We aimed to establish erythroferrone reference interval for Bulgarian population using validated ELISA method.

Methods

151 healthy volunteers were included. In all included participants intima-media thickness (IMT), ankle-brachium index (ABI), and blood pressure were measured, bodi-mass index (BMI) were calculated. Biological specimen (venous blood) was taken in order to evaluate total red blood cells count, erythrocyte indices – MCV, MCH and MCHC, hemoglobin, haematocrit, high sensitive CRP, serum iron and TIBC, serum transferrin, ferritin, haptoglobin, CRP, LDH, CPK, ASAT, ALAT, creatinine, glucose, lipid profile (total and HDL, LDL-cholesterol, triglycerides), hepcidin, TNF-⟨, IL-6. Included volunteers were divided into two age borders – a) from 18 to 50 years old and b) above age of 50. The distribution was as follows: males – total number 103, below age of 50 - 59 (57.3%), females – total number 114, below age of 50 - 69 (60.5%).

Results

For calibration curve we use recombinant human ERFE with level 10 ng/mL, from which after proper dilutions the required clinically relevant values were established. Diagnostic sensitivity was established 0.056 ng/ml. After applying specific statistical analyses and parametric distribution we found 6.3 – 15.7 ng/mL as reference range for Bulgarian population.

Conclusions

The immunological ELISA method we choose for serum erythroferrone quantification showed high specificity and sensitivity during validation process. It is new parameter for Bulgarian clinical laboratory practice and hematology specialty.

Acknowledgement

This project is sponsored by MU-Sofia, as part of Grant Д-57/2019.

doi: 10.1016/j.cca.2022.04.025

M144

Elisa erythroferrone serum quantification method in Bulgarian population

V. Manolov [b], O. Georgiev [f], V. Vasilev [a], V. Pencheva-Genova [f], I. Petrova [e], E. Hadjiev [d], T. Kunchev [e], R. Emilova [c], K. Tzatchev [b]

[a] *Clinical laboratory, "Aleksandrovska" hospital, Sofia, Bulgaria*
[b] *Dept. of Clinical Laboratory, Medical University-Sofia, Bulgaria*
[c] *Dept. of Immunology, NCIPD, Sofia, Bulgaria*
[d] *Dept. of Internal Diseases, Medical University-Sofia, Bulgaria*
[e] *Dept. of Neurology, Medical University-Sofia, Bulgaria*
[f] *Dept. of Propaedeutics of Internal Diseases, Medical University-Sofia, Bulgaria*

Background-aim

Erythroferrone (ERFE) is newly discovered regulator of hepcidin synthesis, produced from erythroblasts. ERFE is involved into iron homeostasis, an important trace element with a dual role in human organism. We aimed to validate ELISA method for quantification of serum hepcidin in Bulgarian population.

Methods

Validation of immunosorbent quantification method for ERFE in serum in Bulgarian population went through several evaluations like analytical scope through calibration curve, limit of detection (LD), low (LLOQ) and upper (ULOQ) limit of quantification, middle point (MPQ) of quantification, intra- and inter-assay precision.

Results

For calibration curve were use recombinant human ERFE with level 10 ng/ml, from which after proper dilutions the required clinically relevant values were established. Each and every standard was measured twice, and corrected against blank reagent sample. The calibration curve was four parametric; logarithmic by axis X, linear by axis Y. LD was evaluated by ten times measured blank reagent sample. We established 0.056 ng/ml, which ensure very high diagnostic sensitivity. Evaluation of LLOQ, MPQ and ULOQ showed CV $<$ 8% and bias $<$ 10%. Trueness of method was evaluated using recovery procedure, which showed area from 96.5% up to 97.6%. Intra-assay precision showed CV $<$ 5%; inter-assay repeatability – CV $<$ 6%.

Conclusions

The immunological ELISA method we choose for serum erythroferrone quantification showed high specificity and sensitivity during validation process. It is a new parameter in Bulgarian clinical laboratory practice.

Acknowledgement

This project is sponsored by MU-Sofia, as part of Grant Д-57/2019.

doi: 10.1016/j.cca.2022.04.026

M145

Serum lipase testing on the Abbott alinity: Indirect reference range estimation reveals differences between diasys and sentinel reagents

H. Hawran [c], G. Hoffmann [a], F. Arzideh [b], M. Orth [d]

[a] *Trillium Fachverlag, Grafrath, Germany and German Heart Center, Munich, Germany*
[b] *University Hospital of Cologne, Germany*
[c] *Vinzenz von Paul Kliniken, Stuttgart, Germany*
[d] *Vinzenz von Paul Kliniken, Stuttgart, Germany and Heidelberg University, Medical Faculty of Mannheim, Germany*

Background-aim

Lipase testing has not been standardized by IFCC but among the different methods available, the spectophotometric method using two coupled reactions and a non-diglyceride substrate to form methylresorufin is widely used by different vendors ("DGGR procedure").

To increase the specificity, colipase and bile acids (desoxycholate and taurodesoxycholate) are contained in the reagent buffers. Unlike to other routine enzyme tests, linearity as well as a good sensitivity and specificity at the upper reference limit still can be improved.

Methods

In our study, we compared the performance of two lipase tests (Diasys and Sentinel) on the Abbott Alinity by parallel testing in about 10.000 consecutive patient samples. Both assays state a reference range <60 U/L.

Results

Bland-Altman analysis revealed an excellent overall correlation of both tests (r = 1.000 (95% CI: 0.9924-1.000); XSentinel = YDiasys + 3 (95% CI: 3.000-3.061). However, Bland-Altman analysis revealed differences between both tests in particular at the upper reference limit.

When reference ranges were estimated by an indirect method, remarkable differences were observed: from the identical set of patients, the Sentinel method revealed a reference range up to 55.0 U/L in males and 55.2 U/L in females while the Diasys method revealed a reference range of 64.0 U/L in males and 56.0 U/L in females.

Conclusions

We conclude, that even when replacing a method with a nearly identical other method on the same analyzer, an indirect method for the estimation of reference limits can be helpful to improve the classification of patient results.

doi: 10.1016/j.cca.2022.04.027

M146

Architect/alinity tumormarker assays: Can results be used mutually interchangeably?

M. Orth [f], K. Lackner [c], A. Hamwi [d], W. Huf [b], J. Kudla [e], W. Sendel [e], M. Hoffmann [a], L. Lennartz [a]

[a] *Abbott GmbH, Wiesbaden, Germany*
[b] *Department of Laboratory Medicine, Krankenhaus Hietzing mit Neurologischem Zentrum Rosenhügel, Vienna, Austria*
[c] *Institute for Clinical Chemistry and Laboratory Medicine, University Hospital, Mainz, Germany*
[d] *Labor Dr. Hamwi, Linz, Austria*
[e] *Labor im Westen, Essen, Germany*
[f] *Vinzenz von Paul Kliniken, Stuttgart, Germany and Heidelberg University, Medical Faculty of Mannheim, Germany*

Background-aim

In tumor markers testing, established guidelines recommend re-baselining when changing the method or instrument platform since these factors might effect the testing results and might blur the patient results. However, this re-baselining is an obstacle of switching from one platform to another since both methods have to be tested in parallel for extended periods of time. If reagent chemistry, detection principle and instrument hardware design are very similar between instrument platforms and the differences between both platforms might be only minuscule. Aim of this study was to examine whether significant differences are in fact present between the established Abbott ARCHITECT and the newly-developed ALINITY assays or whether these tests might be used mutually interchangeably.

Methods

We performed a method comparison study between ARCHITECT and ALINITY assays for ⟨-fetoprotein (AFP), carcinoembryonic antigen (CEA), CA 125, CA 15-3, CA 19-9, prostate-specific antigen (PSA), and free PSA (fPSA) to show equivalence between patient results.

Testing was performed together with the daily routine testing during the validation studies at 5 different sites (2 in Germany, 2 in Austria and 1 in Switzerland). In total, 1.575 samples were tested in parallel with the different tumor marker assays, with approximately 250 samples for each tumor marker.

Results

Strong correlations were seen for AFP (r = 0.999), CA 125 (r = 0.998), CA 15-3 (r = 0.997), CA 19-9 (r = 1.000), CEA (r = 1.000), free PSA (r = 0.993), and total PSA (r = 1.000). Linear regression equations for the different tumor marker had slopes close to 1 (0.96 to 1.01) and an intercept between -0.43 and 1.28.

Conclusions

The excellent agreement between the ARCHITECT and ALINITY results for all tumor markers tested (AFP, CEA, CA 125, CA 15-3, CA 19-9, PSA and free PSA) allows switching from one method to the other without re-baselining.

doi: 10.1016/j.cca.2022.04.028

M147

Evaluation of the randox non-esterified fatty acids assay using an Abbott alinity C analyser

S.P. Peh, J.W.S. Setoh, C.K.M. Ho

Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital, Singapore

Background-aim

Free fatty acids (FFA),also known as non-esterified fatty acids (NEFA), are hydrophobic molecules and transported in the blood bound to the proteins. They provide a source of energy for cardiac and skeletal muscles. One of the main indications for measuring serum FFA levels is the investigation of hypoglycaemia in infants and children to ascertain whether hyperinsulinism or fatty acid oxidation defect is present. The test may also be indicated in patients with suspected inborn errors of metabolism, uncontrolled diabetes mellitus or certain endocrine disorders.In this study, we aimed to study the performance of the Randox NEFA colorimetric assay on Abbott Alinity c analyzer.

Methods

We evaluated the inter-day precision, limit of quantitation (LOQ), linearity, method comparison of the assay and its performance in the Randox International Quality Assessment Scheme (RIQAS).

Results

The coefficients of variation (CV %) for inter-day imprecision over 10 days were 2.51% at 0.48 mmol/L and 1.94% at 1.82 mmol/L. The manufacturer's LOQ of 0.07 mmol/L was verified. The assay demonstrated linearity for measurement between 0.1 and 2.0 mmol/L. There was a mean bias of 3.98% observed in the method comparison between Abbott Alinity c NEFA assay and Abbott Architect c 8000 NEFA assay. External quality assessment results were comparable with peers participating in the RIQAS programme.

Conclusions

We have evaluated the Randox NEFA assay performance (imprecision, LOQ, linearity, method comparison and EQA results) on the Abbott Alinity c analyzer. The assay performance was found to be satisfactory.

doi: 10.1016/j.cca.2022.04.029

M148

Glycated hemoglobin HBA1C evaluation in type 2 diabetic patients according to international recommendations

I. Kihel [b], M. Nachi [a], B. Addad [a]

[a] *biochemistry, medecin department, university of ORAN 1*
[b] *biochemistry, pharmaceutical department, university of ORAN 1*

Background-aim

Hemoglobin A1c (HbA1c) is an essential parameter for monitoring glycemic balance in type 2 diabetes (T2D). This statement is based on the fact that HbA1c allows us to learn about 3 months glycemic balance .The aim of this work is to evaluate the rate of HbA1c in type 2 diabetic patients and to verify the interval compared to international recommendations.

Methods

Our work concerned T2D patients followed in the internal medicine department of the University Hospital Center of Sidi Bel Abbes -Algeria . for each patient An HbA1c assay by NGSP / DCCT certified HPLC technique on a Bio-Rad D10 analyzer was carried and the following variables were collected on file: age, sex, age of diabetes and micro and macroangiopathic complications.

Results

2/3 of the population studied had HbA1c levels outside the recommended target (lower than 7%), this result provides the clinician with a decision-making biological criterion allowing him on the one hand to adapt the therapy according to the age and clinical condition of the patient and to control compliance, on the other hand to insist with patients, on strict compliance with hygienodietetic rules.

110 patients with a mean age of 62 years were included in our study [38-82]. 58% had diabetes for less than 15 years and 42% had it for 15 years or more. 60% of the patients presented macro-vascular complications and 45% of the micro-vascular complications, mainly retinopathies (21%) and neuropathies (16%). According to the recommendations of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), we observed that 20% of the population studied had HbA1c levels corresponding to the general glycemic targets imposed (δ7 %), 18% had HbA1c levels between 7 and 8%, 70% of which had macroangiopathic complications. 11% of T2D patients had an HbA1c level between 8 and 9%. However, HbA1c levels higher than 9% up to 14% were found in 44% of the patients.

Conclusions

We conclude that a large part of our T2D population greatly exceeds the imposed limits, which explains the acute and chronic complications very often observed during follow-up. The real problem of our sample and of the general population remains the application of the dietary hygiene rules. Despite the standardization of HBA1c assay methods, it remains to be verified that the proposed limits can be applied to all populations, regardless of ethnicity or lifestyle.

doi: 10.1016/j.cca.2022.04.030

M149

Performance evaluation of the Roche Cobas 8000 ISE, C502 and C702 modules

L.T.C. Lum, S.Q.R. Lee, T. Binte Hussaini

Sengkang General Hospital

Background-aim

The Cobas 8000 analyzer is a scalable, module-based solution for a wide range of in vitro diagnostic testing. Their clinical chemistry modules include the Cobas ISE module, the high throughput cobas c702 spectrophotometry module and the medium throughput cobas spectrophotometry c502 module. We assessed the analytical performance of 53 general chemistry assays for the Cobas 8000 c702 and c502 in terms of verification of precision, linearity, and method comparison with the predecessor model, Cobas 6000 c501.

Methods

Precision verification was performed according to CLSI EP15-A3, where 5 consecutive replicates of at least 2 different concentrations of quality control materials are processed each day over a 5-day period. The 25 data points collected were analyzed using a one-way ANOVA. Linearity was performed according to CLSI EP06-A. Commercial linearity material or patient sample constituting at least 6 levels of concentrations were tested in triplicates. LoQ was verified by processing a patient sample with a concentration close to the manufacturer's claimed LoQ, 5 replicates a day over 5 days. The total coefficient of variation (CV) obtained from the 25 data points is compared against the manufacturer's claimed %CV at the LoQ. Method comparison was done by processing a minimum of 40 patient samples spanning the assay range on Cobas 8000 and 6000 instruments.

Results

The coefficients of variation (CV) of all 53 general chemistry analytes evaluated fell within 0.2% to 3.3% and are well within the precision claims of the manufacturer. Linearity was observed for all assays over the tested analytical range. Method comparison results show that the two analyzers were comparable, with slopes of 0.93 – 1.08 and correlation coefficients (r) $>$ 0.975 for all evaluated assays.

Conclusions

The overall performance of Cobas 8000 ISE, c702 and c502 met the manufacturer's claims and the analyzer is considered fit for clinical purpose.

doi: 10.1016/j.cca.2022.04.031

M150

Performance evaluation of the Roche Cobas E801 module

L.T.C. Lum, S.Q.R. Lee, T. Binte Hussaini

Sengkang General Hospital

Background-aim

The Cobas 8000 analyzer is a scalable, module-based solution for a wide range of in vitro diagnostic testing. The Cobas e801 is the latest addition to the Cobas 8000 modular analyzer series. It is a high throughput immunochemistry module that performs a broad range of heterogeneous immunoassays using proprietary electrochemiluminescence technology. We assessed the analytical performance of 13 immunoassays on the e801, in terms of verification of precision, linearity, limit of quantitation (LoQ) and method comparison with the predecessor model, Cobas 6000 e601.

Methods

Precision verification was performed according to CLSI EP15-A3, where 5 consecutive replicates of at least 2 different concentrations of quality control materials are processed each day over a 5-day period. The 25 data points collected were analyzed using a one-way ANOVA. Linearity was performed according to CLSI EP06-A. Commercial linearity material or patient sample constituting at least 6 levels of concentrations were tested in triplicates. LoQ was verified by processing a patient sample with a concentration close to the manufacturer's claimed LoQ, 5 replicates a day over 5 days. The total coefficient of variation (CV) obtained from the 25 data points is compared against the manufacturer's claimed %CV at the LoQ. Method comparison was done by processing a minimum of 40 patient samples spanning the assay range on both instruments.

Results

The CVs of the 13 immunoassay analytes evaluated were between 0.7% to 3.4% and well within the precision claims of the manufacturer. Linearity was observed for all assays over the tested analytical range. LoQ was verified against the manufacturer's claims for all assays. Method comparison results show that the two analyzers were comparable, with slopes of 0.96 – 1.06 and correlation coefficients (r) > 0.975 for all evaluated assays.

Conclusions

The overall performance of Cobas 8000 e801 met the manufacturer's claims and the analyzer is considered fit for purpose.

doi: 10.1016/j.cca.2022.04.032

M151

Determining the sample size and rejection limits to detect between reagent lot variation using real-world data in clinical chemistry

K. Sollip [b], K. Soo-Kyung [a], C. Jeonghyun [b], P. Sholhui [a], H. Jungwon [a], J. Tae-Dong [a]

[a] *Department of Laboratory Medicine, Ewha Womans University College of Medicine, Seoul, Korea*
[b] *Department of Laboratory Medicine, Ilsan Paik Hospital, Inje University College of Medicine, Goyang, Korea*

Background-aim

To maintain the consistency of the laboratory test results, between-reagent lot variation should be verified before using new reagent lot in clinical laboratory. Although the Clinical and Laboratory Standards Institute (CLSI) document EP26-A deals with this issue, because so many test items are performed in the field of clinical chemistry, precise statistical evaluation of reagent lot-to-lot variation for each test item is challenging in reality. We aim to investigate a practical way for between-reagent lot variation using the real-world data in clinical chemistry.

Methods

We applied the CLSI document EP26-A using repeatability and within laboratory imprecision (S_{WL}) data of three clinical laboratories for a total of 83 chemistry items (41 general chemistry including 12 urine chemistry and 42 immunochemistry tests). Three criteria were used to define the critical difference (CD) of each test item as follows: the acceptable limits provided by external quality assurance (EQA) agencies, reference change value (RCV) regarding biological variation, and total allowable error. The sample size and rejection limits that could detect CD between-reagent lots were determined for two decision levels with statistical power ε90% and false rejection rate δ2.5%.

Results

The sample size and rejection limits are varied mainly depending on how the CD is defined. In one laboratory, for example, if the CD criteria were set to EQA, the sample size of 32 chemistry tests was only one at each decision level, but when the CD was set to RCV and TE, the sample size of 59 and 40 tests was determined as one. In some case, unrealistic results have been produced. If the CD, repeatability, and S_{WL} of total cholesterol were 7.0%, 1.5%, and 1.9%, the sample size and the rejection limits for the mean difference were 401 and 0.6 times CD, respectively. In other cases, sample size and rejection limits could not be found in the CLSI EP26-A guideline.

Conclusions

The CLSI EP26-A guideline did not provide the answer for all cases. Since the sample size and rejection limits are varied depending on CD, it is important to determine the optimal and practical CD first that suits the individual laboratory conditions. Alternative approaches could be used when the statistical method could not provide the answer.

doi: 10.1016/j.cca.2022.04.033

M152

An evaluation of COBAS C502 apolipoprotein assays

T.C. Aw, C.S. Lau, S.K. Phua, H.P. Gan

Department of Laboratory Medicine, Changi General Hospital, Singapore

Background-aim

Apolipoproteins A-1 (ApoA) and B (ApoB) and lipoprotein(a) [Lp(a)] have been shown to be predictive markers in cardiovascular (CV) events and are increasingly used. Newer assays assessing Lp(a) in molar concentrations rather than mass units (mg/dL) prove that Lp(a) is significantly associated with residual CV disease risk even in statin-treated subjects. We describe our evaluation of the Cobas c502 Apo A, ApoB and Lp(a) assays on our laboratory auto-analyser (Cobas 8000, Roche Diagnostics).

Methods

The c502 ApoA, ApoB and Lp(a) assays are all turbidimetric immunoassays, analysing plasma samples from Li-heparin/EDTA tubes. The performance evaluation included assay linearity (using pooled patients' serum), analytical precision (using 2 levels of Roche control materials) and concordance analysis (n = 30) with samples from another institution using similar assays. Statistical analyses were performed using MedCalc software v18.11.3 (MedCalc, Ostend, Belgium). Our laboratory is a College of American Pathologists (CAP) accredited laboratory. As this work was part of our routine evaluation of new diagnostic assays, it was exempt from institutional review board approval.

Results

The c502 assays were linear for ApoA 0.04-2.4g/L (against a claimed measuring range of 0.2-4.0g/L, LOD of 0.03g/L), ApoB 0.04-4.7g/L (against a claimed measuring range of 0.2-4.0g/L, LOD of 0.03g/L), and Lp(a) 7.0-197.3nmol/L (against a claimed measuring range of 7-240nmol/L, LOD 7nmol/L, LOQ 20nmol/L). Inter-assay precision (CV %) was satisfactory: 1.7/1.5 for ApoA, 2.0/1.4 for ApoB and 2.2/1.2 for Lp(a) respectively. There was good agreement between our results and those of the other laboratory for ApoA, ApoB and Lp(a) with Pearson r = 0.998, 0.998 and 0.989 respectively and Passing-Bablok regression equations of y = -0.00385 + 0.984x, y = -0.023 + 1.029x, and y = 0.638 + 0.829x respectively. The results for these apolipoproteins using CAP 2018 and 2019 PT material was also satisfactory.

Conclusions

The performance of the Cobas c502 ApoA, ApoB and Lp(a) assays are good, within the manufacturer's claims, comparable to the CAP peer group analysis and fit for operational use.

doi: 10.1016/j.cca.2022.04.034

M153

How levels of reference ranges can influence the costs of public healthcare national programmes

C. Grigore [a], M. Totan [b], S.V. Cristea [a], N. Grigore [b]

[a] *Laboratory Department, Academic Pediatric Hospital, Sibiu*
[b] *University Lucian Blaga, Sibiu*

Background-aim

Suboptimal levels of vitamin D in children are related to bone diseases, allergies, asthma, autoimmune diseases. Different guides offers different values for what they consider suboptimal levels of vitamin D related to bone health. According to these levels, the doctors initiate the treatment, so the national healthcare system spend money.

Methods

We determined in the laboratory the 25OH vitamin D using an ELFA method on an automated VIDAS system. We made a retrospective study on 331 children admitted in the hospital, using as reference values for the vitamin D status the guide of the Romanian national programme and the guide of the American society of Endocrinology. The Romanian guide settles the reference values for the optimal level of vitamin D higher then 20 ng/ml, insufficiency between 10-20 ng/ml an deficiency below 10 ng/ml and the American guide considers as optimal the values higher then 30 ng/ml, insufficiency between 20-30 ng/ml and deficiency below 20 ng/ml.

Results

Using the Romanian guide we found 228 children (68.5%), with values higher then 20 ng/ml (normal), 68 (20.5%) with values between 10-20 ng/ml (insufficiency) and 35 (11%) with values below 10 ng/ml (deficiency). Using the American guide we found 134 (40.5%) with values higher then 30 ng/ml(normal), 94 (28%) with values between 20-30 ng/ml (insufficiency) and 103 (31.5%) with values lower 20 ng/ml (deficiency). After screening the children population for vitamin D status, we have to treat 11% of all children, according to the Romanian guide and 31.5% of all the children according to the American guide.

Conclusions

National healthcare systems have to be aware of the differences generated by the interpretation of what we call „normal reference ranges" for their population because they can dramatically influence the costs of public healthcare national programmes.

doi: 10.1016/j.cca.2022.04.035

M154

Performance evaluation of a chemiluminescence (CLIA) immunoassay for quantification of aldosterone- a step towards continuous quality improvement

N. Bano, S.B. Hashmi, L. Jafri, I. Siddiqui, S. Ahmed

Department of Pathology and Laboratory Medicine, The Aga Khan University Karachi, Pakistan

Background-aim

Quantification of serum aldosterone (ALDS) is increasingly required in clinical practice because primary aldosteronism is the most frequent secondary cause of hypertension. Chemiluminescence (CLIA)-based assays are ranked as better substitutes to conventional and laboursome enzyme-linked immunosorbent assay (ELISA) platforms. The aim of this study was to evaluate the performance of CLIA immunoassay and compare it with the previously functional ELISA assay.

Methods

This cross-sectional study was performed at the section of Clinical Chemistry, Department of Pathology and Laboratory medicine, the Aga khan university (AKU), Karachi, over a period of four months from March – June 2019. ELISA was performed on ETI max 3000 using kit from IBL diagnostics whereas for CLIA, LIASON analyzer (Diasorin) was evaluated. Sample ordered in routine clinical course already analyzed using ELISA, quality control material and proficiency testing samples by College of American Pathologists (CAP) were analyzed for performance evaluation of the CLIA assay. The total allowable error was taken as 15.0%. Statistical analysis was done using Microsoft Excel and EP Evaluator version 10.3.0.556 (Data Innovations).

Results

The precision of CLIA for level 1 and level 2 with an observed 0.171 ng/dl and 0.77 ng/dl were within the respective SD goals. The method

was linear over a reportable range of 0.97 to 100 ng/dL. On method comparison with ELISA using Deming regression a slope 1.071, intercept 0.191 and correlation of 0.9583 was obtained suggestive of excellent agreement between the currently evaluated and the previously functional assay.

Conclusions

The CLIA showed acceptable performance. Furthermore, availability of peer group comparison in proficiency testing, less laboursome and time consuming and better accuracy makes it better substitutes to conventional utilized ELISA assays.

doi: 10.1016/j.cca.2022.04.036

M155

Urinary VMA, HVA and 5-HIAA measurement on HPLC with electrochemical detection – A 3-way method comparison study using commercially available reagents

C.H.C. Tan, S.P. Chiong, S.Y. Tan, S.Y. Leo, H.T. Sim, C.P. Yeo

Clinical Biochemistry Laboratory, Department of Clinical Pathology, Singapore General Hospital

Background-aim

VMA (Vanillymandelic Acid), HVA (Homovanillic Acid) and 5-HIAA (5-Hydroxyindole-Acetic Acid) are used in the investigation of neuroblastomas and neuroendocrine tumours. Despite LC-MS/MS being the analytical method of choice, HPLC with electrochemical detection (HPLC/EC) is used by most clinical laboratories as it is less complex, easier to set up, less costly. In view of announcement by Biorad to discontinue their "VMA/HVA/5-HIAA by HPLC Test" kit, our laboratory evaluated commercially available Chromsystems and Recipe kits as alternative choices for service continuation.

Methods

Analytical performance of the Chromsystems and Recipe kits were optimized and validated on our HPLC/EC setup. Comparison of the Chromsystems and Recipe kits against the Biorad kit was performed using 68, 83 and 73 patient urine samples for VMA, HVA and 5-HIAA respectively. Statistical analysis was performed using Analyze-It software.

Results

Results from imprecision and linearity studies for VMA, HVA and 5-HIAA on both Chromsystems and Recipe kits were satisfactory for (CV for total imprecision $<10\%$ and linearity recoveries 80-120%). Limits of detection were validated for Chromsystems (but not Recipe) at 3.0 umol/L for all three analytes. Method comparison showed that Chromsystems and Recipe kits compared well against each other but registered positive bias to BioRad; Altman-Bland bias 1) VMA (Chromsystems: 8.7%, 95%CI: 5.1% to 12.2%; Recipe: 14.2%, 95%CI:7.6% to 20.8%) 2) HVA (Chromsystems: 9.7%, 95%CI:6.1% to 13.3%; Recipe:7.1%, 95%CI:2.5% to 11.7%) and 3) 5-HIAA (Chromsystems: 14.3%, 95%CI: 9.1% to 19.5%; Recipe: 24.2%, 95%CI: 19.3% to 29.0%). Our study observed chromatogram baselines on Chromsystems to be significantly less noisy compared to those from Recipe.

Conclusions

Both Chromsystems and Recipe kits demonstrated equivalent performance in imprecision and linearity and in comparison with the Biorad kit. Chromsystems fared better than Recipe with regards to lower limit of detection and chromatogram quality. Whilst positive bias of the Chromsystems and Recipe results against the Biorad results is unlikely to be of clinical significance, laboratories should review respective reference intervals and communicate with clinical stakeholders before transiting to an alternative kit.

doi: 10.1016/j.cca.2022.04.037

M156

Verification of the performance of anti-calcium dobesilate- enzymatic creatinine kit and evaluation the calcium dobesilate drug interference in China based on a multicenter real-world study

X. Guo, L. Hou, D. Wang, L. Qiu

Department of Clinical Lab, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing

Background-aim

Calcium dobesilate produced significantly negative interference during the detection of enzyme creatinine. Thus, Peking Union Medical College Hospital and Maccura (Maccura Biotechnology Co., Ltd., Chengdu, China) have developed the world's first anti-calcium dobesilate interference creatinine reagent. The aim of this study was to 1) verify the performance of this new creatinine reagent, 2) investigate the proportion of creatinine interfered by calcium dobesilate based on a multicenter real word study in China.

Methods

This study was a multicenter study from 23 hospitals in different regions of China. Precision of the anti-calcium dobesilate interference creatinine assay was verified by Clinical and Laboratory Standards Institution (CLSI) EP-15 A using two serum pools (82.24μmol/L and 308.27 μmol/L). Accuracy of creatinine was verified by isotope dilution mass spectrometry (ID-MS/MS) method using five frozen serum pools. The interference test was performed by an invitro method. In real-world surveys, creatinine concentrations were measured by both in-use creatinine kit in hospitals and the anti-interfering kits imultaneously.Take the deviation of the two methods more than 10% as the standard to further determine the calcium dobesilate concentration in samples. Subsequent case verification was performed to finally confirm the number of interfered samples.

Results

The within-laboratory CV% of new creatinine assays on analyzers from 23 hospitals, varied from 1.20% to 4.07% for low concentration serum pool and 0.35%~3.43% for high concentration serum pool, respectively. The average bias of 5 serum pools of the 23 detection systems varied from 2.6% to 6.2%. Interference experiments showed that the deviation of the creatinine test did not exceed 10% when the calcium dobesilate drug concentration was below 200μg/mL. In real-world surveys, 67,469 samples were tested in 23 centers, and 1,191 samples were measured for calcium dobesilate concentration. Of these, 265 were detected with drug-containing, accounting for 0.39% of all patients.

Conclusions

The precision, accuracy and anti-interference ability of the anti-interference reagents can satisfy clinical needs. In China, the prevalence of calcium dobesilate interfering with enzymatic creatinine should be taken seriously.

doi: 10.1016/j.cca.2022.04.038

M157

Applying the new model equation for the estimation of glomerular filtration in systemic treated cancer patients

B. Možina, A. Grošel

Institute of Oncology Ljubljana, Department of Laboratory Diagnostics, 1000 Ljubljana, Slovenia

Background-aim

The evaluation of the renal function in cancer patients in which treatment with carboplatin and/or cisplatin is indicated, with different treatment aims, must be as accurate as possible in order to avoid a potentially inadequately successful treatment and/or the significant adverse effect of systemic chemotherapy.

Methods

The reference method of evaluating renal function, glomerular filtration (GF), is calculated on the basis of clearance of 51Cr-EDTA, which is both time-consuming and costly. Until recently we used the estimation of GF with the Wright formula (Wright JG, et al., Br.JCancer, 2001) to determine renal function.

In 2017, J Clin. Oncol. published an article entitled “New Model for Estimating Glomerular Filtration Rate in Patients with Cancer” (Janowitz T, et al.). The New Model equation was formulated, proving to be the most reliable method of estimating renal function (GF) in patients with cancer.

Results

At the laboratory of the Institute of Oncology Ljubljana (OI) we performed an evaluation of the New Model equation on 160 samples of patients with cancer.

The New Model calculation provides on average -2% (from -25% to + 20%) lower results in relation to the BSA-adjusted CKD-EPI (Bland-Altman plot).

The New Model calculation, regardless of whether we determine creatinine with the Jaffe or the enzymatic method, shows a poorer correlation with the Wright calculation than BSA-adjusted CKD-EPI. The scattering of results over the entire area is characteristically greater. The New Model calculation provides on average -8% (from -35% to + 20%) lower results in relation to the Wright calculation (Bland-Altman plot). The BSA-adjusted CKD-EPI calculation shows a good correlation with the Wright calculation, and gives on average -5% (from -20% to +10%) lower results (Bland-Altman plot), which is similar to the findings of the authors of the above-stated publication (J Clin. Oncol., Janowitz et al).

Conclusions

Based on our findings we determined that the New Model calculation is the most reliable method of estimating renal function (GF) in patients with cancer with stable renal function where treatment with carboplatin and cisplatin is indicated. Therefore, we propose the use of this method at the OI as well as in oncological patients who are being treated outside the OI in Slovenia.

doi: 10.1016/j.cca.2022.04.039

M158

Validation of various equations for calculated serum LDL cholesterol in Korean

R. Choi [a], M. Park [a], Y. Oh [a], S.H. Kim [a], S.G. Lee [a], E.H. Lee [b]

[a] *Department of Laboratory Medicine, Green Cross Laboratories, Yongin, Republic of Korea*
[b] *Green Cross Laboratories, Yongin, Republic of Korea*

Background-aim

Low-density lipoprotein cholesterol (LDL) can be measured directly (LDL_{direct}) with several different measurement methods or calculated (LDL_{cal}) from a lipid profile that includes measurements of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG). The aim of this study was to validate and compare multiple equations for LDL_{cal} with LDL_{direct} in a Korean adult population.

Methods

We retrospectively reviewed clinical lipid profile test results (TC, HDL, TG, and LDL_{direct}) performed between July 2017 and September 2018 in Korean adults from the laboratory information system of Green Cross Laboratories. LDL was calculated using 11 different equations suggested by Friedewald, DeLong, Rao, Hattori, Anandaraja, Ahmadi, Pauvilai, Chen and Zhang, Vujovic, de Cordova, and Martin and was compared with LDL_{direct} measured using a homogenous enzymatic method.

Results

During the study period, 5,204 lipid profile results were obtained from 4,568 Korean adults (2,204 men and 2,364 women) aged 18.0–99.1 years. Ranges of lipid concentrations were as follows: 71-849 mg/dL for TC, 6.0-136 mg/dL for HDL, 18-295 mg/dL for LDL_{direct}, and 9-4406 mg/dL for TG. Among the 11 equations, the Vujovic equation [LDL_{cal} = TC – HDL – (TG/6.58)] showed the highest intraclass correlation coefficient (ICC, 0.938) followed by DeLong [LDL_{cal} = TC - (HDL + 0.16 x TG)] with ICC of 0.926 and Martin [LDL_{cal} = TC – HDL – (TG/different adjustable factors based on TG and non-HDL)] with ICC of 0.920. The Vujovic equation also showed the lowest mean systemic difference (-10.8 mg/dL) and median absolute error (9.5%) among equations. For samples with high TG concentration (>400 mg/dL, n = 308), the Martin equation showed the highest ICC (0.885) followed by that of Vujovic (ICC = 0.818). The Martin equation showed the lowest mean systemic difference (-6.2 mg/dL) and median absolute error (9.0%) in samples with high TG, followed by the Chen equation (-8.1 mg/dL and 12.7%, respectively).

Conclusions

Considering that the accuracy of equations varied according to TG concentration, future studies are needed to validate the accuracy of LDL_{direct} and equations for LDL_{cal} in association with clinical implications in a Korean population.

doi: 10.1016/j.cca.2022.04.040

M159

High-throughput fully automated laboratory solutions - alinity vs cobas - are results interchangeable

C. Bertholet, M. Lamtiri-Laarif, R. Gadisseur, L. Vranken, S. Delcour, E. Cavalier

Department of Clinical Chemistry, University Hospital of Liège, Liège, Belgium

Background-aim

Laboratory medicine plays a key role for diagnosis, monitoring and follow-up of patients. To ensure result comparability when changing from one automated solution for laboratory medicine a complete validation is required to ensure continuous high analytical and quality standard. The objective was to compare the results of the new Alinity family solution to the Roche Cobas solution for 68 routine parameters (37 Clinical chemistry CC and 31 immunoassay IA), including thyroid markers, tumor markers, cardiac markers, fertility hormones and anemia, lipids and liver panels.

Methods

Patient pools (serum and Li-Heparin Plasma plus urine pools) were tested in duplicate with the two automated laboratory solutions, Alinity Family and Roche Cobas systems according to concordance correlation coefficient ⟩c (Lin, 1989 & 2000) which evaluates the degree to which pairs of observations fall on the 45° line through the origin. According to McBride (2005) the Strength of agreement was considered as poor, moderate, substantial and almost perfect if ⟩c was <0.90, between 0.90–0.95, between 0.95–0.99 and > 0.99, respectively. All 68 assays were tested in duplicate in both methods.

Results

Results were consistent between 1st vs 2nd rep and 1st rep vs mean. Therefore for all further comparison only 1st rep results were used for comparison. Among CC parameters, strength of agreement was almost perfect for 4 parameters, substantial for 21 parameters, moderate for 3 parameters and poor for 9 parameters. Regarding IA parameters, strength of agreement was almost perfect for 1 parameter, substantial for 9 parameters, moderate for 6 parameters and poor for 15 parameters.

Conclusions

Strength of agreement between alinity and cobas was almost perfect or substantial for 57% of CC parameters, whereas it was 32 % for IA parameters. Reasons for discrepancies are mainly linked to difference in standardization or recognition of different epitopes. Adaptation of reference ranges will thus be needed for some analytes.

doi: 10.1016/j.cca.2022.04.041

M160

Vitamin d levels in a healthy South African population

P.I. Machingura [b], R.T. Erasmus [a], T.E. Matsha [b]

[a] *Department of Pathology, Chemical Pathology Division, National Health Laboratory Service (NHLS) and University of Stellenbosch, Tygerberg Hospital, Cape Town, South Africa*
[b] *SAMRC/CPUT/Cardiometabolic Health Research Unit, Department of Biomedical Science, Faculty of Health & Wellness Sciences, Cape Peninsula University of Technology, Cape Town, South Africa*

Background-aim

The methods for the determination Vitamin D have changed, yet the reference intervals (RIs) have not been revised for the new method. The aim of this study was to determine RIs for Vitamin D measured by the paramagnetic particle chemiluminescence.

Methods

Three hundred and seventy seven (377) healthy adults (men = 116) aged between 20 and 82 years were selected from the ongoing Vascular and Metabolic Health study. Vitamin D levels were measured using the paramagnetic particle chemiluminescence test on the Beckman DXI analyser (Beckman DXI, Beckman Coulter, South Africa). The Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines were used to determine the RIs.

Results

The mean age of participants was 46.50 years and standard deviation 15.47 years. Vitamin D levels did not differ significantly between men and women, respectively 24.78 ± 8.07 and 23.41 ± 7.83 ng/mL, $p = 0.12$. In the overall sample the RI (2.5th to 97.5th percentiles) ranged from 11.10 to 43.30 ng/mL. In women the RI ranged from 9.90 to 41.00 ng/mL whilst in men it was 12.70 to 47.10 ng/mL.

Conclusions

Vitamin D reference interval lower limit observed in this current study was lower than and almost half of the recommended cut off levels for vitamin D deficiency recommended by the manufacturer which is based on the 2011 Endocrine Clinical Society Practice Guidelines for vitamin D deficiency but similar to the cut off for vitamin D deficiency recommended by the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets.

doi: 10.1016/j.cca.2022.04.042

M161

Establishment of reference intervals of vitamin a and e for Chinese elderly based on liquid chromatography tandem mass spectrometry method and analysis of their effects on common biochemical indicators

Y. Yin, D. Wang, C. Ma, L. Qiu, S. Yu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing

Background-aim

Vitamin A (VA) and Vitamin E (VE) are significant to sustain life activities and keep a good physical condition. However, majority of population suffered from deficiency of micronutrients, especially in elderly people. The purpose of this study is to establish reference intervals (RIs) for elderly people based on liquid chromatography tandem mass spectrometry (LC-MS/MS) method and analysis of their effects on common biochemical indicators.

Methods

Participants included 356 apparently healthy individuals who aged 65 years old and over, underwent the healthy checkups were randomly

selected in the study (197 males and 159 females) with an average of 67 (65, 71) years old. We measured both VA and VE using self-established LC-MS/MS method. Effect of gender on the level of VA and VE was evaluated and RIs were established. The parameter method was used to estimate the RIs for VA and VE. Beyond that, the relationship between VA, VE and other biochemical indicators were analyzed.

Results

Females had significant higher level of VE than males in the elderly ($P < 0.05$). However, no significant difference was observed in VA by gender. The RI of VA for total elderly subjects was 0.283-0.730 mg/L. RIs of VE were 4.39-15.63 mg/L, 4.51-16.14 mg/L and 4.41-14.67 mg/L for total elderly population, females and males, respectively. The multiple linear regression results showed VA had increased level of ALT, GGT, Urea, Glu and UA level ($P < 0.05$), while VE had increased level of TC and LDL-C and reduced DBil' level ($P < 0.05$).

Conclusions

RIs for VA and VE based on LC-MS/MS method in Chinese elderly individuals were established.

doi: 10.1016/j.cca.2022.04.043

M162

Establishment of an improved liquid chromatography tandem mass spectrometry method for measuring catecholamine and their metabolites in urine and methodology comparison with high performance liquid chromatography and electrochemical detection

Y. Yin, S. Yu, J. Yu, M. Li, L. Qiu

Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing

Background-aim

Quantitation of urine catecholamine [epinephrine(E), norepinephrine(NE), dopamine(DA)] and their metabolites [metanephrine (MN), normetanephrine(NMN) and 3-methoxytyramine (3-MT)] play crucial roles in screening practices for pheochromocytoma and paraganglioma. This study aimed to establish a simple and rapid method for quantification of catecholamine and their metabolites in urine by liquid chromatography tandem mass spectrometry (LC-MS/MS). Meanwhile, comparing urine catecholamine and their metabolites levels based on high performance liquid chromatography and electrochemical detection (HPLC-ECL) with LC-MS/MS analysis.

Methods

One hundred microlitres sample was used and subjected to anion exchange solid phase extraction (SPE) followed by the detection of eluates under positive electro spray ionization and multiple reaction monitor modes. The performance verification of LC-MS/MS was evaluated. Urine samples for comparison from 107 patients with suspected PPGL were retrospectively included in this study conducted at Peking Union Medical College Hospital from May to June in 2019. Passing–Bablok regression analysis, Bland–Altman plots and interclass correlation coefficients (ICC) were used to evaluate the concurrence among urine E, NE and DA levels determined using HPLC-ECL.

Results

The total analysis time was 4min. The linearity of the methods were 0.25-500ng/mL(E), 0.25-500ng/mL(NE), 0.5-1000ng/mL(DA), 0.25-500ng/mL(MN), 0.25-500ng/mL(NMN) and 0.25-500ng/mL(3-MT), all $r > 0.999$. Limit of detection were 0.1ng/mL for E, NE, MN, NMN and 3-MT, 0.2ng/mL for DA, respectively. Good reproducibility was obtained with within laboratory coefficient variation (CV) of 3.8%~5.2%(E), 3.9%~4.3%(NE), 2.8%~3.8%(DA), 1.7%~5.2%(MN), 1.9%~5.4%(NMN) and 3.2%~5.5%(3-MT). Recovery were excellent within 94.9% to 102.7% for six analytes. The method is rapid, reliable and simple. Spearman's correlation coefficient of HPLC-ELC and LC-MS/MS were 0.334, 0.813 and 0.812 for E, NE and DA, respectively (all $p < 0.001$). The Bland-Altman plot showed that the average bias (%) for HPLC-ELC and LC-MS/MS were 61.1%, 24.4% and 6.2% for E, NE and DA, respectively. ICC between HPLC-ECL and LC-MS/MS were calculated, which were 0.34, 0.97 and 0.84 for E, NE and DA, respectively.

Conclusions

The self-established LC-MS/MS method for urine catecholamine and their metabolites quantification showed excellent performance with its rapid, and reliable analysis results. Good consistence between HPLC-ELC and LC-MS/MS methods in measuring DA was found. However, significant inconsistencies between HPLC-ELC and LC-MS/MS methods for measuring E and NE indicate that different measures cannot be used interchangeably.

doi: 10.1016/j.cca.2022.04.044

M163

Multi-site evaluation of the new IFCC-traceable ortho-clinical diagnostic vitros ALTv and LDHi slides: Can traceability improve performance and allow reference interval harmonization between vitros and Beckman platforms?

J.E. Raizman [b], B. Goudreau [a], A.K. Füzéry [b], A.K. Tsui [b]

[a] *Alberta Precision Laboratories, Edmonton, Alberta, Canada*
[b] *Department of Pathology and Laboratory Medicine, University of Alberta, Edmonton, Alberta, Canada*

Background-aim

Ortho Diagnostics recently released two new slides, lactate dehydrogenase (LDHi) and alanine aminotransferase (ALTv), traceable to IFCC reference methods. Historically, method differences between VITROS and other wet chemistry platforms produced biases that prevented unified reference intervals. The aim of this study was to evaluate whether LDHi and ALTv traceability could improve performance and allow alignment of reference intervals.

Methods

ALTv and LDHi were evaluated on VITROS 250, 350 or 4600 analyzers across 2 to 10 sites. Patient samples (n = 63-105) were selected across the full measurement range and measured on both old and new slides. Fresh aliquots were then measured on the Beckman DxC IFCC-traceable ALT and LDH methods. Inter-laboratory precision studies for ALTv and LDHi were performed using BioRad QC material run twice per day for 5 days. Total allowable error was defined as 15% for both analytes.

Results

Patient sample results were similar between ALTv and old slides (slope = 1.021, y-intercept = -8.381, r = 0.995, bias = -4.8%). However, there was improvement in bias compared to Beckman, from an average of 24% activity on the old slides, to 17% on ATLv. When restricted to values around the medical threshold of 50 U/L, ALTv had 24% lower activity than the old slide, but comparison to Beckman improved from a 46% difference on the old slide to 11% on ALTv. For LDH, we found 19% average higher activity on LDHi compared to the older slide (slope = 1.197, y-intercept = -1.941, r = 0.999). This trend was consistent in comparisons to Beckman where LDHi had, on average, 21% higher activity, which was worse than the tight 1.3% average bias on the old slides. Inter-laboratory precision on ALTv and LDHi was excellent with CVs < 5% across low and high concentrations.

Conclusions

Although there was improvement in correlation between the IFCC-traceable ALTv method and Beckman ALT, surprisingly, LDHi performed worse than the older slides, yielding a significant positive bias. Thus, harmonization of reference intervals was achieved with ALTv, but not LDHi. Despite IFCC-traceability, LDHi required a correction factor to adjust for differences between manufacturers.

doi: 10.1016/j.cca.2022.04.045

M164

Evaluation of two methods for fecal calprotectin testing: Chemiluminiscence and ELISA

A. Gallego, S. Fuentes, S. Martín, J.M. Maesa, A. León

Department of Clinical Biochemistry, Virgen Macarena Hospital, Sevilla

Background-aim

Calprotectin is a protein that provides in faeces an indicator of the severity of intestinal inflammation.

Its determination is useful for the differentiation between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

The objective of this work is to evaluate the results of fecal calprotectin by two different methodologies, Liaison Calprotectin, DiaSorin (CHEMILUMINISCENCE) and Buhlmann fcal (ELISA).

Methods

44 samples were analyzed in our hospital complex and calprotectin levels (mcg / g stool) were compared by two different methods; Buhlmann fcal (ELISA) and Liaison Calprotectin (CHEMILUMINISCENCE) according to the manufacturer's specifications .

The results were analyzed by Passing-Bablock linear regression, Bland Altman diagram and Pearson correlation coefficient.

Results

Both methods, ELISA and CHEMILUMINISCENCE, present similar results, the correlation being

r = 0.831 (0.709-0.905).

In the Passing-Bablok regression, the confidence interval (95%) of the slope (0.6736) includes 1 (0.544-1.021), as well as the confidence interval (95%) of the ordinate at the origin (-2.63) that includes zero (-17,499 - 4,411), which indicates that there are no statistically significant differences in both methods.

The observation of the Bland Altman scatter plot shows a systematic bias of 38.0. Its CI (95%) contains the 0 (-91.041 – 167.046) with which there are no systematic differences in both.

Conclusions

According to our results, both methods are interchangeable, although chemiluminescence shows greater dispersion of the results with respect to the ELISA.

In addition, we can point out that the Liaison Calprotectin is a fast, versatile and highly automated method that allows a high number of samples to be analyzed routinely.

doi: 10.1016/j.cca.2022.04.046

M165

Evaluation of norudia glycated albumin assay on multiple analytical platforms

S. Yoo[c], S. Kim[b], W. Choe[a], H. Park[e], H. Kim[d]

[a] *Department of Laboratory Medicine, Eulji University School of Medicine, Nowon Eulji University Hospital, Seoul*

[b] *Department of Laboratory Medicine, Inje University College of Medicine, Ilsan Paik Hospital, Goyang*

[c] *Department of Laboratory Medicine, Inje University College of Medicine, Sanggye Paik Hospital, Seoul*

[d] *Department of Laboratory Medicine, Korea University Medical Center, Korea University Guro Hospital, Seoul*

[e] *Department of Laboratory Medicine, Seoul National University Boramae Medical Center, Seoul*

Background-aim

Norudia glycated albumin (GA) assay was evaluated for the analytical performance and the assay applicability on multiple analytical platforms.

Methods

The evaluation included precision, linearity, reference interval, and comparison with Lucica GA assay. A multicentre study was done to compare the results of Norudia GA assay on five kinds of widely used automated clinical chemistry analysers.

Results

Within-laboratory precisions for GA% presented 1.3-3.3% and 0.8-2.6% for low- and high-level control materials, respectively, on different analysers. GA assay was linear in 0.0 to 780.0 μmol/L of GA. The claimed reference range (12-16 GA%) was verified. Norudia GA showed a good GA% correlation with Lucica GA (correlation coefficient 0.999). GA% from each analyser showed a good correlation with the consensus mean of the results of five analysers (correlation coefficient 0.997 to 0.999).

Conclusions

Norudia GA assay can successfully be implemented in all the tested platforms, with a good GA% correlation.

doi: 10.1016/j.cca.2022.04.047

M166

Evaluation of serum KL-6 assay as a clinical diagnostic marker of interstitial lung disease in Korean patients

H.S. Kim, H. Cho, J.J. Lee, H. Chae, E. Oh, Y. Kim

Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Background-aim

Interstitial lung disease (ILD) refers to a group of parenchymal lung disorders that affect the lung interstitium. ILD can be caused by various etiologies and is diagnosed based on clinical, radiological and histological findings. Krebs von den Lungen-6 antigen (KL-6), a large glycoprotein originated from regenerating type II pneumocytes, has attracted attention due to its potential as a useful marker for predicting the diagnosis, monitoring and prognosis of ILD. And this marker has recently been introduced as a clinical test in Korea. This study aimed to evaluate the performance of the assay as well as the diagnostic value of serum KL-6 level for ILD.

Methods

This study analyzed a total of 108 residual serum samples from consecutive Korean patients with various lung disorders including 20 patients with ILD, 68 patients as disease control, and 20 healthy controls. Serum KL-6 concentration was measured using Nanopia® KL-6 latex-enhanced immunoturbidimetry assay (Sekisui Medical Co. LTD. Tokyo, Japan). Method validation studies including precision and linearity analysis as well as ROC analysis were performed.

Results

The within-laboratory precision was 7.15% and 3.29% using quality control materials at average concentrations of 395 U/mL and 972 U/mL, respectively. The assay was linear between 50-5,000 U/mL. On ROC analysis, the area under curve was 0.979 and the ideal cut-off value was 500 U/mL. When using this cut-off value of 500 U/mL, a value that is also recommended by the manufacturer, the sensitivity and specificity of the assay for detecting ILD 95.0% and 90.9%, respectively. In addition, serum KL-6 level of ILD patients was higher than that of non-ILD patients (median [range], 1,244 [358.7 to 5,553.7] versus 221.6 [96.0 to 824.7]) with statistical significance ($P < 0.0001$).

Conclusions

Based on our study, serum KL-6 detects ILD with accuracy and can be regarded as a useful biomarker for diagnosis of ILD, especially where only a few markers exist for ILD diagnosis and monitoring.

doi: 10.1016/j.cca.2022.04.048

M167

Establishing biological reference ranges for select biochemical parameters in adult Indian population

P. Chavan [a], V. Bhat [c], S. Ojha [b], M. Poojary [b], N. Sharma [a]

[a] *Composite Laboratory, Actrectmc, Navi Mumbai*
[b] *Department of Transfusion Medicine, Actrectmc, Navi Mumbai*
[c] *Microbiology Laboratory, Actrectmc, Navi Mumbai*

Background-aim

Laboratory tests are an important and integral part of the clinical decision making. Interpretation of these test results is based on biological reference ranges. These reference ranges are derived from the healthy population for the intended demography. They use 95% confidence interval of the values obtained from this population to describe the dispersion of variables in healthy individuals. They may vary significantly in different populations and cultures depending on the dietary preferences, race, geography as well as socio-economic status. Many of these reference intervals provided by various manufacturers for their reagents and methods are based on European or American population (Caucasian) studies and recommend laboratories to establish their own reference intervals. However, very few Indian studies are available on this subject. Aim of this study was to establish 95% reference interval for hematological and select biochemical parameters in adult Indian population and to correlate the above with respect to physiognomic parameters like gender, age and weight.

Methods

Blood samples from 1762 voluntary blood donors found fit for donation (F:362, M:1360) were analyzed for biochemical parameters (Serum creatinine, Serum Urea, Serum Protein, Serum Albumin, Serum Lactate Dehydrogenase, Serum Creatinine Kinase). Reference ranges were calculated using non-parametric method. The mean for the same was compared statistically with that of ranges in current use. Spearman's Rank correlation with age and weight was performed for all parameters.

Results

Biological reference ranges obtained were as follows: Sr. Protein (F: 5.3-10.1; M: 5.5-10.3g/dl), Sr. Albumin (F:2.8-5.4; M:3.1-6 gm/dl), Sr. Creatinine (F:0.6-1.3; M: 0.7-1.5mg/dl), Sr. Urea (F:11.2-30; M: 12-34 mg/dl), Sr. LDH (F:109.6-260; M:105.2-269 U/l), Sr. CKI(F: 36-210; M: 53-355 U/l). Significant p value was observed when difference between means were compared with those of established ranges. Spearman's Rank Correlation Coefficient rho was found to be insignificant in all parameters for age and weight.

Conclusions

The significantly different values observed in our study for biochemical parameters in apparently healthy adult Indian population may be attributed to differences in genetic composition as well as dietary preferences in our population. However, studies with larger sample size are needed to validate and establish reference ranges for the Indian population.

doi: 10.1016/j.cca.2022.04.049

M168

Automation of harboe method for the measurement of plasma free hemoglobin

T.H. Lee [c], H. Chung [c], J. Chung [b], M. Hur [c], S. Hwang [a], Y.K. Song [d], D.H. Lee [d]

[a] *Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, South Korea*
[b] *Department of Laboratory Medicine, Dongguk University Ilsan Hospital, Ilsan, South Korea*

[c] *Department of Laboratory Medicine, Konkuk University Medical Center, Seoul, South Korea*
[d] *Department of Laboratory Medicine, National Cancer Center, Ilsan, South Korea*

Background-aim

Although plasma free hemoglobin (fHb) test is important for assessing intravascular hemolysis, it is still dependent on the gold standard Harboe method using manual and labor-intensive spectrometric measurements at the wavelength of 380-415-450 nm. We established an automated fHb assay using a routine chemistry autoanalyzer that can be tuned to a wavelength of 380-416-450 nm.

Methods

The linearity, precision, accuracy, correlation, and sample carryover of fHb measurement using TBA2000FR method and manual Harboe method were evaluated, respectively. fHb values measured by manual Harboe method were compared with those measured by our new automated TBA2000FR method.

Results

fHb measurements were linear in the range of 0.05~38.75 μmol/L by TBA2000FR, and 0.05~9.69 μmol/L by manual Harboe method. Imprecision analysis (%CV) revealed 0.9~2.8% for TBA2000FR method and 5.3~13.6% for the manual Harboe method. Comparison analysis showed 0.9986 of correlation coefficient (TBA2000FR = 0.970 x Harboe +0.12). In analytical accuracy analysis, the manual Harboe method revealed about 4 times higher average total error % (12.2%) than the TBA2000FR automated method (2.8%). The sample carryover was -0.0016% in TBA2000FR method and 0.0038% in Harboe method.

Conclusions

In the measurement of fHb, the automated TBA2000FR method showed better performance than the conventional Harboe method. It is expected that the automated fHb assay using the routine chemistry analyzer can replace the gold standard Harboe method which is labor-intensive and need an independent spectrophotometry equipment.

doi: 10.1016/j.cca.2022.04.050

M169

Evaluation of a new fully automated chemiluminescent immunoassay for measurement of glutamic acid decarboxylase autoantibodies

C. Adamantidou, D. Gruson, M. Khourssaji

Cliniques Universitaires Saint LUC

Background-aim

Glutamic acid decarboxylase (GAD) is a neuronal enzyme involved in the synthesis of the neurotransmitter gamma-aminobutyric acid. Antibodies directed against the 65-kd isoform of GAD are the major pancreatic islet autoantibodies and one of the most important serological markers for the diagnosis of insulin-dependant diabetes mellitus. However, most of the anti-GAD assays available till now remain manual or semi-automated. The aim of our study was to determine the reliability of a novel fully automated chemiluminescence immunoassay.

Methods

We evaluated the Maglumi® anti-GAD automated immunoassay. We first validated the reference interval claimed by the manufacturer with serum samples from 25 healthy individual. Method comparison was performed against our routine method, the Euroimmun GAD Enzyme Linked Immunosorbent Assay (ELISA), with a set of seventy patients' samples.

Results

The anti-GAD levels in the observed in the group of healthy volunteers were ranging from 1 to 7.7 IU/L (median was 5.1 IU/L) and were compatible with the manufacturer threshold of 17 IU/mL. With the ELISA method the anti-GAD concentrations in patients' samples were ranging from 1 to 280 IU/L. The two assays were significantly correlated ($r = 0.91$, $p < 0.001$). The linear regression between the ELISA and the automated immunoassay showed a slope of 5.99 and an intercept of -16.96. Most importantly, and considering to the lack of standardization between the two methods, we observed a kappa coefficient of 0.96 demonstrated and excellent clinical agreement between methods for negative and positive results.

Conclusions

Our study confirms the reliability of the Maglumi anti-GAD automated immunoassay on the basis f a strong agreement with a reference ELISA method. The automation of anti-GAD testing can facilitate, through a reduced turnaround time of analysis, a faster diagnosis of insulin-dependant diabetes mellitus.

doi: 10.1016/j.cca.2022.04.051

M170

Evaluation of reference intervals of some biochemical markers in adolescents

O. Melnichuk[a b], E. Semikina[b], T. Blinova[b], E. Filyanskaya[b], I. Dzhgarkava[b]

[a] *Laboratory department, Pirogov Russian National Research Medical University, Moscow*
[b] *National Medical Research Center for Children's Health*

Background-aim

Laboratory test results should be compared to reference intervals (RIs) to distinguish between healthy and potentially diseased people. RIs mainly depend on age, gender, ethnicity and also laboratory analysis technology. Our data show RIs of biochemical markers of adolescents from seven regions of the Russian Federation.

Methods

Venous blood samples were taken from 1277 healthy adolescents (654 female and 623 male). For investigation of biochemical markers serum samples were analyzed with Unicell DxC analyzer (Beckman Coulter, USA) in central laboratory. RIs were calculated according to CLSI guidelines C28-A3c for the following parameters: alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), alkaline phosphatase (ALP, U/L), total protein (TP, g/L), bilirubin total (TBIL, μmol/L), enzymatic creatinine (CR-E, μmol/L), urea (mmol/L), total cholesterol (CHOL, mmol/L), glucose (GLU, mmol/L), iron serum

(μmol/L). Statistical evaluation of results was performed with the SPSS 20. RIs are presented as 95% intervals (2.5th-97.5th percentiles).

Results

RIs were calculated separately for male and female (age 12-17 years), gender – biochemical marker (RI): female – ALT (8.13-26.91), AST (16.39-36.30), ALP (83-367), TP (65.04-82.06), TBIL (4.97-22.13), CR-E (45-71), Urea (3.2-6.1), CHOL (3.02-5.69), GLU (3.75-5.84), iron serum (11.70-30.03). Male – ALT (9.39-30.78), AST (17.90-40.26), ALP (149-359), TP (65.20-80.93), TBIL (5.59-23.49), CR-E (45-85), Urea (3.51-6.47), CHOL (3.23-5.50), GLU (3.84-5.92), iron serum (12.60-32.60).

Conclusions

This data analysis enabled a new determination of RIs for cohort in the age group of 12-17 years in the Russian Federation.

doi: 10.1016/j.cca.2022.04.052

M171

Comparison of liaison, atellica IM-1600, cobas E801 automated analyzers for serum calcitonin measurement

J. Song, Y. Cho, S. Lee, J. Kim

Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea

Background-aim

Calcitonin, a peptid hormone that controls calcium reabsorbing, has a high diagnostic value because it rises specifically in medulary thyroid cancer patients. In light of this, we analysed concordance of the results of serum Calcitonin test by LIAISON analyzer (Diasorin, Saluggia, Italy), Atellica IM 1600 analyzer (Siemens Healthcare Diagnostics Inc., New York, USA), Cobas e801 modular analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Methods

Serum Calcitonin measurement was conducted using 3 different analyzers on residual part of 100 specimens that were collected from december, 2019 to january, 2020 in Severance hospital. Between every possible pairs of analyzers, Pearson's correlation coefficient, slope and y-intercept were assesed via Passing-Bablok regression. The agreements of classification according to clinically relavent cut-off of 10pg/ml were also evaluated with cohen's kappa coefficient. The statistical analysis was performed using Analysis-it and the the level of statistical significance was set at $p > 0.05$.

Results

When Cobas e801 versus Liaison, Atellica IM-1600 versus Liaison, Cobas e801 versus Atellica IM-1600 were compared, correlation coefficients were 0.994, 0.987, 0.997, respectively; The slopes were 1.176, 1.113, 1.001 and y-intercepts were -0.9341, 0.9366, 1.814, respectively. The agreements of classification at cut-off of 10pg/ml showed high classification concordance with kappa of 0.80, 0.81, 0.87, respectively, in the same order mentioned above.

Conclusions

All three devices, Liason, Atelica IM-1600 and Cobbas e801 showed good correlations with each others in the Calcitonin concentration measurement. But when compared to Liason, the Cobbas e801 and Atelica IM-1600 tended to show positive bias. For the classification concordance according to cut-off of 10 pg/ml, The agreement was slightly higher in between the Atellica IM-1600 and the Cobbas e801 compared to in between Liason and Cobas e801 and between Liason and Atelica aIM-1600.

doi: 10.1016/j.cca.2022.04.053

M172

Establishment of reference intervals for nonfasting lipid profile: Our experience in Indian subcontinent

G.K. Saharia [b], M. Mangaraj [a]

[a] *AIIMS BHUBANESWAR*
[b] *ALL INDIA INSTITUTE OF MEDICAL SCIENCES (AIIMS) BHUBANESWAR*

Background-aim

There is increasing evidence to support the routine use of nonfasting lipid testing. For most patients, nonfasting lipid testing is appropriate as it is evidence-based, safe, valid and convenient. Physiologically, we spend most of our lives in the nonfasting state but the cut off levels of non-fasting lipid profile for cardiovascular risk have not been defined yet due to non-availability of any reference interval for the same. Aim-To estimate the reference interval for nonfasting lipid profile in a population of Odisha province of India.

Methods

After approval from Institutional Ethics Committee, the study participants were recruited by cluster sampling from different villages under Khurda district of Odisha, India where AIIMS Bhubaneswar is situated. As per IFCC guidelines for health associated reference values, subjects with renal failure, congestive heart disease, chronic respiratory diseases, liver diseases, malabsorption syndromes, obesity, hypertension, diabetes, taking alcohol, tobacco, oral contraceptives etc. and pregnant ladies were excluded. From each of these villages, subjects above 18 years of age were selected randomly using standard methods to get a total of 2000. All the samples were screened for haemoglobin by Sysmex XP 100 to rule out anemia and a total of 650 samples found to be anemic. The assays were done for total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and high-density lipoprotein (HDL) in rest 1350 healthy subjects using Beckman Coulter AU5800 automated analyzer in the laboratory of Department of Biochemistry and the concentration of very low-density lipoprotein (VLDL) was calculated. Reference intervals (overall and specific for age and sex) were determined using non-parametric methods.

Results

The mean age of 1350 healthy individuals is 38 years and 47% are males. The reference interval (2.5th to 97.5th percentile) for TC ranged from 56.63 to 209.46 mg/dL. Similarly, for TG, LDL, HDL and VLDL it ranged from 33.32 to 248.44 mg/dL, 41.75 to 146.94 mg/dL, 18.77 to

66.72 mg/dL and 1.88 to 57.84 mg/dL respectively. Age group and gender wise reference intervals were also calculated for all the parameters.

Conclusions

As many countries are currently changing their guidelines towards measuring nonfasting lipid profile for cardiovascular risk prediction there is a great need for reference ranges including apolipoproteins which is not estimated in our study. Lipid values obtained in this study can be used as the reference value, based on which clinical correlation can be made as Indian dietary and ethnic variations may influence the worldwide reference interval.

doi: 10.1016/j.cca.2022.04.054

M173

Total imprecision of 8 high sensitivity cardiac troponin assays within sex-specific reference limits: Clinical implications

K. Schulz [a], S. Amy [a], P. Kavsak [d], A. Jaffe [c], J. Ordóñez-Llanos [b], P. Collinson [e], B. Lindgren [a], R. Ler [a], A. Sexter [a], A. Chapman [f], F. Apple [a]

[a] *Hennepin Healthcare Research Institute*
[b] *Hospital de la Santa Creu i Sant Pau*
[c] *Mayo Clinic*
[d] *McMaster University*
[e] *St. George's Hospital*
[f] *University of Edinburgh*

Background-aim

Clinical utilization of high sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) have assisted in ruling out of myocardial infarction (MI) and injury in early presenters to the emergency department (ED) predicated on concentrations between the limit of detection (LoD) and the sex-specific 99th percentile upper reference limits (URLs). Understanding the imprecision of assays at low concentrations (ng/L) is important for early rule out and rule in.

We examined the total imprecision (percent coefficient of variation [%CV]) of 2 quality control (QC) materials between the LoD and URLs of hs-cTn assays.

Methods

Quality control materials used in the 'clinical Scorecard based on Comparison Of high-sensitivity (hs) cardiac troponin (cTn) I and T assays for Ruling out acutE myocarDial infarction' (SCORED) study were analyzed. Assays studied were: hs-cTnI (Abbott ARCHITECT i2000, Beckman Coulter Access 2, Siemens Atellica, Ortho-Clinical Diagnostics VITROS 5600, ET Healthcare Pylon, Medience PATHFAST); hs-cTnT (Roche Cobas e411, ET Healthcare Pylon).

Results

Mean hs-cTnI and hs-cTnT concentrations for a serum pool, prepared at a concentration approximating the LoD, varied between 1.5 to 11.5 ng/L; differences were expected due to lack of assay standardization. %CVs ranged from 8.5% to 34.9%, across 27- 48 days. For one assay (Abbott) %CVs for 3 lots varied between 10.2% and 21.1%. Mean concentrations for a commercial control material below the sex-specific URLs varied between 8.0 to 46.8 ng/L. %CVs were lower than the serum pool and ranged from 4.3% to 15.3% across 20 -23 days.

Conclusions

Our findings show %CVs for 6 hs-cTnI and 2 hs-cTnT assays on the same QC materials. QC values near the LoD assist in ruling out MI and QCs within the normal range assist in defining normality. %CVs (total imprecision) improved with increasing concentrations. Variation in the assays mean values is expected due to the lack of standardization. Our data support that assay imprecisions are acceptable around the LoD and need to be monitored in clinical practice. We encourage manufacturers to provide QC material at low concentrations approximating the LoD for this purpose.

doi: 10.1016/j.cca.2022.04.055

M174

Determination of reference range (based on CLSI) leukocyte parameters for Indonesian subject

A.Z. Arjana [a], N. Devita [b], S. Irianti [a], T. Ratnaningsih [a]

[a] *Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada*
[b] *Faculty of Medicine, Universitas Islam, Indonesia*

Background-aim

Complete blood count is a basic test required in almost all clinical cases and important for making the diagnosis. Each laboratory is recommended to establish the reference range locally due to the variation of population characteristics and geographic areas. This study aims to determine the reference range for leucocyte and differential count of leucocyte parameters in Indonesian adult subjects.

Methods

This is a cross-sectional observational study. Samples were obtained from general check-up patients in Dr. Sardjito General Hospital. Blood samples were collected in K2-EDTA tubes. All samples were analyzed using automatic hematology analyzer CELL-DYN Ruby, which had been validated before. Subjects who had exclusion criteria or became outliers were removed. The reference range was analyzed using Medcalc software according to the CLSI C28-A2 recommendation.

Results

A total of 265 subjects (130 men and 135 women) aged 21 – 40 years old included in this study. There is significant differences between men and women group at the level of neutrophil, lymphocyte, eosinophil, and basophil ($p < 0,05$), but no significant differences at the level of leucocyte and monocyte ($p > 0,05$). Reference ranges for each men and women were stated respectively: Neutrophil (43,74-74,32; 48,43-77,95)x103µ/L, lymphocyte (14,81-44,39; 14,05-41,54)x103µ/L, eosinophil (0,25-8,49; 0,27-5,77)x103µ/L, and basophil (0,26-1,73; 0,35-2,03)x103µ/L. The reference range for leukocyte and monocyte were same, namely 4.54-11.15x103µ / L and 3.24-9.46 x103µ / L.

Conclusions

This study has determined the reference range for leucocyte parameters in Indonesian adult subjects using automatic hematology analyzer CELL-DYN Ruby.

doi: 10.1016/j.cca.2022.04.056

M175

Blood smear pitfall of platelet count confirmation examination: A case study

N. Devita [b], A.Z. Arjana [a]

[a] *Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada*
[b] *Faculty of Medicine, Universitas Islam Indonesia*

Background-aim

Manual platelet estimation is one of the methods used when automated platelet estimates are very low or very high. Confirmation is necessary to ensure the results issued by the automatic blood counts. Some recommendations recommend using a blood smear that is stained with Wright-Giemsa stain. This case shows the failure of this stain in detecting platelets.

Methods

This case shows the discrepancy between the results of examining platelet numbers using an automatic complete blood instrument with a manual method with blood film stained with Wright-Giemsa stain. Automatic blood count used is Sysmex XN-1000, Sysmex XN-550, Ruby, and ADVIA 2120. Examination conducted using appropriate tool usage instructions. The manual technique used is painting wright and platelet count manually with Rees Ecker method.

Results

A 34-year-old female patient came to Sardjito General Hospital to carry out surgery. The patient's blood is drawn for surgery. The complete automated blood test results with Sysmex XN-1000 showed a platelet number of 572 x 10^3 cells / μL. Peripheral blood films were obtained and stained with Wright-Giemsa stain. The results obtained an estimate of 76 x 10^3 cells / μL. This discrepancy is then followed up by repeated checks with another tool, Ruby. The results obtained value 566 x 10^3 cells / μL. Repeated blood smears were performed to ensure manual results. The results obtained are similar values that are 88 x 10^3 cells / μL. Examination using Sysmex XN-550 showed 543 x 10^3 cells / μL. Confirmation of the results is done using the Advia 2120 tool at the same time to see the function of other platelets. The results obtained value of 513 x 10^3 cells / μL. Manual calculation using the Rees Ecker method yields a platelet count of 599 x 10^3 cells / μL.

Conclusions

The difference in these results is possible because the Wright-Giemsa stain is not able to enter and color all platelets. The results of the examination in the tool showed conformity to the platelet manual calculation method with the Rees Ecker Technique.

doi: 10.1016/j.cca.2022.04.057

M176

Plasma free hemoglobin measurement using hemolysis indices from cobas C702 and atellica CH930 analyzers

C. Yonggeun [a], R. John Hoon [b], L. Sang-Guk [a], K. Jeong-Ho [a]

[a] *Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea*
[b] *Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea*

Background-aim

Determination of plasma free hemoglobin (pfHb) levels using hemolysis index (H-index) equipped on automated analyzer enables timely diagnosis of hemolytic disease over the conventional manual method using standalone spectrophotometer. In this study we evaluated the accuracy of H-index of recently introduced analyzers in estimating pfHb level.

Methods

A total of 176 plasma samples requested for the measurement of pfHb in Severance hospital were used in comparison study. For clinical reporting, Lambda 365 UV/Vis (PerkinElmer) was used and pfHb levels were calculated with formula proposed by Fairbanks. Then, H-index of remnant samples were quantified by both Atellica CH930 (Siemens) and Cobas c702 (Roche) analyzers. Linear regression curves of H-indices against pfHb were made by Passing and Bablok method. Agreement and kappa values between the interpretational categories based on our institutional cutoff value of 33.2 mg/dL were also calculated. Lastly, we assessed correlations of the difference between each H-indices and pfHb level with the optical density (OD) at 415 nm, 450 nm, or 700 nm to explore possible origin of the bias.

Results

H-indices from both analyzers and pfHb level showed good numerical relationship; slope of 1.01 and 0.98, intercept of -2.91 and -1.81, and r^2 of 0.900 and 0.903, for Cobas c702 and Atellica CH930, respectively. Also, the interpretational categories matched well; agreement of 0.898 and 0.892, and | of 0.734 [0.618-0.851] and 0.727 [0.611-0.843], in the same order as mentioned above, respectively. Meanwhile, for both analyzers, some samples with inferior H-indices compared with corresponding pfHb were well observed on dot plots, which also raised the number of false negative cases by H-indices. Among the observed 3-wavelengths, OD at 450 nm was the most correlated variable with the bias in H-indices; $r = -0.474$ ($p < 0.001$) for Cobas c702 and $r = -0.466$ ($p < 0.001$) for Atellica CH930.

Conclusions

This is the first evaluation report of the clinical feasibility of H-indices from Cobas c702 and Atellica CH930 as to estimate pfHb. Our results suggest H-indices from both automated analyzers could reliably substitute conventional spectrophotometry method in detecting hemolytic disease. However, care should be taken in the interpretation of H-index measured in severely icteric samples for the possible presence of negative bias.

doi: 10.1016/j.cca.2022.04.058

M177

Evaluation of automated immunoassays for urine free cortisol

H.W. Cho, Y. Cho, S. Lee, J. Kim

Laboratory medicine, Yonsei University College of Medicine, Seoul, Korea

Background-aim

Cortisol is a primary glucocorticoid hormone synthesized and secreted by adrenal cortex. Its functions include metabolic regulation and immunosuppression. Cortisol levels show a diurnal pattern and reg-

ulated by a negative feedback loop in hypothalamus-pituitary-adrenal cortex axis. Biologically active form of free cortisol is excreted into the urine. 24-h urine free cortisol (UFC) is a useful indicator of adrenal status and a screening tool for Cushing's syndrome. This study aimed to assess the correlation between the results of three different 24-h UFC assays.

Methods

24-h UFC samples were measured on a rolling basis. 40 urine samples were measured for 24-h UFC by Unicel DxI800 (Beckman Coulter, Fullerton, CA, USA) and Alinity i system (Abbott Laboratories, Abbott Park, IL, USA) simultaneously and 50 were measured by Unicel DxI800 and Atellica IM 1600 (Siemens Healthcare Diagnostics Inc., New York, USA) simultaneously. Using Unicel DxI800 as a reference method, we assessed the other two different instruments of Alinity i system and Atellica IM 1600 for measuring 24-h UFC using the Passing-Bablok regression to obtain a slope and y-intercept, Pearson correlation coefficient, and Bland Altman difference plots. Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK).

Results

Compared to Unicel DxI800, Alinity i system and Atellica IM 1600 yielded correlation coefficients (r) of 0.960 and 0.934, respectively. The Alinity i system showed negative bias from the Unicel DxI800 with slope of 0.499 (95% CI: 0.391 to 0.569) and intercept of -1.542 (95% CI: -2.161 to -0.791). The Atellica IM 1600 showed positive bias from the Unicel DxI800 with slope of 1.184 (95% CI: 1.017 to 1.333) and intercept of 2.003 (95% CI: 0.901 to 2.944).

Conclusions

Both the Alinity i system and Atellica IM 1600 showed low correlations for 24-h UFC concentration with the Unicel DxI800. Negative proportional and constant error were observed for Alinity i system and positive proportional and constant error were observed for Atellica IM 1600. Therefore, it is needed to perform the follow-up 24-h UFC examinations using the same instrument or adopt of different reference intervals to maintain comparability among these instruments.

doi: 10.1016/j.cca.2022.04.059

M178

Evaluation of full-automated pretreatment step versus semi-automated methods for tacrolimus and cyclosporine measurements

W. Alomaim [d], K.M. Sumaily [a], J. Abdel Jawad [d], N. Otaibi [c], S.A. Dawoud [a], R. Alyaeesh [c], G. Alsanie [d], S. Mahmoud [c], M. Shebani [c], A. Alhamad [c], J. Rodriguez [c], W. Tamimi [b]

[a] *Clinical Biochemistry Unit, Medical Laboratories, King Saud University Medical City, King Saud University, Riyadh, Saudi Arabia*
[b] *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical city, King Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*
[c] *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical City, National Guard, Riyadh, Saudi Arabia*
[d] *Department of Pathology and Laboratory Medicine King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia*

Background-aim

Measuring and monitoring of blood tacrolimus (Tac) and cyclosporine (Cyclo) levels are very crucial in the management of organ transplantation. Currently, whole blood samples are received in the lab and special pre-treatment extraction and precipitation step is performed manually on the samples which delay the turn-around-time (TAT) for reporting. In order to improve faster reporting and true randomly access of these assays we evaluate fully automated analyzer versus the semi-automated pre-treatments assays.

Methods

A whole blood samples from 25 transplanted patients were received and were performed on Architect (Abbott) and Dimension EXL/RXL (Siemens) immunoassay analyzers for both assays. The results were compared with liquid chromatography mass spectrometry LC/MSMS (Thermo Fisher Scientific). Data from twelve consecutive proficiency testing samples from College of American Pathologists (CAP) were used to compare the accuracy and precision. EP evaluator software was used to calculate the correlation coefficient (R) , bias, accuracy and precision.

Results

The correlation coefficient (R) for Architect and Dimension versus LCMSMS were 0.9190 (P value = 0.2976) and 0.8959 (P value = 0.09548) for Tac respectively, 0.9421 (P value <0.00001) and 0.9358 (P value <0.00001) for Cyclo respectively. The difference between the Architect and Dimension against the LCMSMS were within allowable error of 78.3% and 56.5% respectively for Tac; and 30.4% and 17.4% respectively for Cyclo. The linearity ranges for Tac and cyclo on LCMSMS were 1-44 ng/mL and 26-1287 ng/mL respectively. However, the CAP samples showed much better agreement (100%) between each method and LCMSM with R of 0.9995 and 0.9975 for Tac respectively and 0.9992 and 0.9978 for cyclo respectively. The agreement between the full-automated pretreatment versus semi-automated pretreatment immunoassay methods for Tac and Cyclo measurements was 88% and 96% respectively with R of 0.9282 (P value = 0.09638) and 0.8901 (P value = 0.020346) respectively. The two immunoassays showed positive biases relative to LC-MS/MS for cyclo but not for Tac.

Conclusions

The full-automated pretreatment step Dimension analyzer has demonstrated reliable and reproducible performances of tacrolimus and cyclosporine assays compared with other non-automated pretreatment step immunoassay analyzer and with LCMSMS which will improve faster laboratory routine measurement and reporting.

doi: 10.1016/j.cca.2022.04.060

M179

Verification of the erythrocyte sedimentation rate measured with two different methods

R. Kogovšek, P. Meško Brguljan

Clinical Biochemistry and Hematology Department, University Clinic for Respiratory Diseases and Allergy Golnik, Golnik, Slovenia

Background-aim

Erythrocyte sedimentation rate (ESR) test is one of the widely performed laboratory tests. It is considered as nonspecific test, usually ordered with other tests to detect and monitor the course of inflammatory conditions, infections or certain malignancies. The aim of the study was to evaluate the comparability of two modified Westergren methods

for ESR determination; manual Becton Dickinson (BD) Seditainer™ stand and automated Analyzer BD Sedi-40.

Methods

Paired 60 venous blood samples were collected into BD Seditainer® tubes (5 and 1,8 mL). 5 mL tube was used for manual method. A Tube was placed on a Seditainer stand and zero level of scale was aligned to the bottom of the meniscus. After 60 minutes erythrocyte level was read. According to converted scale, the results are equivalent to Westergren method. 1,8mL tube was placed on BD Sedi-40 Analyzer. The results were automatically read after 30 minutes. All measured data were statistically evaluated using Medcalc software (V.18.2.1).

Results

Non-normal distribution was shown with the Kolmogrov-Smirnov test (P < 0,0001). Median ESR measured by manual method was 17,5 mm/h (10 – 31) and for automated 15 mm/h (9 – 30,5). Spearman's rank correlation coefficient displayed a very good association between both variables (rho 0,984; P < 0,001). Passing-Bablok regression analysis yielded the equation y = 1,09 (95% CI 1,02 - 1,04)x + 0,05 (95% CI -0,57 - 0,89). Based on a Bland-Altman analysis, the mean bias was 2,2 mm/h (95% CI 1,4 - 3,1) with a 95% limit of agreement -4,3 to 8,8.

Conclusions

In this study, we demonstrated that both methods, manual BD Seditainer™ stand and automated Analyzer BD Sedi-40, correlated very strongly. We showed that a bias of 2,2 mm/h exists between the two methods, with a small proportional difference demonstrated by regression. There are several advantages of automated method according to manual one, such as higher standardisation, reduced volume of blood needed and reduced turnaround time. For more relevant estimation of automated modified method and their suitability for routine clinical use, comparison with the gold standard Westergren method should be made.

doi: 10.1016/j.cca.2022.04.061

M180

Performance evaluation of the measurement of complete blood count parameters between Mindray BC 6000 and BT pro 2401 hematology analyzers

S. Kader

Karapınar Public Hospital Biochemistry Laboratory Konya /, Turkey

Background-aim

Today, different trademarks of Hematology Analyzers have been used in blood analysis and but the compatibility of these devices with each other is unknown. In this study, the aim was to investigate the agreement between the results of complete blood count parameters values with BC 6000 hematology analyzer (Mindray, China) and BT PRO 2401 hematology analyzer (Izmir, Turkey).

Methods

This prospective study was included in 51 EDTA-anticoagulated samples submitted to our biochemistry laboratory for routine testing of CBC (complete blood cell). Statistical correlations CBC parameters values, analyzed in each analyzer as a single analysis from each sample were evaluated using two-way random intraclass correlation coefficient (ICC) and regression analysis. We compared the accuracy and precision of CBC count methods; i.e. BC-6000 Plus (optical (O) and impedance (I)) and BT PRO 2401 (optical (O) and impedance (I)).

Results

There was agreement between the two devices in Wbc, Neu, Mon, Neuperc, Lymperc, Monperc, Hct, Mcv, Mch, RdwSD, Plt, Mpv and pct parameters as intraclass correlation coefficient (ICC) < 90. However we found disagreement in Bas, Eos, Eosper, Basper, Rbc, Hgb,Mchc hemoglobin, RdwCV and Pdw measurements between Mindray and Bt-pro 2401 (ICC > 90).

Conclusions

In terms of white blood cells and neutrophil analysis which suggests that they can be used interchangeably, perfect agreement between them. However, instruments did not ensure satisfactory interchangeability and did not facilitate a substitution of one analyzer by another.

doi: 10.1016/j.cca.2022.04.062

M181

Comparison of two TSH-receptor antibody assays in graves' disease

B. Krhin, A. Oblak, A. Bicek, S. Gaberscek, K. Zaletel

Dept. of Nuclear medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia

Background-aim

The measurement of TSH-receptor antibodies (TRAb) is essential for management of Graves' disease (GD) which includes initial diagnosis, evaluation of treatment response and follow-up. In active GD, TRAb are usually increased, whereas in GD in remission, Hashimoto's thyroiditis (HT) or other thyroid disorders TRAb are negative. The aim of our study was to evaluate the performance of two different TRAb assays.

Methods

Patients' (n = 152) sera were evaluated by two automated immunoassays: "Kryptor Brahms TRAK Human" (Thermo Scientific, Germany) which is currently routinely used in our department, and new "Immulite 2000 XPi TSI" (Siemens Healthineers, Germany) which is declared by ability to specifically detect only TSH-receptor stimulatory antibodies (TSAb). Both assays were performed according to the manufacturer's instructions. Results were evaluated according to manufacturer's positivity cut-offs (1.8 IU/L and 0.55 IU/L for Kryptor Brahms TRAK Human and Immulite 2000 XPi TSI, respectively).

All patients were clinically evaluated by two independent thyroid specialists who established the diagnosis of thyroid disease on the basis of clinical course, thyroid ultrasound and the results of laboratory tests. Active GD was diagnosed in 101 patients. In 51 patients, clinical outcome confirmed GD in remission, HT or other non-autoimmune thyroid disease.

Results

Correlation between the Kryptor Brahms TRAK Human (A) and Immulite 2000 XPi TSI assay (B) gave a following Passing-Bablok linear regression fit: B = 0.996 * A – 0.169 (n = 152), where a significant

deviation form linearity was detected (p = 0.02), and B = 0.860 * A – 0.012 (n = 89) for concentrations with no dilutions and above limit of quantitation, and where no significant deviation form linearity was detected (p = 0.79).

Using Bland-Altman difference calculation (n = 152), Kryptor Brahms TRAK Human assay gave in average 0.9 IU/L higher results compared to Immulite 2000 XPi TSI assay and showed relatively high scatter of results (from -8.5 to +10.4 IU/L; 97.5 percentile).

In our patients, Immulite 2000 XPi TSI assay showed 92.8% diagnostic sensitivity, 92.7% diagnostic specificity, and 92.8% diagnostic accuracy, while Kryptor Brahms TRAK Human assay exhibited 64.9% diagnostic sensitivity, 87.3% diagnostic specificity, and 73.0% diagnostic accuracy.

Conclusions

Based on our results, Immulite 2000 XPi TSI assay has better diagnostic performance than Kryptor Brahms TRAK Human assay in management of GD.

doi: 10.1016/j.cca.2022.04.063

M182

Comparative study of the albumin dosage by the purple bromocresol method on Siemens® dimension RXL and bromocresol green on COBAS® Integra 400

N. Ould Bessi [b], S. Bounoua [b], M.E. Mehni [c], A. Chikouche [a]

[a] *Biochemistry Laboratory, Department of Medicine, Algiers-1 University, Pierre and Marie Curie Center. Algiers, Algeria*
[b] *Biochemistry Laboratory, Department of Pharmacy, Algiers-1 University, Pierre and Marie Curie Center. Algiers, Algeria*
[c] *Biochemistry Laboratory, Department of Pharmacy, Mouloud Mammeri university, Tizi-ouzou, Algeria*

Background-aim

Albumin is the most abundant protein in human serum. It is present in the vascular and extravascular compartment. It performs several biological functions; In the vascular compartment, it maintains oncotic pressure, any decrease in its value will be responsible for the appearance of edema. It also performs the function of specific and non-specific transporter (hormones, fatty acids, drugs, etc.).

Its dosage is particularly important for the evaluation of nutritional status and liver function. Several albumin assay methods are used routinely: colorimetric, turbidimetric, nephelometric and electrophoretic. The result must be reliable and accurate because of its importance in diagnosis orientation and in making therapeutic decision.

Two methods are used in our laboratory, the bromocresol purple method (BCP) and the bromocresol green method (BCG). The clinical importance of the results of these assays makes it necessary to make a comparison and an evaluation of the interchangeability of the results of the two techniques.

Methods

We used heparinized plasmas taken among those received daily in the laboratory. 115 samples were included in the study.

Albumin was dosed on each sample on the same day by two methods:

- Bromocresol purple method (BCP) on SIEMENS® DIMENSION RXL.
- Bromocresol green method (BCG) on COBAS® INTEGRA 400.

The COFRAC protocol was followed for the comparison of the two methods using:

- The student T test;
- Bland and Altman diagram;
- The linear regression line.

MedCalcStatistical Software was used for the statistical study.

Results

The Bland and Altman diagram has shown that the BCP method underestimates the albumin concentrations compared to the BCG method with an average difference of 8.4 g /L which is outside the limits of acceptability set by the SFBC. The regression line gave the equation: Y (COBAS) = 1.036X (SIEMENS) + 7.2135.

After correcting the values of the BCP method by the equation of the regression line, the Bland-Altman diagram showed an average of 0.1 g / L of difference and the new equation of the regression line was :Y = 0.9961X + 0.03134.

Conclusions

The correction by the equation made it possible to bring the two populations of results closer together and to provide clinicians with consistent results regardless of the method used.

doi: 10.1016/j.cca.2022.04.064

M183

Establishment of reference intervals for thyroid function tests in Chitwan district, Nepal

D. Mahaseth [b], J.P. Sah [b], R.K. Yadav [b], F. Mandal [a], A. Nigam [a], A. Magar [a], A.A. Magar [a]

[a] *International Reference Laboratory (IR-Lab), Bharatpur-10, Chitwan , Nepal*
[b] *School of Health and Allied Sciences, Pokhara University, Pokhara-30, Kaski, Nepal*

Background-aim

Reference intervals are the most important tool used for interpretation of pathology reports which are widely affected by different factors such as age, sex, lifestyle, ethnicity, developmental stage, nutrition and other environmental factors. Hence, it is obvious that it should be established for every population. The aim of this study was to determine the reference values for thyrotropin (TSH), thyroid hormones (free thyroxine, fT4; free triiodothyronine, fT3) in the population of Chitwan district of Nepal.

Methods

A total of 265 euthyroid subjects were enrolled in this study for establishment of reference intervals at International reference Laboratory (IR-Lab), Bharatpur-10, Chitwan, Nepal. The serum level of fT3, fT4, and TSH from these subjects were measured by using electrochemiluminescence immunoassay method (CLIA, Maglumi-800, Republic of China). We analyzed the data using SPSS version-20.

Results

Among 265 subjects, the reference intervals derived for fT3, fT4, and TSH were 3.01 ± 0.37 pg/mL, 1.45 ± 0.17 ng/dL, 2.48 ± 1.24μIU/ml respectively. We found a significant difference ($p < 0.05$) in TSH values between different age and gender groups.

Conclusions

Our finding is quite different from other reports even though the instrumental as well as reagent set up used in their research are same. This is because of using different population within same country. Therefore, it is concluded that each laboratory should establish its own reference values for proper diagnosis and treatment of disease.

doi: 10.1016/j.cca.2022.04.065

M184

Verification of the reference interval of a new prognostic biomarker for heart failure, GDF-15, on the cobas® E411 automated system

N. Ould Bessi [a], W. Aksas [a], N. Benadjal [a], N. Ferrat [a], M.E. Mehni [b], A. Djouadi [a]

[a] *Biochemistry Laboratory, Department of Pharmacy, Algiers-1 University, Pierre and Marie Curie Center. Algiers, Algeria*
[b] *Biochemistry Laboratory, Department of Pharmacy, Mouloud Mammeri university, Tizi-ouzou, Algeria*

Background-aim

Heart failure (HF) is a severe disease in which the heart is unable to provide enough blood flow to meet the needs of the body. The number of HF patients is constantly increasing worldwide due to the aging of the population and the improvement of the management of coronary artery disease and high blood pressure which are the main etiologies. The prognosis for this disease is poor, hence the need for appropriate and early management.

In the last decade, alongside the classic natriuretic peptides, new circulating cardiac biomarkers, reflecting different aspects of the molecular interactions involved in HF, have been researched. Among these markers, the Growth Differentiation Factor (GDF-15) emerges as a molecule which provides information on the cardiac and extracardiac pathways involved in cardiovascular disease and which is related to the incidence, progression and prognosis of HF.

This marker will be dosed in our laboratory by electrochemiluminescence on Cobas © e411.

The objective of our work is to verify the reference interval of the GDF-15 test on the Cobas © e411 analyzer, in a sample of the Algerian population.

Methods

- For the verification of the reference interval of GDF-15 in our population, we followed the IFCC / CLSI recommendations. It is a transference of a reference interval reported by a manufacturer.
- Apparently healthy controls, over the age of 20, were recruited on the basis of an anamnesis.
- A biochemical assessment was made for the controls on the cobas © integra400 automated system.

Results

The reference interval was verified with 22 controls, including 11 women and 11 men of different age groups.

The range observed was 409.7 - 1593 pg / ml which is included in the range given by the manufacturer which was 400-3076 pg / ml.

Conclusions

The values found in a sample of the Algerian population are included in the interval reported by the manufacturer, this interval can therefore be used for the interpretation of GDF-15 results.

doi: 10.1016/j.cca.2022.04.066

M185

QIP-MS: A reliable method for detection of M-proteins traceable to the international serum standard DA470K

K.B. Tan [c], D. Sakrikar [b], J. Ashby [a], S. North [a], L.J. Ouverson [b], G. Wallis [a], B. Du Chateau [b], S. Harding [a], D. Barnidge [b]

[a] *The Binding Site Group Ltd, Birmingham, United Kingdom*
[b] *The Binding Site Group, Rochester, MN*
[c] *The Binding Site Group, Singapore*

Background-aim

Assays used in clinical laboratories include controls traceable to an established standard (e.g. ERM-DA470k for routine immunoglobulin (Ig) measurement). Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS) is a novel method for the detection of Igs. We describe the inclusion of an ionization control to ensure reproducible assay performance and establish traceability to DA470k using immunoglobulin / ratios.

Methods

QIP-MS was performed on ERM-DA470k and normal pooled serum UNP001 using modified sheep polyclonal antibodies (anti-IgG, -IgA, and -IgM) covalently attached to blocked magnetic microparticles. Sera were incubated with microparticles, washed, and bound Igs were eluted and reduced to generate heavy and light chains. A Shimadzu MALDI-TOF-MS and a SCIEX LC-MS were used to generate mass spectra and determine the ratio of the area under the curve (AUC) for polyclonal light chain molecular mass distributions. Ig elution buffer contained a low molecular weight protein as an ionization control to confirm MALDI spot crystallization and establish traceability to ERM-DA470k using Hevylite / ratios and LC-MS results.

Results

Elutes from 3 immunoprecipitations (anti-IgG, -IgA, and IgM) were analyzed by MALDI-TOF-MS and LC-MS. Molecular mass distributions of and light chains were observed in all mass spectra. The / AUC ratios by MALDI-TOF-MS and LC-MS for ERM-DA470k were: IgG = 2.01 & 1.58, IgA = 1.18 & 0.91, IgM = 1.64 & 1.20; for UNP001: IgG = 1.77 & 1.52, IgA = 1.40 & 0.91, IgM = 1.73 & 1.11. The / ratios observed using MALDI agreed with Hevylite ratios within an acceptable limit of ± 15%. In the presence of 10 mg/L purified polyclonal IgG

(simulating hypogammaglobulinemia) an ionization control was able to confirm MALDI spot viability. An ionization control could also detect the accidental presence of agents that could disrupt MALDI spots and interfere with ionization.

Conclusions

ERM-DA470k can be used to evaluate UNP001 as a master calibrator for QIP-MS; we show commutability of the calibrator material for the determination of IgG, IgA, and IgM / ratios. An ionization control is an excellent determinant of the viability of the MALDI matrix spot.

doi: 10.1016/j.cca.2022.04.067

M186

Automated processing of sample preparation for homocysteine analysis using LC-MS/MS

S. Jun [a], K. Lee [b,c], J. Song [b,c]

[a] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam*
[b] *Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul*
[c] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam*

Background-aim

Accurate measurement of serum homocysteine, a nonessential, sulfur-containing amino acid involved in one carbon metabolism, is important in the diagnostic evaluation of its related diseases including homocysteinemia. The homocystenine analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) among a variety of methods provides advantages in terms of sensitivity and specificity. However, this analysis has been limited by time consuming manual sample preparation because the more tests, the more time it takes. We developed and evaluated an automated sample preparation protocol for the quantification of homocysteine in serum by LC-MS/ MS.

Methods

After whole preparation steps were alnalyzed to classify each step that can be automated, each sample transfer into a deep-well plate, addition of internal standard and dithiothreitol solution, mixing and protein precipitation by addition of trichloroacetic acid solution except to centrifugation of plate with centrifuge at 3700G for 10min were performed by the Freedom EVO (Tecan, Männedorf, Switzerland). The deproteinized supernatant transfer the another deep–well plate and addition of methanol were also performed by the Freedom EVO. After all preparation steps were complete, each sample was analyzed on an UPLC system. Analytical performances were evaluated including precision, linearity, lower limits of detection (LLOD) and quantification (LLOQ), carryover test and method comparison.

Results

Within-run precisions and between precision were 3.88-4.4% and 0.6~1.8%, respectively. The LLOD and LLOQ were 1.0 mol/L and 5.0 mol/L. Linearity was acceptable in the range of 0-100 mol/L (R2 > 0.998). Carryover was not observed. The test results appled using automated sample preparation were in good agreement with those using manual sample prepraration (correlation coefficients: 0.989).

Conclusions

Automation of homocysteine quantification by LC-MS/MS offers important improvements with respect to both the routine laboratory workflow and the analytical quality. In addition to improved reproducibility, our automation protocol avoids the risk of labeling errors, minimizes the hands-on time and reduces direct handling of infectious material.

doi: 10.1016/j.cca.2022.04.068

M187

Efficacy of using fastgene mirna enhancer with clinical samples

A. Kikuchi [a], A. Naruse [a], T. Sawamura [a], K. Nonaka [b], K. Takagi [c]

[a] *Department of Research and Development, Daiyukai Research Institute for Medical Science, Ichinomiya, Japan*
[b] *Department of Surgery, Daiyukai General Hospital, Ichinomiya, Japan*
[c] *Department of Urology, Daiyukai Daiichi Hospital, Ichinomiya, Japan*

Background-aim

miRNA analysis can be performed on a variety of clinical samples. However, some samples do not produce a Cp value using quantitative reverse transcription-polymerase chain reaction (RT-qPCR). Since the quality and quantity of miRNAs affect downstream analysis, obtaining reliable data requires high quality samples. Thus, we used the FastGene miRNA Enhancer (FG Enhancer; Nippon Genetics) in combination with two commercially available total RNA and miRNA extraction kits to improve the quality of RNAs isolated from a variety of clinical samples.

Methods

Clinical samples included white blood cells from 500 L of peripheral blood and 10 mg of stool that was collected from eight healthy subjects and formalin-fixed paraffin-embedded tissues from eight colon cancer patients. Method A optimized the FastGene RNA Premium Kit (Nippon Genetics) for total RNA extraction. Method B used the HighPure miRNA Extraction Kit (Roche) to isolate miRNA. All the reagents were used with one column. We added FG Enhancer to the 70% ethanol solution and substituted the Binding Enhancer solution with FG Enhancer in Method A and B, respectively. Subsequently, we followed the kit instructions for both protocols. The isolates were subjected to RT-qPCR and Cp values analyzed using a stem-loop primer targeting miR-21.

Results

Method A and Method B (absence/presence of FG Enhancer) exhibited the following Cp values (mean ± standard deviation): white blood cells, 27.40 ± 1.35/22.78 ± 0.92 and 30.86 ± 0.79/23.19 ± 0.94; stool, 32.07 ± 1.36/30.36 ± 1.18 and 36.55 ± 0.35/29.44 ± 0.97; and formalin-fixed paraffin-embedded tissues, 22.92 ± 0.63/20.60 ± 0.68 and 27.34 ± 0.83/21.10 ± 0.70.

Conclusions

We have demonstrated that the addition of FG Enhancer improved the efficiency of miRNA extraction.

doi: 10.1016/j.cca.2022.04.069

M188

Evaluation of the ischemia modified albumin assay on the Atellica IM analyzer

S. Kim [b], J. Seo [b], Y. Nam [a], K. Lee [a], S.H. Song [b], J. Song [a]

[a] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea*
[b] *Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea*

Background-aim

Ischemia modified albumin (IMA), produced when circulating serum albumin contact ischemic heart tissuess, is a sensitive biomarker of myocardial ischemia. Some papers were already published on the usefulness of IMA to diagnose myocardial ischemia and so on. The IMA kit (MedicalSystem Biotechnology Co., Ltd, Ningbo, China) is based on an albumin cobalt binding method. The aim of our study was to evaluate the analytical performance of a new IMA assay on the Atellica IM analyzer (Siemens Healthineers, Erlangen, Germany).

Methods

Analytical performance including precision, linearity, carryover, comparison and was evaluated. In addition, verification of reference intervals was assessed. Statistical analyses were performed. Statistical analyses were performed Excel (Microsoft Co., Redmond, WA, USA) and Analyse-it Ultimate Edition (Analyse-it Software, Ltd., Leeds, United Kingdom).

Results

The coefficients of variation for within-run and within laboratory precision were less than 2.00% at all two levels (70.3 U/mL and 99.6 U/mL). The calibration curves were linear in the range of 13.6-163.6 U/mL. There was no significant carryover. The results between two different Atellica IM anayzers were in good agreement with correlation coefficient of 0.9757. There were no healthy samples outside the reference interval provided by manufacturer.

Conclusions

The IMA kit on the Atellica IM analyzer showed good precision, linearity, and good correlation. Therefore, it is suitable for the measurement of IMA levels in clinical laboratories.

doi: 10.1016/j.cca.2022.04.070

M189

Biological variation estimates for hemogram parameters in blood samples obtained from healthy adults

O. Melnichuk, E. Svetalkina, N. Mayanskiy

Laboratory department, Pirogov Russian National Research Medical University, Moscow

Background-aim

Analysis of hemogram is used to evaluate health status in different cases such as infection, anemia, inflammation. To ensure safe application of the hemogram parameters requires reliable biological variation (BV) data.

Methods

Hemogram parameters were measured in venous blood samples obtained from healthy adults (blood donors) with the help of a Sysmex XN analyzer. BV was defined for 18 parameters . For each parameter, we calculated within-subject BV (CVI), between-subject BV (CVG), and reference change value (RCV); these three coefficients are percentages; for CVI and CVG, 95% CIs are given. The age and donation interval are presented as median (P25; P75) Statistical evaluation of the results was performed with the help of SPSS 20.

Results

In total, samples from 21 individuals (6 females. 15 males) of a median age of 35 (30; 48) years were analyzed. Each individual provided three samples; a median period between sample donations was 4.5 (1.3; 8.4) months No gender differences were found for any CVI, CVG, or RCV. BV estimates for hemogram parameters were as follows (CVI, CVG, RCV): HGB: 2.9 (2.1-3.7), 8.1 (5.1-10.0), 8.1; RBC: 2.9 (2.1-3.8), 10.7 (8.7-12.6), 8.4; HCT: 2.9 (2.0-3.9), 7.3 (4.8-9.9), 8.6; MCV: 1.9 (1.3-2.4), 6.5 (5.6-7.4), 5.3; MCH: 1.7 (1.3-2.2), 6.4 (5.3-7.5), 5.3; MCHC: 1.9 (1.4-2.4), 3.0 (2.7-3.3), 6.0; RDW-CV: 3.3 (1.9-4.78), 10.8 (9.4-12.3), 9.4; PLT: 9.8 (7.1-12.5), 25.3 (19.9-30.7), 27.8; MPV: 7.1 (5.1-9.1), 11.5 (3.8-19.2), 19.8; RET: 15.3 (9.8-20.7), 29.6 (15.8-43.5), 43.8; IRF: 50.2 (29.2-71.2), 73.2 (40.1-106.2), 140.3; LFR: 3.9 (2.5-5.3), 7.2 (5.9-8.5), 17.4; WBC: 13.2 (10.2-16.2), 20.9 (12.7-29.2), 36.9; LYMPH: 14.7 (11.8-17.6), 25.7 (19.1-32.2), 42.1; MONO: 13.4 (9.1-17.8), 30.6 (25.3-36.0), 42.6; NEUT: 17.6 (13.8-21.3), 31.5 (20.0-42.9), 49.3; EO: 28.7 (18.4-38.9), 66.6 (52.7-80.6), 82.4; BASO: 23.7 (17.8-29.5), 40.7 (35.4-45.9), 66.1.

Conclusions

Our study provides updated BV estimates for hemogram parameters in adults.

doi: 10.1016/j.cca.2022.04.071

M190

Evaluation of suitability of clinical chemistry biological reference intervals in selected Kenyan laboratories preparing for ISO 15189-2012 accreditation

R.N. Maina, D. Mengo

Kenya Accreditation Service (KENAS)

Background-aim

Reference Interval (RI) is range of test values expected for a population in in which 95% of the individuals are presumed to be healthy. ISO 15189 requires laboratories to define RIs, document the basis for the RIs and communicate this to the users. Most laboratories in Kenya use RIs published in text books and reagent kit inserts for the interpretation of laboratory results. Most of these RIs are derived from non-local populations and may not be suitable for use in Kenya. Review of RIs adopted by different laboratories in Kenya is needed to highlight the need to establish local reference ranges.

Methods

Two hospital laboratories handling blood donors were selected in each of the four regions in Kenya (central, Western, Eastern and Coastal Kenya). The selected laboratories had adopted reference intervals from Roche (central), Human diagnostics (Eastern), Beckman coulter (coast Kenya) and locally published (Kericho, Kenya) for Western Region. Blood was collected and analyzed for clinical chemistry [ALT, AST, bilirubin total, chloride and creatinine] from 20 healthy blood donors (each gender) in each laboratory). Data was analyzed using excel based RI verification tool from pSMILE.org following method described in Clinical Laboratory and Standards Institute (CLSI) EP28-A3c. The RI was validated if ε 90% of the 20 results fell within the proposed reference range. Participants included in this study signed informed consent.

Results

RIs for all analytes (both genders) for Kericho, Kenya were validated: ALT(10-52U/L), AST (14-43U/L), Creatinine (0.1-1.2mg/dl), Bilirubin total (0.3 -2.3 mg/dl) and Chloride (100-112mmol/L). The following RIs were no acceptable as <90% of the results were within the proposed Reference Interval: Bilirubin (0.3-1.3 mg/dl) for Beckman Coulter; ALT (0-40U/L), AST (0-36U/L), chloride (60-140 mmol/L) and bilirubin (0.6-1.3 mg/dl) for Roche; Bilirubin (0.1-1.2 mg/dl), (ALT 0-42U/L) and (AST 0-37U/L) for human.

Conclusions

Validation of locally established RIs (Kericho, Kenya) and not those from reagent package inserts may indicate the latter are not suitable for local population and may lead to erroneous clinical decisions. The laboratories should always validate RIs from such sources before adopting them.

doi: 10.1016/j.cca.2022.04.072

M191

Essential step in the detection of creatine deficiency syndromes: Reference values for creatine and guanidinoacetate

M.B. Badal Cogul, J. Robles Bauzá, J. Miquel Bauçà, B. Lasheras Vicens, R. Guerrero Márquez, A. Pardo Riera, G. Pérez Esteban

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

Creatine is essential for energy metabolism and stored in tissues with high energy demand. Two enzymes participate in its biosynthesis: glycine amidinotransferase (AGAT), which catalyzes the formation of guanidinoacetate (GAA); and guanidinoacetate methyltransferase (GAMT), which produces creatine from GAA.

Alterations in the creatine transporter or the enzymes AGAT or GAMT may translate into creatine deficiency, which can lead to intellectual disability in children, speech delay and behavioral problems. Increases or decreases in GAA levels help distinguish among etiologies of this inborn error of metabolism.

Measurement of GAA and creatine in urine represents the first step for the diagnosis of creatine deficiency syndromes. Our aim was to establish age- and sex-dependent reference values for creatine and GAA in our region, and compare them with previous literature.

Methods

Retrospective observational study between Jan 2013 and Jun 2020. Data were collected from the Laboratory Information System (GestLab). Individuals with neurological pathology related with a significant increase/decrease in creatine and GAA levels were excluded, along with those due to poor sample preparation or previous diet.

Two age groups were studied: <6 and ε6 years old, for both genders. Each group included a minimum of 550 individuals. For the establishment of reference limits, percentiles 1 and 99 were calculated.

Both biomarkers were measured by high-pressure liquid chromatography (Shimadzu Scientific US).

Values were compared using the Student's t-test (alpha error was set at 5%). Results were compared with literature.

Results

1380 individuals were included. No sex-differences were seen, so only age-dependent reference limits are presented. For children with age <6 years: the creatine and GAA upper and lower limits were 2.00-0.03 and 173.80-13.10, respectively. In case of people with age ε 6 years: the creatine and GAA upper and lower limits were 1.76-0.03 and 138.50-11.50, respectively. Statistic differences were seen between age-dependent groups for each biomarker (p-value <0.001).

Conclusions

Our intervals are in agreement with those previously reported. A decrease is seen in both GAA and creatine levels with age, although no differences were seen between boys and girls.

Implementation of these values will allow a more accurate management of creatine deficiency syndromes.

doi: 10.1016/j.cca.2022.04.073

M192

Preterm pre-eclampsia: Predictive capacity of different commercial tests (PLGF, SFLT-1/PLGF ratio)

J.A. Delgado RodrÍguez[a], J.M. BauÇa[a], M.B. Badal Cogul[a], M.A. Ballesteros Vizoso[a], D. Morell GarcÍa[a], M.D.M. MÀs Morey[b], M. Vila CortÉs[b], M.I. Pastor GarcÍa[a]

[a] *Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain*
[b] *Department of Obstetrics and Gynecology, Hospital Universitari Son Espases, Palma de Mallorca, Spain*

Background-aim

Circulating levels of anti/angiogenic factors may be used as biomarkers for the diagnosis of preeclampsia (PE). The aim of this study was to compare the diagnostic capacity of two immunoassays for the measurement of placental growth factor (PlGF) by PerkinElmer (PkE) and Roche (Rc), and the soluble fms-like tyrosine kinase-1 (sFlt-1) by Rc for early PE (defined as <34 weeks of pregnancy) and preterm PE (defined as <37 weeks of pregnancy).

Methods

Retrospective observational study performed on data extracted from the laboratory information system (GestLab). We included pregnant women with a suspicion of PE according to American College of Obste-

tricians and Gynecologists's criteria with a measurement of PlGF (PkE), PlGF (Rc) and sFlt-1/PlGF (Rc) before 37 weeks of pregnancy. Only results for the first measurement were considered for the performance of ROC curves and the calculation of optimal cut-off values. Areas under the curve (AUCs) were compared using the method described by DeLong. Sensitivity (Se) and specificity (Sp) were calculated for each biomarker.

Results

Fifty-one pregnant women were included, from which 32 presented preterm PE (13 with early PE). AUCs (and 95% CI) for early PE were 0.85 (0.690-0.951), 0.850 (0.686 -0.949) and 0.857 (0.694-0.953) for PlGF (PkE), PlGF (Rc) and the ratio sFlt-1/PlGF, respectively. In turn, AUCs (and 95% CI) for preterm PE were 0.740 (0.598-0.853), 0.743 (0.602-0.855) y 0.755 (0.614-0.864), respectively. No statistical differences were seen among the 3 biomarkers for both diagnoses.

The optimal cut-off values (and 95% CI) for early PE were PlGF (PkE) $<$60pg/ml [Se: 0.846 (0.537-0.973); Sp: 0.667 (0.431-0.845)], PlGF (Rc) $<$100pg/ml [Se: 0.846 (0.537-0.973); Sp: 0.667 (0.431-0.845)] and sFlt-1/PlGF $>$38 [Se: 0.846 (0.537-0.973); Sp: 0.667 (0.431-0.845)]. For preterm PE: PlGF (PkE) $<$115pg/ml [Se: 0.781 (0.596-0.901); Sp: 0.667 (0.431-0.845)], PlGF (Rc) $<$150pg/ml [Se: 0.781 (0.596-0.901); Sp: 0.684 (0.435-0.864)] and sFlt-1/PlGF $>$27 [Se: 0.750 (0.562-0.879); Sp: 0.789CI:0.539-0.930)].

Conclusions

The three biomarkers show a similar diagnostic capacity, which is higher for early PE. The inclusion of sFlt-1 does not increase the diagnostic value over PlGF, while it implies higher health spending.

doi: 10.1016/j.cca.2022.04.074

M193

Clinical performance of the standard F COVID-19 AG FIA for the detection of SARS-COV-2 infection

S. Jirungda [a], P. Khamphrom [b], K. Klaprajun [b], T. Prachakul [a], J. Kraisin [c]

[a] *Biomolecular Laboratory, Department of Medical Technology and Clinical Pathology, Saraburi Hospital, Thailand 18000*
[b] *Clinical Chemistry Laboratory, Department of Medical Technology and Clinical Pathology, Saraburi Hospital, Thailand 18000*
[c] *Clinical Hematology Laboratory, Department of Medical Technology and Clinical Pathology, Saraburi Hospital, Thailand 18000*

Background-aim

The Coronavirus disease 19 (COVID-19) pandemic continues to spread globally. An accurate, rapid, and simple diagnostic tool for detection of contagious individuals with SARS-CoV-2 infection is therefore an essential supplement for molecular testing. This study aimed to assess the clinical performance of the STANDARD F COVID-19 Ag FIA.

Methods

A rapid SARS-CoV-2 detection assay, the STANDARD F COVID-19 Ag FIA (SD Biosensor®, Republic of Korea), was compared with a real-time RT-PCR assay, Allplex 2019-nCoV Assay (Seegene®, Republic of Korea), for the detection of SARS-CoV-2 from 423 COVID-19 suspected cases and contacted individuals at Saraburi Hospital, Saraburi, Thailand during 22 and 29 July, 2021. A pair of nasopharyngeal swabs was obtained; one was tested by the Standard F COVID-19 Ag FIA and the other by real-time RT-PCR assay.

Results

Of these 423 samples, 74 (17.5%) were detected to be positive, and 349 (82.5%) were negative from SARS-CoV-2 by the STANDARD F COVID-19 Ag FIA. It was determined that 78 (18.4%) were detected to be positive, and 345 (81.6%) were negative by the real-time RT-PCR assay. Overall, the specificity, sensitivity, positive predictive value and negative predictive value of the STANDARD F COVID-19 Ag FIA was 98.8%, 89.7%, 94.6%, and 97.7%, respectively.

Conclusions

The rapid antigen test for SARS-CoV-2 observed comparable sensitivity and specificity with the real-time RT-PCR assay. Therefore, the routine usage of the rapid antigen screening assay for SARS-CoV-2 infection maybe practical when high volumes of samples are tested in a hospital setting.

doi: 10.1016/j.cca.2022.04.075

M194

Establishment of community based fructosamine reference interval for apparently healthy adults in Addis Ababa, Ethiopia

T. Getahun [e], D. Bikila [e], Z. Geto [a], H. Wossen [e], T. Lejisa [e], Y. Tolcha [e], C. Bashea [e], M. Meles [e], G. Ashebir [e], O. Mohammed [a], M. Kassaw [c], A. Kebede [d], L. G/egzeabher [b], S. Kinde [b], F. Challa [e], A. Tsegaye [b]

[a] *Department of Biomedical Science, College of Medicine and Health Science, Wollo University, Wollo, Dessie, Ethiopia*
[b] *Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia*
[c] *Food Science and Nutrition Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia*
[d] *National Capacity Building Directorate, Ethiopian Public Health institute, Addis Ababa, Ethiopia*
[e] *National References Laboratory for Clinical Chemistry, Ethiopian Public Health Institute, Addis Ababa, Ethiopia*

Background-aim

Reference intervals are fundamental tools to interpret laboratory results in diagnostic, prognostic, and efficient utilization of overall healthcare resources particularly in resource-limited countries like Ethiopia. The absence of locally derived reference intervals from apparently healthy subjects for the local population will impact on the physician, laboratorians, and researchers. To establish Reference Interval for fructosamine for apparently healthy adults in Addis Ababa, Ethiopia.

Methods

A cross sectional study was conducted in four selected sub-cities (Akaki, Kirkos, Arada, Yeka), Addis Ababa, Ethiopia from December 2019 to June 2020. The study was including study participants with 18 and above years of age. Socio-demographic data were obtained from the national reference interval study database. Blood sample was collected and analyzed by using Cobas 6000(c 501) instrument. Both Kolmogorov–Sminorv, and Mann-Whitney test were analyzed to check the data normality distribution and partitions, respectively. Reference inter-

vals, lower 2.5th percentile and 97.5th percentile for the upper, (overall, and specific for sex, age, and body mass index) were determined using non-parametric methods.

Results

Out of the total 344 participants, 256 (121 males, 135 females,) study participants aged 18-60 years participated in this study. Reference interval was established for fructosamine from 203 - 321umol/L.

Conclusions

This study established reference intervals for fructosamine for adult population using the current available equipment platform. The established reference interval is applicable for the current practice in the country to diagnosis, treatment, and monitor of diabetics mellitus.

doi: 10.1016/j.cca.2022.04.076

M195

Hemoglobin A1C by Abbott diagnostics afinion 2 versus refence laboratory method – Method correlation studies

D. Syed [a], E. Richards [a], S. Simpson [b]

[a] *Laboratory Department, Ryan Health Network, New York, NY 10025 USA*
[b] *Nursing Department, Ryan Health Network, New York, NY 10025 USA*

Background-aim

Introduction: Ryan Health Network comprises of six major community health centers and various clinics in shelters and schools including community based Mobile units. Overall, Ryan Health provides comprehensive health services at 17 locations. It is a nonprofit organization and receives funding both from public and private organizations. All locations are in the Borough of Manhattan in New York City and perform waived tests under Limited Service Laboratory permits. Ryan Health has a very active Diabetic Education Program. Currently all the patients enrolled in the program get their glucose levels tested upon their visit to the respective center using Roche Diagnostics AccuChek Inform II Glucose Monitoring System while Hemoglobin A1c, a better long-term marker of glucose level over time in the body, was sent to the reference laboratory. This delayed the clinical intervention if needed on the day of the visit to better control patient's glucose level for good metabolic control. To provide our patients better care at the time of visit we decided to bring this test in-house. We chose Afinion 2 based on price, time it takes to test a patient, test cost, instruments maintenance and time it takes to test a patient. Besides all this we also wanted to ensure that a} . the results obtained would be comparable to the reference laboratory results, b). Since we would be using the capillary blood, we wanted to ensure that the result obtained by using Finger stick capillary blood will match those obtained by the reference laboratory on lavender top (LT) tube, c) that there was a good correlation between the Afinion 2 system and the reference laboratory results d). that there was no significant difference among the results obtained on all Afinion Test systems placed at different locations throughout Ryan Network as some of our patients could visit different locations. In this exercise we tried to answer the above questions.

Methods

Afinion HbA1c is a fully automated boronate affinity assay. The Afinion 2 HbA1c test cartridge contains all the reagents necessary for the determination of the assy. The respective samples whether capillary blood or the venous collected in LT tube were obtained with patient's consent, when necessary, with the appropriate sampling devices. 1.5 uL of the respective sample was then collected with the integrated device before the test cartridge was placed in the cartridge chamber of the Afinion 2 Analyzer. The sample then went through series of step involving release of hemoglobin from the erythrocytes, precipitation of hemoglobin, transferred to a blue boronic acid conjugate which bound to cis diols of the glycated hemoglobin. The reaction mixture then was soaked through a filter membrane and all the precipitated hemoglobin, conjugate -bound and unbound remained on the membrane. Any excess was moved with a washing reagent. If needed duplicate samples collected at the same time were sent to the reference laboratory. Once the test was complete the result would be displayed and then printed.

Results

Results and Data Analysis:
All experiments were completed, and the results collected and tabulated. They were analyzed using EP Evaluator 5. The correlation between the Finger stick Capillary blood analyzed on Afinion2 results and those obtained by the reference laboratory showed a good correlation between the two as revealed by the following calculated correlation parameters, Correlation coefficient = 0.9973, slope of line Deming 0.978 (0946-1.010), Intercept (Deming) = -0.05 (-0.30 to 0.20), SEE (Deming) = 0.17, Bias -0.22, X mean = 7.46 ± 2.31, and Y mean 7.25 ± 2.26 within allowable error limits of 0.5% or 10%. The comparison results of all five instruments are tabulated in table 1. There was no significant difference in HbA1c results among the 5 instruments as depicted in Figure 1. All data fit in the inner circle.

Conclusions

Acceptance criterion between the reference laboratory results and those obtained inhouse on the Afinion 2 was set in consultation with the clinicians to be between ± 0.0 – 0.3. Our correlation studies revealed that there is no significant difference between the Afinion 2 HbA1c results and the reference lab HbA1c results. This is true for both types of samples, the lavender top venous blood and the capillary(fingerstick) venous blood. Instrument to instrument bias was also not significant as depicted in figure 1. We feel confident that Afinion 2 has produced comparable, accurate and reliable results to our reference laboratory without having to adopt to new reference ranges. Availability of HbA1c testing inhouse has greatly improved the control of Diabetes mellitus in patients enrolled in our Diabetic Education Program. For results within the acceptance criterion, we also observed that the test cartridge after sampling should be placed in the chamber of the Afinion 2 within 40 seconds.

doi: 10.1016/j.cca.2022.04.077

M196

Performance of next generation clinical chemistry assays

M. Berman, S. Gawel

Core Diagnostics, Abbott Labs, Illinois, USA

Background-aim

Sigma metrics are valuable for benchmarking and comparing different laboratory assays or comparator systems. A Sigma metric compares the precision and bias performance of the assay to the laboratory total

allowable error (TEa) goal. This study evaluated the Sigma performance of 10 ARCHITECT and 10 Alinity c system clinical chemistry assays across several therapeutic areas. In addition, reference standards were used to establish accuracy and bias to ensure traceability to an established reference.

Methods

A sigma metric was calculated for the following assays – Alanine Aminotransferase2, Aspartate Aminotransferase2, Alkaline Phosphatase2, Creatinine2, Total Bilirubin2, Gamma Glutamyl transferase2, Iron2, Lactate Dehydrogenase2, Triglyceride2 and Urea Nitrogen2. The data were plotted together on a single method decision chart. The sigma metric was calculated using the equation: sigma = (TEa – [bias])/precision. The TEa goal was based on a recognized standard (e.g. Clinical Laboratory Improvement Amendments (CLIA), Rili-BAEK); precision was based on the within-laboratory standard deviation (SD) or percent coefficient of variation (%CV) for the sample with a concentration closest to a medical-relevant concentration level as estimated from a 20-day study performed per Clinical and Laboratory Standards Institute (CLSI) EP05-A2; bias was based on the difference from the predicted value of a reference.

Results

The next generation clinical chemistry assays showed excellent precision (</= 3.5%) and bias (</= 5.5%) for a sample near the medical decision point. A majority of the ARCHITECT and Alinity c clinical chemistry assays demonstrated at least 6-sigma performance and all of the assays had at least 4-sigma performance at a medical-relevant concentration level.

Conclusions

Sigma metrics are a powerful tool for selecting the most sensitive assays and allowing for comparison of assay performance across platforms and vendors. Sigma metrics of 6 or higher signify world-class assay performance, a key objective for Abbott during the assay design process. The exceptional performance of the ARCHITECT and Alinity c next generation clinical chemistry assays tested in this study reflect this commitment to world-class assay design and performance.

doi: 10.1016/j.cca.2022.04.078

M197

Performance evaluation of three automated high performance liquid chromatography HBA1C analysers

M.H. Lim, B.M.Q. Koh, C.P. Yeo

Clinical Biochemistry Section, Department of Clinical Pathology, Singapore General Hospital, Singapore

Background-aim

With a steadily rising prevalence over the last few decades, Diabetes Mellitus (DM) has become a major public health problem worldwide. Poorly controlled DM can lead to complications in a multitude of organs and increase the risk of premature death, bringing about substantial economic costs. A key strategy to combat the disease involves improving the accessibility of care to DM patients through primary care-oriented programs to facilitate early detection and ongoing monitoring, and prevent the development of diabetic complications. Due to its stable nature and ability to reflect long-term glycaemic control, glycated haemoglobin (HbA1c) has become an important laboratory test that is increasingly preferred to blood glucose for the diagnosis and monitoring of DM. We evaluated three ion-exchange high performance liquid chromatography (HPLC) HbA1c analysers Bio-Rad D10 (D10), Bio-Rad D100 (D100) and Tosoh G11 (G11) as a replacement for our current Siemens DCA Vantage analyser in a primary care setting.

Methods

Imprecision, linearity and carryover were assessed in accordance with our laboratory's method evaluation protocol, adapted from relevant Clinical and Laboratory Standards Institute (CLSI) guidelines. 120 patient samples with HbA1c concentration between 4.7% to 14.0% were analysed on the three analysers and our existing central (Roche Cobas e601) and satellite laboratory (Siemens DCA Vantage) analysers. Accuracy was evaluated using samples with assigned values provided by the Health Sciences Authority Singapore HbA1c External Quality Assurance Program. The Royal College of Pathologists of Australasia (RCPA) allowable limits for HbA1c was set as the acceptance criteria to assess method differences and accuracy. As either capillary or EDTA samples may be used for HbA1c analysis in our primary care facilities, the mean difference between the Haemoglobin Capillary Collection System (HCCS) and EDTA tube was studied using samples collected from 53 volunteers. Turn-around-time (TAT) for each analyser was assessed by recording the time taken to analyse a batch of 20 samples. All statistical analyses were done on Excel using the Analyse-it software v5.90.

Results

Total imprecision across the three analysers range between 0.9-1.5%, with within-run imprecision between 0.6-1.2%. Manufacturers' analytical measuring ranges were verified and no significant carryover was observed. Relative mean difference observed for D10, D100 and G11 against Cobas e601 were 1.98%, 0.03% and 0.33% respectively, while that stood at -0.89%, -2.84% and -2.53% against DCA Vantage. On Passing-Bablok regression, the gradient of the slope ranged from 0.95 to 1.00, with an intercept between -0.03 to 0.19. Spearman correlation coefficient recorded between 0.97 to 0.99. For accuracy assessment, the bias observed ranged between -1.10% to 4.34% across the three analysers. Minimal differences were (-0.21% to 0.34%) seen between the HCCS and EDTA samples on all three analysers. The time taken to analyse a batch of 20 samples on the D10, D100 and G11 were 68, 18 and 21 minutes respectively.

Conclusions

All three analysers achieved an optimal imprecision goal of δ2% and demonstrated good accuracy. Comparison studies exhibited acceptable differences with our existing Cobas e601 and DCA Vantage analysers. In addition, the HPLC platforms offered the advantage of detection of haemoglobin variant interference over the immunoassay methods. All three analysers were found to be suitable alternatives to replace our Siemens DCA Vantage platform, though the D100 and G11 demonstrated superior TAT over the D10.

doi: 10.1016/j.cca.2022.04.079

M198

Performance verification of CA 19-9 on the Siemens advia centaur analyzer

N. Bano, S. Ahmed, L. Jafri

Aga Khan University, Karachi, Pakistan

Background-aim

CA 19-9 antigen has been identified in patients with colorectal, pancreatic, bile duct, hepatocellular, stomach, and esophageal cancers. Method standardization directly affects result accuracy, which in turn affects patient's outcome. Regulatory authorities as Clinical and Laboratory Standards Institute (CLSI), recommends method validation studies to quantifiably characterize system performance, assess potential for error and identify method-to-method differences. So this analytical study was executed to evaluate the two immunoassay methods Siemens Advia Centaur and Immulite 2000 for CA 19-9 (Carbohydrate Antigen) measurement.

Methods

A method validation study was performed at section of Chemical Pathology, Department of Pathology & Laboratory Medicine, AKU. Serum samples were simultaneously analyzed on the two immunoassays for CA19-9 to evaluate imprecision, linearity and method comparison. Precision was done by analyzing 3 control level 20 times. For assessing linearity and analytical measurement range 4 samples covering the entire measurement range of the respective instruments were analyzed in triplicates. For method comparison 40 serum samples from patients and 3 specimen of proficiency testing material by College of American Pathologist were analyzed. Statistical analysis was done using Microsoft Excel and EP Evaluator version 10.3.0.556 (Data innovations, LLC).

Results

The coefficient of variation (CV) for the control levels on Advia Centaur were 3.6%. Centaur was linear over a range of 1.2 to >700 U/ml while Imnmulite was linear from 2.5 to 1000 U/ml. Centaur depicted better extended analytical measurement range i.e. 140000 compared to 100000 U/ml of Immulite. Manually performed dilution verification & automatic dilution,results found within acceptable limits. For method comparison the allowable systematic error was 15.0% with slope 0.998 intercept 0.544 and correlation of 0.9988.

Conclusions

Advia Centaur showed better performance specifically for analytical sensitivity, while it is less time consuming making it a comparable substitutes to convetional utilized Immulite 2000 method.

doi: 10.1016/j.cca.2022.04.080

M199

Enhancing refiner – Improving performance of reference interval estimation for skewed distributions

T. Ammer[a], A. Schützenmeister[d], H.U. Prokosch[a], M. Rauh[b], J. Zierk[b,c], C.M. Rank[d]

[a] *Chair of Medical Informatics, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany*

[b] *Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Germany*

[c] *Center of Medical Information and Communication Technology, University Hospital Erlangen, Erlangen, Germany*

[d] *Roche Diagnostics GmbH, Penzberg, Germany*

Background-aim

Precise reference intervals are essential for the interpretation of laboratory test results in medicine. The current gold standard is to determine the central 95% range of test results from apparently healthy subjects (direct method). However, this method is practically, logistically and ethically challenging. Recently, we published a novel indirect method for the estimation of reference intervals using real-world data (RWD), refineR. In the published simulation study, refineR outperformed another indirect method (kosmic) and the direct method with N = 120. For RWD, the algorithm achieved results comparable to the direct methods. However, performance of refineR for heavily skewed distributions has not been studied so far.

Methods

We provide an update to the refineR algorithm that accounts for challenges associated with skewed distributions. First, we adapted the definition of the region of test results that characterizes the main peak by utilizing the peak of the input data in combination with a linear fit to both sides of it. Second, we introduced a more fine-grained search region for lambda. Third, we adapted the cost function by introducing a factor to account for measurement imprecision in RWD and a term to account for the number of selected bins within the data peak. Last, for skewed, shifted distributions, we added the option to use the modified Box-Cox transformation. The updated version was applied to the simulated test cases published for refineR 1.0 and to two simulated heavily skewed distributions (C-reactive protein, CRP and Immunoglobulin E, IgE).

Results

The updated refineR algorithm substantially improves the performance of reference interval estimation for heavily skewed distributions, with a considerable reduction in median percentage error from 50% (refineR 1.0) to 11% (enhanced version) for CRP and from 31% (refineR 1.0) to 19% (enhanced version) for IgE. Performance for the previously published simulation studies was comparable to that of refineR 1.0 (overall median percentage error of 1.64% (enhanced version) compared to 1.53% (refineR 1.0)), still outperforming kosmic and the direct method with N = 120.

Conclusions

We present an update to the refineR algorithm, which improves the estimation of reference intervals from RWD for skewed distributions.

doi: 10.1016/j.cca.2022.04.081

M200

Evaluation of eight commercially available clinical chemistry assays on the ALINITY® C system

H. Li, M. Li, J. Wang, P. Sun, H. Guo, P. Yin

Abbott Core Diagnostics, Shanghai, China

Background-aim

Expansion of instrument analyte menus is often needed to accommodate country specific needs. To address specific needs in China, eight clinical chemistry assays have been modified for evaluation on the Alinity c system. These analytes are used in the investigation of several diseases including lipid metabolism disease, liver disease, cardiovascular disease, kidney disease, malignant tumor, and glucose metabolism disease.

Methods

The following analytes were evaluated: Cystatin C (CysC), Homocysteine (HCY), Retinol binding protein (RBP), Adenosine deaminase (ADA), Small dense Low-Density Lipoprotein (sd-LDL), Leucine aminopeptidase (LAP), ®-hydroxybutyric acid (®-Hb), and sialic acid (SA). Reagents from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT Alinity c system. Precision, accuracy, LoQ, and linearity range were evaluated with guidance from CLSI documents EP15-A3, EP06-Ed2 and EP17-A2. Correlation to Jinya assays from BSBE on ARCHITECT c system was performed using human serum/plasma/urine samples across the measuring range of each analyte.

Results

In the studies, all assays demonstrated good performances. Within run CVs ranged from 0.7% to 8.2%; linearity correlations were greater than 0.9967; and correlation coefficients were greater than 0.988. LoQs were 0.03 mg/L for CysC, 0.44 μmol/L for HCY, 0.8 mg/L for RBP, 0.34 U/L for ADA, 0.21 mg/dL for sd-LDL, 0.08 U/L for LAP, 0.02 mmol/L for ®-Hb, and 3.89 mg/dL for SA.

Conclusions

These initial results are very promising. All the assays tested exhibit good precision, accuracy, linearity, anti-interference, and LoQ performances on Alinity c system. In addition, they also have excellent correlation with Jinya assays from Beijing Strong Biotechnologies Inc. (BSBE) on ARCHITECT system.

doi: 10.1016/j.cca.2022.04.082

M201

Is it reliable to establish the critical difference for laboratory tests based on the opinion of the clinician?

S. Kim [c], J. Lim [a], J. Chung [b], J. Chang [c], H.S. Lim [d]

[a] *Department of Laboratory Medicine, Chungnam National University Hospital, Daejeon, 35015, Korea*
[b] *Department of Laboratory Medicine, Dongguk University Ilsan Hospital, Goyang, 10326, Korea*
[c] *Department of Laboratory Medicine, Inje University Ilsan Paik Hospital, Goyang 10380, Korea*
[d] *Department of Laboratory Medicine, Seoul Clinical Laboratories, Yongin, 16954, Korea*

Background-aim

Clinicians aim to answer the question, “How reliable are these results?” while comparing laboratory results to medical decision levels, assessing if differences in serial data from a patient are significant, and making other patient care decisions. Laboratorians also want to know, “Does my measuring process fulfill the accuracy goals for clinical performance?” Although there are no commonly agreed criteria for determining the degree of a difference in laboratory results, the hierarchy of approaches to establishing criteria for analytical performance was proposed by a consensus conference of ISO Technical Committee 212, the International Federation of Clinical Chemistry and Laboratory Medicine, the World Health Organization, and the Clinical Chemistry Section of the International Union of Pure and Applied Chemistry, in order of preference. Although acceptance criteria based on well-designed, clinical outcomes studies are the highest level for evaluating comparability testing, the clinical outcome studies are difficult to conduct; therefore, there are few examples in laboratory medicine. The second-highest strategy is to conduct a questionnaire survey of doctors to identify their expectations for analytical quality, which would offer them confidence in patient management. However, since this approach still requires a rigorous methodology to define its analytical correlation with clinical scenarios, there are few studies. Therefore, we decided to set up a critical difference through a clinical doctor survey on the most frequently conducted health examination items.

Methods

The survey was conducted on doctors who dealt with health examination laboratory tests including members of the Korean Society of Medical Health Screening through an online survey platform for a total of 25 days from August 12 to September 5, 2021. A questionnaire for clinicians was conducted in two scenarios for the ‘important clinical judgment concentration’ of 27 common health examination items. Scenario 1 assumes that men in their 50s without underlying diseases undergo regular medical checkups once a year. Respondents selected this year’s results that they thought were “clinically meaningful change” from last year’s results which were within the normal range. Scenario 2 assumed that a 50-year-old man without an underlying disease visited the hospital a month later for a re-examination, saying he had abnormal figures after undergoing a checkup at another hospital. Respondents selected the results of this hospital that they thought were ‘clinically meaningful change” from the other hospital’s results. Medically useful criteria for the analytic performance of laboratory tests (MUCV) were calculated according to the Skendzel (1985) method.

Results

A total of 290 doctors, including 104 family medicine and 93 internal medicine doctors, responded. Of these, 22.4% answered that they only perform health check-ups, and 37.6% said they devoted more than half to health check-ups. In two scenarios, MUCV was calculated for 27 inspection items. In scenario 1, MUCV was in the range of 6.09% (for Hb 15.0 mg/dL) to -54.44% (for TSH 1.80 uIU/mL) according to the test items. In scenario 2, MUCV was in the range of 2.07% (for Hb 15.0 mg/dL) to 66.43% (for TSH 0.10 uIU/mL) according to the test items. The MUCV in scenario 1 tended to be larger than the MUCV in scenario 2. For example, MUCV was -7.40% and 6.09% for Hb 15.0 mg/dL in Scenario 1 vs MUCV was -3.46% for Hb 12.8 mg/dL and 2.07% for Hb 17.2 in Scenario 2.

Conclusions

The MUCV of the same test item was different according to the two scenarios. No matter how well-designed research is based, it seems not useful to set up a MUCV based on a clinician’s questionnaire.

doi: 10.1016/j.cca.2022.04.083

M202

Increase operation efficiency by setting beta-hydroxybutyrate in automatic chemistry analyzer

C. Kaewsin, I. Chaowaleard

Clinical Chemistry and Endocrine Section, Department of Clinical Pathology and Medical Technology, Rajavithi Hospital

Background-aim

Beta-Hydroxybutyrate (BHB) is the most predominant ketone presents during diabetic ketoacidosis (DKA). Determination of serum BHB is useful for diagnosis and monitoring of severe diabetic complications. BHB determination using point-of-care testing (POCT) is rapid and widely used but required several manual operations which waste staff manpower. This study aimed to evaluate the analytical performance of commercially BHB reagent on automatic chemistry analyzer by compared the outcome with current method.

Methods

Assay parameter of commercially BHB reagent (Stanbio Laboratory) was setup in an open channel of Alinity c automatic chemistry analyzer (Abbott Core Laboratory). Precision and accuracy studies were performed using quality control materials (low, normal, and high levels). The precision of assay was performed based on the Clinical and Laboratory Standards Institute (CLSI) EP5-A2 guideline and acceptable criteria are coefficient of variations (CV) $<4.600\%$ for within-run and $<6.133\%$ for between-run precision. The %bias was calculated to determine accuracy and acceptable criteria is bias $<18.4\%$. The linearity standard at the concentrations of 0, 0.5, 1, 2, 4, and 8 mM were used to evaluate linearity of the assay. Proficiency testing was analyzed by the Randox International Quality Assessment Scheme (RIQAS) clinical chemistry program. Process mapping was drawn to compare working step between automatic chemistry analyzer and current method. The working hour of current method was calculated.

Results

The results showed that CVs for within-run and between-run precision were 0.520-2.624% and 0.649-3.014%, respectively, which not exceeding acceptable criteria. The bias at low, normal and high BHB levels were 9.380%, 2.250% and 4.120%, respectively. Linearity range was 0 - 8 mM. Six consecutive RIQAS evaluation results shown excellent performance. Using BHB assay on automatic chemistry analyzer was able to reduce 70% working step and 360 working hours per year.

Conclusions

We successfully setup an analytical performance of BHB in the open channel of Abbott Alinity c automatic chemistry analyzer. Performing BHB on automatic chemistry analyzer improve operation efficiency by reducing workload 12.5% of one staff when compared with current workload.

doi: 10.1016/j.cca.2022.04.084

Clinica Chimica Acta 530 (2022) S297–S324

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Microbiology, InfectionDisease

W056

Utility of nucleic acid amplification test in the detection of tuberculosis in biological fluids from presumptive tuberculosis patients in a cardiovascular center in the Philippines

O.P. Balisan, L. Cale-Subia, A. De Luna

Philippine Heart Center

Background-aim

Tuberculosis (TB) is currently ranked as the eighth leading cause of mortality in the Philippines. It also ranks as one of the leading causes of morbidity in the country. Recent studies suggest accuracy and high specificity of Nucleic Acid Amplification Test (NAAT) for TB, which confer to its ability to rule-in the disease. In this study, the utility of NAAT was evaluated in the detection of TB in biological fluids from presumptive TB patients.

Methods

A total of 85 biological fluid specimens (e.g. pleural, pericardial, peritoneal, cerebrospinal fluid, etc.) from presumptive TB patients were examined microscopically by using Ehrlich Ziehl Neelsen (ZN) staining method, and also inoculated on Löwenstein Jensen (LJ) medium for culture. We compared NAAT and ZN smears, and the sensitivity, specificity, positive predictive value and negative predictive value were calculated according to LJ culture method accepted as the gold standard.

Results

Using the LJ culture as the gold standard for detection of TB, NAAT showed sensitivity of 66.67% compared to 9.09% for ZN smears, specificity of 93.67% compared to 98.65% for ZN smears, with a positive predictive value (PPV) of 44.44% and negative predictive value (NPV) of 97.37%.

Conclusions

It is concluded that NAAT is an efficient and consistent diagnostic test for TB detection. NAAT showed more sensitivity compared to ZN smear microscopy in the screening of TB in biological fluids from presumptive TB patients. However, due to a comparably lower sensitivity than previous studies reviewed, the utility of NAAT alone may not be sufficient. The combined use of culture and NAAT is superior than utilizing either alone.

doi: 10.1016/j.cca.2022.04.794

W057

Study of antibiotic activity in urine of pediatric patients and its influence on the urine culture

R. Rubio Sánchez, E. Lepe Balsalobre, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville

Background-aim

Urinary infection is very common in pediatrics and represents a diagnostic challenge, since the only manifestation is usually the presence of fever without an obvious focus. The objective of this study was to determine the presence of antibiotic activity in urine samples of pediatric patients and assess their possible influence on the clinical management of these patients.

Methods

Observational and prospective study conducted between September 2018 and February 2019. 138 urine samples from patients under 5 years of age who went to the Emergency Department of our hospital for febrile syndrome were included.

All the samples were carried out a biochemical study using a test strip and those with a pathological result also underwent a urinary sediment study. The use of antibiotics prior to sampling was evaluated by measuring antibiotic activity in the urine.

Antibiotic activity was determined by bioassay, impregnating a filter paper disk with urine that was placed on a Mueller-Hinton agar plate inoculated with antibiotic-sensitive Bacillus subtilis. After 24 hours of incubation at 35-37 ° C, growth inhibition around the disk was considered as evidence of antimicrobial activity in the urine.

Results

The bioassay was positive in 18 samples, of which there was only one microbiological growth in the culture; all these patients had taken

antibiotics before taking the sample. The bioassay was negative in 120 samples, with microbiological growth in 15 of them; 12 of these patients had taken antibiotics before taking the sample.

Conclusions

The presence of antibiotics in urine may explain the low performance of the urine culture in the analyzed samples. In many cases there is an antibiotic administration prior to taking the sample that could interfere with the result of the cultures.

The biochemical parameters of the urine, such as the presence of more than 25 leukocytes per field and / or positive nitrites, should alert the possibility of bacterial infection and be taken into account in the management of patients.

The absence of antibiotic activity in 12 of the patients´ urine with previous administration of antibiotics reveals the importance of considering their pharmacokinetics. This could be due to a concentration of the drug below the limit of detection of the bioassay, the absence of urinary excretion or the complete elimination at the time of taking the sample.

doi: 10.1016/j.cca.2022.04.795

W058

Salmonella infection among the suspected enteric fever patients

S. Santosh Kumar Gupta

Pokhara University, Kaski, Nepal

Background-aim

The main aim of research is to find out the prevalence of enteric fever and resistance pattern of Salmonella in blood samples from patients visiting hospital.

Methods

A prospective study was carried out at Everest Hospital, Kathmandu, Nepal from April 2015 to December 2015 on patients having a febrile episode lasting for ≥ 3 days presenting them at Everest Hospital requesting for blood culture and susceptibility testing.

Cases included in the study were patients defined by physicians as probable case of enteric fever with fever (38°C and above) that has lasted for at least three days and showing clinical signs and symptoms of enteric fever.

A total of 692 blood samples from patients following case definition of suspected enteric fever were included in the study. Samples with improper labeling, insufficient blood volume, and inappropriate collection and transport were rejected.

Results

A total of 692 enteric fever suspected febrile cases were included in which 371(53.6%) were male and 321(46.4%) were female. Among the total 692 blood samples collected for culture, the highest number of cases 210 (30.3%) were from the age group 21-30 years, followed by 150 (26.4%) cases from the age group 11-20 years.Out of the 692 enteric fever suspected cases investigated, 40 (5.8%) samples were found to be culture positive and 652 (94.2%) were found to be culture negative. The overall incidence of enteric fever cases was 58 per 1000 cases

Conclusions

Enteric fever remains an important public health problem in Nepal. The study revealed that the prevalence of enteric fever was found to be the most common among males than in females and highly observed among 11-20 years of patients. The prevalence of Salmonela enterica serovar Typhi was found to be relatively higher than Salmonella enterica serovar Paratyphi A, among significant growth obtained from blood culture. In this study, increased resistance of the isolates was recorded towards amoxycillin and the results also suggest that chloramphenicol and cotrimoxazole were most effective antibiotics. This study was conducted with a motive to evaluate the emerging threat of antibiotic resistance especially in first generation quinolone and fluoroquinolone group of antibiotics toward the causative agents of enteric fever, isolated during the course of study from the patients requesting for blood culture having febrile symptoms. During the course of study high rate of nalidixic acid-resistant S. Typhi and S. Paratyphi A but low rate of resistance with remaining quinolone antibiotics has been recorded. So, higher rate of nalidixic acid resistance as well as resistant pattern with other antibiotics suggests, great improvisation should be done while administering antibiotics otherwise it may cause bizarre problem in the effective empirical treatment of enteric fever.

doi: 10.1016/j.cca.2022.04.796

W059

Identification of volatile biomarcators of infection by giardia sp. in pediatric patients with persistent diarrhea

E. Lepe Balsalobre , R. Rubio Sánchez, M.d.M. Viloria Peñas, A. Moro Ortiz

Virgen de Valme University Hospital, Seville

Background-aim

Pediatric chronic diarrhea syndrome is a very common pathology that is difficult to diagnose, especially when etiology is infectious (persistent diarrhea). For this reason, laboratory techniques for diagnosis have been diversified, with gas chromatography - mass spectrometry (GC-MS) very useful.

The objective of this study was to detect the presence of volatile biomarkers of Giardia sp. infection in pediatric patients with persistent diarrhea.

Methods

17 stool samples from pediatric patients with symptoms of persistent diarrhea were analyzed. Of these, 7 had infection with Giardia sp. and 10 had diarrhea of non-parasitic cause.

The static head space extraction method was performed. The analyzes were carried out on a gas chromatograph coupled to a triple quadrupole mass spectrometer.

Results

25 volatile compounds were determined: 8 esters, 6 alcohols, 6 terpenes, 2 sulfur compounds, 1 amide, 1 acid and 1 aldehyde. The esters were found in only 2 of the 10 patients with diarrhea of non-parasitic etiology, but in all patients with Giardia sp. Acetic acid and alcohols (1,3-dimethoxy-2-propanol and 1,4-dimethoxy-2,3-butanediol) only appeared in patients with giardiasis. Sulfur compounds (dimethyl disul-

fide and dimethyl trisulfide) were only found in patients with diarrhea without parasitic etiology.

Conclusions

The method of extraction of volatile compounds has been shown to have sufficient and effective sensitivity to discriminate between stool samples of patients not infected with the parasite from those that were.

Sulfur compounds come from the metabolism of L-cysteine (essential amino acid for the survival of Giardia sp.). The absence of these compounds in patients with giardiasis is due to the fact that the synthesis is impeded by the high demand of its precursor. The absence of sulfur compounds may be an indicator of infection with Giardia sp.

Three biomarkers of Giardia sp. infection are proposed that were present in patients infected by the parasite, but not in patients with diarrheal processes without giardiasis: acetic acid, 1,4-dimethoxy-2,3-butanediol and 1,3-dimethoxy-2-propanol.

doi: 10.1016/j.cca.2022.04.797

W060

Enhanced identification of group b streptococcus in infants with suspected meningitis

A. Geteneh [d], M. Kiros [f], D. Alemu [e], H. Andualem [b], W. Abebe [g], T. Alemayehu [g], Y. Alemu [c], D. Hailu [a], T. Kasa [c], W. Mihret [a], A. Mulu [a], A. Mihret [a]

[a] *Bacterial and Viral disease research directorate, Armauer Hanson Research Institute, Addis ababa, Ethiopia*
[b] *immunology unit, Department of Medical laboratory sciences,Debre Tabor University, Debre Tabor, Ethiopia*
[c] *Medical Microbiolgy unit, Department of Medical laboratory science,Jimma university, Jimma, Ethiopia*
[d] *Medical Microbiolgy unit, Department of Medical laboratory sciences, Woldia university, Woldia, Ethiopia*
[e] *Medical Microbiology unit, Arbaminch college of health sciences, Arbaminch, Ethiopia*
[f] *Medical Microbiology unit, Department of Medical laboratory sciences,Debre Tabor University, Debre Tabor, Ethiopia*
[g] *Pediatrics unit, Department of Pediatrics, Addis ababa university, Addis ababa, Ethiopia*

Background-aim

Meningitis is an infection of the meninges, characterized by an onset of fever, headache, neck stiffness, and photophobia over a period of hours to days. In Ethiopia, meningitis due to an infectious agent is among the top ten causes of death among infants. The rate of maternal and neonatal Group B streptococcus (GBS) colonization is high that contribute to meningitis. However, there is study gap to rule out GBS meningitis in Ethiopia where its magnitude is unknown. Therefore, this study was aimed to determine the magnitude of GBS in infants with suspected meningitis.

Methods

Hospital based cross sectional study design was implemented for identification of GBS in infants with suspected meningitis at Tikur Anbessa specialized hospital by using PCR targeting cfb gene encoding the Christie-Atkinson-Munch-Peterson factor (CAMP) from June 2018 to October 2018.

Results

Among 72 infants with suspected meningitis in Tikur Anbessa Specialized Hospital, the magnitude of GBS was 63.9 % (46/72). Out of the 46 GBS positive infants, 10.9% (n = 5) of them died. The late onset of GBS (LOGBS) disease was noted to have poor outcome with 3 LOGBS out of 5 GBS positive deaths occurred.

Conclusions

Although the infant mortality rate in Ethiopia declines from time to time, death due to Group B streptococcus remains high. The cfb gene targeted PCR contributes a lot for identification of GBS in culture negative CSF samples and hence this more sensitive technique needs to be conducted at least at the referral hospitals.

doi: 10.1016/j.cca.2022.04.798

W061

Outbreak of NDM-5 producing carbapenem-resistant klebsiella aerogenes among neonates

P. Fen, Z. Hong, X. Qi

Shanghai Children's Hospital

Background-aim

Carbapenem-resistant Enterobacteriaceae (CRE) has emerged as a public health problem and gives rise to epidemic outbreak in hospital surroundings. This study is aim at investigating carbapenem-resistant Klebsiella aerogenes (CRKA) isolated from neonates over two -month period in a pediatric hospital, Shanghai.

Methods

Fifty CRKA isolates were collected between January and February, 2019. Antimicrobial susceptibility of the strains was determined by Vitek 2 Compact and disk diffusion method. The ®-lactamases and outer membrane porin genes including OmpK35 and OmpK36 were characterized by polymerase chain reaction (PCR) and DNA sequencing. Conjugation experiments were performed to determine the transferability of the plasmids. The plasmids were typed based on their incompatibility group using the PCR-based replicon typing method. Multilocus sequence typing (MLST) was performed for the genetic relationship.

Results

All CRKA strains showed high resistance to resistance to cephalosporins and carbapenems, but still susceptible to fluoroquinolones, aminoglycosides, polymyxin B and tigecycline. Forty six of fifty isolates carried blaNDM-5 genes, with other ®-Lactamase genes including blaCTX-M-1, blaTEM-1, blaSHV-11, and blaEBC being detected. Loss of OmpK35 and OmpK36 genes were observed in 14% (7/50) and 28% (14/50), respectively, with 5 isolates lacking both OmpK35 and OmpK36. Plasmids carrying blaNDM-5 were successfully transferred to the Escherichia coli recipient and plasmid typing shown that IncX3 were the prevalent among CRKA isolates. MLST analysis demonstrated that ST14 were detected in these strains.

Conclusions

There appeared the outbreak of NDM-5 producing CRKA belonging to ST14 among neonates in Shanghai. Then closer attention should be paid to the other resistance mechanism of loss of outer membrane porin in CRKA.

doi: 10.1016/j.cca.2022.04.799

W062

A case report of human toxocariasis

P. Chen

Department of Laboratory Medicin, National Taiwan University Hospital Yun-Lin Branch, Taiwan

Background-aim

Adult Toxocara canis is parasitic in the small intestine of the final host dog. After male and female adults are mated, each female releases approximately 100,000 eggs each day. These eggs are excreted from the body via feces of the dogs, and polluting the environment. The cells in the pregnant egg further develop into the third stage larva in approximately three weeks, and the eggs are infectious. Human mites are one of the child-preserving hosts of canine mites, and the larvae of canine mites cannot develop into adult worms in the host of the larvae, but reside in the larval form in the larval host and migrate in the host of the larvae, further protecting the young host, causing lesions. It is an important zoonotic parasitic disease.

Methods

A 43-year-old female patient with itchy skin, occasional nausea and vomiting, dizziness and fatigue, initially consulted the department of dermatology for treatment of hemorrhoids, but after two weeks, the symptoms did not improve and the patient visited our hospital for outpatient treatment. It was found that the right eye was swollen due to pain. In order to examine the presence of parasitic infection, the patient was hospitalized for further observation and treatment. During hospitalization, routine blood test results were normal, biochemical test results were within the normal range, IgE: 344 (IU/ml) (normal value: 0~100 IU/ml), Toxoplasma IgG Antibody: <1.6 IU/mL, Toxoplasma IgM Antibody: Negative, and fecal parasite test results were negative. Colonoscopy and gastroscopy revealed no abnormalities. The patient still showed suspected traces of the insect crawling in the skin, and therefore tissue from the suspected lesion was further collected, but the results were normal. After being hospitalized for several days, and during the ward round of the physician, it was found that the skin had traces of insect crawling. The skin-like specimen was pulled out from the skin and the test result indicated hair.

Results

According to the professional literacy of parasitology, Toxocara canis has the characteristics of Cutaneous pili migrans. Because the larvae do not develop further in the human body, it is impossible to make a diagnose based on tissue samples and stool examinations. Canine ascariasis currently relies on the use of canine larvae excretory antigen (TcES) for diagnosis by ELISA or Western blotting. Therefore, the patient's blood sample is taken for ELISA, and the test result indicated T. canis sero-antibody detection: Positive (1:1024). Therefore Toxocara canis infection was diagnosed in the patient and the physician administered the anti-helminth drug Mebendazole for treatment.

Conclusions

Toxocara canis is common in temperate and tropical regions, especially in areas with poor sanitation, where humans are susceptible to food or sediment contaminated with eggs of Toxocara canis. When the larva hatches in the human intestine, it enters the intestinal wall and migrates to other organs.

Human toxocariasis is a very serious epidemiological problem in many countries. Large-scale seroepidemiological studies have shown that the positive rate of canine aphid infection in Asia is 10.3%-32%; in the United States, the positive rate 4.6%-7.3%; in Europe, the positive rate is 3.2%-33.1%; in South America, the positive rate is about 39%; in African countries, the positive rate is 30%-44.6%, but in São Tomé and Príncipe in West Africa, the number of students near the capital is as high as 99.8%; the positive rates of adult aborigines and schoolchildren in Taiwan are 44% and 76.6% respectively; the above findings indicate that canine mites are an important zoonotic parasite that threatens the health of the world.

Therefore, it is necessary to pay attention to personal hygiene, home sanitation and avoid eating water or food contaminated with eggs to protect the health of themselves and their families. Including (1) Routinely deworming dogs at home; (2) Regularly disinfecting dogs and cats with hot water, and washing hands after handling the dog or cat's excrement; (3) Paying attention to environmental cleanliness, washing hands before meals and after using the toilet; (4) Washing hands before handling food, cooking all food, not eating raw vegetables, eating fruits and washing them; (5) Paying attention to drinking water and boiling drinking water before drinking. (6) Multi-exercise to enhance the body's immunity. (7) When infection is confirmed, implementing treatment with pharmacotherapy, eating high-fiber foods to increase bowel movements, and performing timely excretion of the worms.

In addition, healthcare providers are also reminded to pay special attention to the diagnosis and treatment of parasitic diseases rarely observed by Chinese people. The symptoms and detailed examination of patients should be carefully inquired about before the possibility of parasitic infection is ruled out.

doi: 10.1016/j.cca.2022.04.800

W063

Reactivity of synthetic peptides of dengue virus with sera of its victim in eastern Nepal: A diagnostic approach

R.K. Shreewastav

Department of Biochemistry, Nobel Medical College Teaching Hospital, Biratnagar

Background-aim

The cases of dengue fever have been reported more frequently In Nepal these days. It is an infection caused by any one of four dengue viruses (DENV-1, DENV-2, DENV-3 and DENV-4). There is antigenic similarity between dengue and other flavivirus in an endemic area like Nepal. Therefore the detection of anti dengue antibodies is a quite challenging and difficult task because of the limitation of commercially available kits as they are based on mixtures of inactivated virus preparations or recombinant envelope proteins. Unavailability of suitable antigens is the main hurdle towards the establishment of dengue diagnosis

because all the four dengue virus serotypes are genetically different with the same pathogenecity and antigenecity. The aim of this study is to develop peptide based diagnosis, which can detect all strains of dengue virus without showing cross reactivity with any other non-specific virus.

Methods

Few peptide sequences of NS1 and Envelop proteins were selected by screening whole protein sequences of Envelope and NS1 protein from all the strains of dengue virus. Peptides were selected on hydrophilicity, secondary structures, antigenicity index, amphipathicity, using Bcepred /DNAstar software for B cell and T cell prediction. These peptides were synthesized by Fluorenylmethoxycarbonyl Chemistry. A total of 124 serum samples (between 2 and 10 days after onset of fever) were selected from different Hospitals from eastern Nepal. These serum samples were dengue positive on the basis of clinical findings and NS1 antigen detection by commercial card test kit.

Results

We have tested 124 dengue positive sera confirmed for NS1 antigen. RR2 showed 80.6% sera positive for IgM and 48.38% positive for IgG. RR3 detected 75% IgM and 51.6% IgG positive sera. RR4 also detected 77.41% IgM and 45.96% IgG positive sera. RR5 showed 51.6%% sera positive for IgM and 42.74% positive for IgG. RR6 detected 69.35% IgM and 40.32% IgG positive sera. RR8 detected 72.58% IgM and 41.12% IgG positive sera.

Conclusions

This study reveals the use of synthetic antigenic peptides for the easy and accurate diagnosis of dengue infection in developing country like Nepal, where degnue is endemic. The study also demonstrates that these synthetic peptides can be used as a reliable tool for the use in sero diagnosis of dengue.

doi: 10.1016/j.cca.2022.04.801

W064

Performance evaluation of standard F Strep A AG FIA test and Sofia Strep A FIA test for children of acute pharyngitis

S. Kim, S. Lee

Gyeongsang National University Changwon Hospital, Changwon

Background-aim

Group A streptococci (GAS) is the most common cause of bacterial pharyngitis. Rapid and accurate diagnosis of bacterial pharyngitis is essential for optimal antibiotic treatment. Clinical performance of STANDARD F Strep A Ag FIA (SD Biosensor, Korea), a recently developed rapid antigen detection test (RADT), and Sofia Strep A FIA Test (Quidel, USA), a currently widely used RADT worldwide, was evaluated for patients with pharyngitis.

Methods

Three-hundred seventy-two patients with sore throat visiting five pediatric clinics in Changwon, Korea were subjected to throat swabs three times during 2018-2019. Two swabs were used for both RADTs and the other swab was delivered to Gyeongsang National University Changwon Hospital (GNUCH) for bacterial culture. Bacterial culture was regarded as a standard test. This study was approved by IRB of GNUCH and all participants agreed on written consent.

Results

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value of STANDARD F Strep A Ag FIA were 95.0%, 95.2%, 88.1%, and 98.1%, respectively compared to bacterial culture. Positive agreement and negative agreement with Sofia Strep A FIA Test revealed 95.1% and 95.6% respectively.

Conclusions

STANDARD F Strep A Ag FIA exhibited an excellent clinical performance and a good agreement with Sofia Strep A FIA Test. Discrepant result might be due to spectrum effect, bacterial numbers of GAS, or delayed transport.

doi: 10.1016/j.cca.2022.04.802

W065

Epidemiology and diagnosis of scabies in [LDQUO] Hassani Abdelkader [RDQUO] hospital, Sidi-Bel-ABBèS, Algeria

Y. Merad [b], M. Belkacemi [a]

[a] *Hemobiology department, Abdelkader Hassani hospital*
[b] *Parasitology-Mycology department, Abdelkader Hassani hospital*

Background-aim

Scabies is a contagious skin disease caused by Sarcoptes scabiei var hominis an obligate ectoparasite that completes its entire life cycle on humans.

There have been no reports on the infection rates of scabies in Sidi-Bel-Abbès hospital.

Methods

A cross-sectional study was conducted to estimate the prevalence of this infection among suspected cases, between December 2018 and December 2019. A total of 61 patients were examined.

The diagnosis was confirmed by microscopic identification of mites, larvae, ova, or scybala (faeces) in skin scrapings.

Results

Prevalence of scabies was 34,4% (21 positive cases), among positive cases 57,1% (n = 12) were females, 52,4% (n = 11) were children, and 81% (17 cases) were from urban settings.

The variables showing statistically significant association with scabies were familial contact with suspected scabies (p = 0,001), nocturnal itch (p = 0,001) and the pruritus location like: abdomen, forearm, thigh, back and buttock, with p < 0,05 respectively.

Conclusions

Prevalence of scabies is relatively high and should not be underestimated.

Diagnosis is difficult because of low mite burdens, atypical manifestations, and the potential for confusion with other skin diseases.

Standardized laboratory diagnostic test would be a major contribution to update the epidemiological data

doi: 10.1016/j.cca.2022.04.803

W066

Comprehensive performance assessment of malaria microscopists and laboratory diagnostic service capacity in districts stratified for malaria elimination in Ethiopia

A. Abebe

Ethiopian Public Health Institute, Addis Ababa

Background-aim

Successful malaria elimination calls for rapid and accurate tracking of cases so that the personnel can promptly be treated before the occurrence of transmission. Therefore, this study assessed the competency of malaria microscopists working in health facilities and Laboratory Diagnosis Capacity.

Methods

A cross-sectional study was conducted from February to June 2018 in 20 malaria elimination targeted districts from six regional states in Ethiopia. A maximum of 5 malaria-microscopists per each facility, available in the facility during the study period, were conveniently included in the study. Structured questionnaires used for personal and facility interview, and 10 Giemsa stained malaria slide panels were administered to each study participant for the performance assessment on malaria microscopy.

Results

In this assessment, 17 district hospitals, 71 health centers (HCs) and 18 private clinics (PCs) were included. Of the 18 PCs, only 10(55.6%) had license& registration certificate. Of the facilities, 91.5%(97/106) use light microscopy, 2.83%(3/106) use RDTs and 2.9%(3/106) use both microscopy and RDT to detect malaria. Accessible and appropriate storage of Giemsa was reported by 58.8%(10/17) hospitals, 81.7%(58/71) HCs & 72.2%(13/18) private clinics. Of 1896 malaria positive & 474 negative slides administered to 237 participants, 318 slides reported falsely negative (false negativity rate: 42.7%) & 47 reported falsely positive (false positivity rate: 2.9%). The participants achieved “good” grade [Agreement(A): 84.6%, Kappa(K): 0.6] on parasite detection and “Poor” agreement (A: 43.8%; K: 0.11) on species Identification. No or slight agreement seen on differentiation of P. falciparum from other species (A: 28.41%; K:0.29). Above 95% of participants, (201/237), did not count or used only plus system of parasite count which is totally unacceptable per the current WHO guideline.

Conclusions

Low Performance of malaria microscopists particularly in species identification & poor to moderate capacity laboratories in the current study. This is really a great obstacle to fulfill the plan of malaria elimination. Therefore, FMoH in collaboration with partners supposed to provide comprehensive In-service training of professionals with fulfillment of laboratory needs is critical to have gold standard service.

doi: 10.1016/j.cca.2022.04.804

W067

antimicrobial susceptibility pattern of common bacterial that cause wound infection in the paediatric surgical patients at yekatit 12 hospitals, Addis Ababa, Ethiopia

T. Mahmud [c], T.G. Tadesse [b], K.D. Tullu [a]

[a] *Department of Medical Laboratory Science, Collage of Health Sciences, Addis Ababa University*
[b] *Ethiopian Biotechnology Institute*
[c] *Yekatit 12 hospitals*

Background-aim

Wound infections contribute significantly to morbidity and mortality in surgical patients. A number of factors contribute to wound infection. Factors that increase the risk of wound infection include patient conditions such as; age, obesity, malnutrition, smoking, and the state of the wound which includes nonviable tissue in the wound, foreign bodies, long surgical procedures, and others. However microorganisms are the major causes with bacteria being the most prevalent. Severe and poorly managed infections can lead to gas gangrene and tetanus which may cause long-term disabilities. Chronic infection can cause septicemia or bone infection which can lead to death. Bacterial wound infections are a common finding in open injuries. Determination of the common bacterial antibiotics patterns is important for providing a guide for antibiotic selection and appropriate management. The main objective of the study was to identify the bacteria that cause wound infections and to determine their antimicrobial patterns in the pediatric surgical wards at Yekatit 12 Hospital, Addis Ababa, Ethiopia.

Methods

A cross sectional study was conducted from December 2017 to April 2017 at Yekatit 12 Hospital, Addis Ababa, Ethiopia. A total 150 clinical specimens were collected from study participant. All wound samples were cultured on Blood agar and Mac Conkey agar. All culture positive samples were characterized by gram stain and biochemical tests using the standard procedure. Antimicrobial susceptibility test was performed using Kirby-Bauer method. All demographic, laboratory and risk factors data obtained were entered and analyzed using SPSS version 20.

Results

The burden of wound infection at Yekatit 12 Hospital was 82%. From 150 [100%] specimens; 137 [91.3%] bacteria were isolated. Of which 90 [65.7%] were gram positive and 47 [34.3%] were gram negative bacteria. Staphylococcus aureus was the most prevalent followed by Pseudomonas aeruginosa, Proteus spp, coagulase negative staphylococcus, Beta hemolytic streptococcus, Klebsiellaspp, Non lactose fermenters, and Enterococcus faecalis. Patients who had mixed infections were 8.67% of the total participants. Staphylococcus aureus was highly sensitive to ceftriaxone but resistant to ceftazidime. Methicillin Resistant Staphylococcus aureus formed 50.6% of the Staphylococcus aureusisolates. BHS was highly sensitive to amoxicillin clavulanate and resistant to cefuroxime. Escherichia coli was sensitive to ciprofloxacin but resistant to amoxicillin clavulanate, cefuroxime, ceftriaxone, imipenem and cef-

tazidime. Klebsiellaspp was sensitive to all the antibiotics that were tested. Proteus mirabilis was sensitive to all the antibiotics except ceftazidime. The Non lactose fermenters were only sensitive to imipenem, ciprofloxacin and cefoxitim. Pseudomonas aeruginosawas highly sensitive to ciprofloxacin and imipenem but less sensitive to ceftazidime and resistant to ceftriaxone. Ceftriaxone, cefuroxime, flucloxacillin and amoxicillin clavulanate were widely used beside other antibiotics for either prophylaxis or treatment of the wound infections.

Conclusions

The prevalence of wound infection remains high despite wide use of antibiotics in the paediatric surgical wards. Resistance to new antibiotics like imipenem was observed.

Recommendations: Due to high resistance of the organisms to antibiotics, sensitivity tests should be regularly carried out to enhance rational use of antibiotics and antibiotic choice should be made based on the sensitivity patterns. Treatment guidelines for use of antibiotics should be formulated based on the hospital Formulary and the sensitivity patterns. This should be reviewed occasionally to ensure rational use of antibiotics

doi: 10.1016/j.cca.2022.04.805

W068

Prevalence of bacterial vaginosis, aerobic vaginitis, and their drug resistance pattern among pregnant women attending antenatal care in ayder comprehensive specialized hospital, Mekelle, Ethiopia

T. Gebrehiwet, M. Saravanan

Department of Medical Microbiology and Immunology, Division of Biomedical Sciences, College of Health Science, Mekelle University, Mekelle-1871, Ethiopia

Background-aim

Bacterial Vaginosis (BV) and Aerobic Vaginitis (AV) are the most common form of vaginitis in women of reproductive age and are serious health problems associated with gynecologic complications and increase risk of pregnancy losses, maternal and neonatal morbidity, and mortality. Therefore the objective of this to determine the prevalence of bacterial vaginosis, aerobic vaginitis and their drug resistance pattern among pregnant women attending ANC at Ayder Comprehensive Specialized Hospital, Northern Ethiopia.

Methods

A hospital-based cross-sectional study will be conducted from October to May 2019. Socio-demographic and clinical data were collected using a structured questionnaire. A total of 422 vaginal swabs were collected, transported, and processed using standard microbiological procedures. The antimicrobial susceptibility profile of aerobic bacterial isolates was performed using the disc diffusion technique as per the standard Kirby-Bauer method. The results were analyzed using SPSS version 22. The associations of different variables were statistically tested using univariate and multivariate regression analysis and the magnitude of association was measured by odds ratio at 95% CI: and P-value < 0.05 was considered as statistically significant.

Results

The overall prevalence of bacterial vagnosis was 85(20.1%). Bacterial vaginosis was statistically significant associated with symptoms of bacterial vaginosis (white homogenous vaginal discharge) ($p < 0.001$). The overall prevalence of aerobic vaginitis was 34(8.1%) while sever aerobic vaginitis was 11(2.6%) and significantly associated with secondary school ($p = 0.022$) and house wife ($p = 0.012$). A total 44 bacterial isolates were recovered, of which 30(68.2%) of the isolates were gram positive and 14 (31.8%) isolates were gram negative bacteria. The overall drug resistance rate of gram positive bacteria was observed against Penicillin (100%), followed by Erythromycin (86.7%). Ampicillin and Amoxicillin/Clav had the highest overall resistance rate (100%) against gram negative bacteria followed by Tobramycin (57.1%)

Conclusions

The prevalence of bacterial vaginosis was high among symptomatic than asymptomatic, and the majority (91.2%) of AV diagnosed pregnant women were also asymptomatic. Therefore, screening of vaginal bacterial infections in pregnant women should be implemented and Antimicrobial susceptibility test should be done for selection of appropriate antimicrobial agent and reduce the emergence of Multi-Drug resistance (MDR).

doi: 10.1016/j.cca.2022.04.806

W069

Multicenter study of common pathogen epidemiology in hospitalized children with acute respiratory tract infection in winter from 2017 to 2018, China

L. Meng[c], J. Zhou[b], X. Shao[a]

[a] *Department of clinical laboratory Children's Hospital of Soochow University, Soochow, China*
[b] *Department of clinical laboratory, Hangzhou Children's Hospital, Hangzhou ,China*
[c] *Department of Clinical Laboratory, Shanghai Children's Hospital, Shanghai Jiaotong University, Shanghai, China*

Background-aim

Acute respiratory tract infections (ARTIs) are a leading cause of the highest number of morbidity and mortality in children under the age of 5 years in developing countries. This study aimed to analyze the epidemiological characteristics of pathogens among children hospitalized with ARTI during the winter, with the aim of providing a reliable basis for clinical diagnosis and treatment.

Methods

A total of 563 hospitalized children with ARTIs in the children's hospitals of Shanghai, Hangzhou, and Soochow were enrolled between November 2017 and February 2018, and nasopharyngeal aspirates were collected. Real time PCR assays were performed to detect 14 common pathogens, including Mycoplasma pneumoniae (MP), Chlamydia pneumoniae (CP), Legionella pneumophila (LP), Chlamydia trachomatis (CT), respiratory adenovirus (ADV), influenza virus A and B (IFV-A and IFV-B), human parainfluenza virus types 1–3 (HPIV 1-3), human rhinovirus (HRV), respiratory syncytial virus (RSV), and human metapneumovirus A and B (hMPV-A and hMPV-B).

Results

Of the 563 specimens obtained from the patients, 467 (82.95%) were positive for at least one pathogen. RSV was the most commonly detected pathogen (48.66%), followed by HRV (21.49%). The detection percent for each of the respiratory pathogens varied considerably by age. RSV was the most common pathogen detected in the children aged less than 6 months. Co-infections were found in 20.6% of the patients. Of these coinfections, the combination of RSV and HRV was the most common.

Conclusions

Our finding demonstrate that the detection percent of respiratory viruses and atypical bacteria in ARTI children was relatively high during the winter in three children's hospitals. The pathogen incidence varied depending on patient age and ARTI manifestation. It is necessary to perform a combined detection of pathogens for ARTI children to improve the diagnosis and treatment.

doi: 10.1016/j.cca.2022.04.807

W070

Clinical and microbiological analysis of infections among children in a neonatal and pediatric intensive care unit

N. Duan, H. Li

Peking University First Hospital

Background-aim

The prevalence of microorganisms in neonatal and pediatric intensive care unit (NICU/PICU) is constantly changing and therefore empirical antibiotics may not be effective because of the resistance of these microorganisms. The aim of this study was to analyze the relative frequency and microbiological characteristics of positive cultures of blood, respiratory tract and urine in children admitted to the NICU and PICU during 2016-2019.

Methods

In this retrospective study, we evaluated 541 positive culture samples from 5063 different cultures (blood, respiratory tract and urine) from children in NICU and PICU in a large-scale general hospital in 2016-2019. All samples were evaluated for type of microorganisms and sensitivity to antibiotics.

Results

In NICU, positive cultures among blood, respiratory tract and urine culture samples were reported at 3.3% (101/3021), 4.7% (7/127), and 71.7% (43/60), respectively. Staphylococcus epidermidis was the most common species in blood cultures (n = 21, 20.8%), Acinetobacter baumannii was the most common species in respiratory tract cultures (n = 3, 42.9%) and Enterococcus faecium was the most frequent species in urine cultures (n = 30, 69.8%). In PICU, positive cultures among blood, respiratory tract and urine culture samples were reported at 8.4% (110/1316), 50.6% (178/352), and 54.5% (102/187), respectively. Acinetobacter baumannii was the most common species in blood (n = 24, 21.8%) and respiratory tract (n = 66, 37.1%) cultures while Enterococcus faecium was the most frequent species in urine cultures (n = 31, 30.4%). In the tested isolates, 75.3% cases of Acinetobacter baumannii (70/93) were carbapenem-resistant Acinetobacter baumannii (CRAB) and highly resistant to antibiotics that had been prescribed empirically. We also found the sensitivity of Enterococcus faecium was 0 to penicillin or ampicillin, 13.1% to (8/61) nitrofurantoin and 100% to linezolid and quinupristin/dalfopristin.

Conclusions

In our study, the most common species in the NICU and PICU were Acinetobacter baumannii (blood and respiratory tract samples) and Enterococcus faecium (urine samples). With carefully evaluating and analyzing common types of microorganisms, it is important to reduce the related pathogen infections and choose the appropriate antibiotic for treatment in our hospital.

doi: 10.1016/j.cca.2022.04.808

W071

Investigation of serial tests of quantiferon-tb gold in-tube and quantiferon-tb gold-plus in contacts to patients with active tuberculosis

S. Yoon [c], H. Moon [c], A. Yi [b], H. Kim [c], H. Chung [c], M. Hur [c], Y. Yun [c], G.H. Yoo [a]

[a] *Department of Internal Medicine, Konkuk University School of Medicine, Seoul, Korea*
[b] *Department of Laboratory Medicine, Green Cross Laboratories, Yongin, Korea*
[c] *Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, Korea*

Background-aim

The detection and management of individuals with latent tuberculosis infection (LTBI) are important steps to control and decrease tuberculosis. The contact with patients with active tuberculosis is one of the important routes of LTBI. Interferon-© (IFN-©) release assays (IGRAs), in particular the QuantiFERON-TB Gold in-tube assay (QFT, QIAGEN, Germantown, USA) has been widely used for the investigation of contacts to active tuberculosis. However, high reversion rates and low reproducibility of QFT have also been documented. The new version of QFT, QuantiFERON-TB Gold-Plus (QFT-Plus, QIAGEN) might provide higher sensitivity in early infection in contacts of patients with active tuberculosis, owing to its ability to assess both CD8+ and CD4+ T cell-mediated immunities (CMIs). Since there is sparse data on the serial tests of QFT and QFT-Plus in contacts of patients with active tuberculosis, we investigated the results of serial tests of QFT and QFT-Plus at short interval in contacts of patients with active tuberculosis.

Methods

From December 2016 to November 2018, 69 contacts of patients with active tuberculosis (57 households and 12 occupational exposures; median age (interquartile range, IQR), 35 (27-50) years old) were enrolled at a university-affiliated medical center (Seoul, South Korea). QFT and QFT-Plus were tested within 8 weeks of first contact (first assessment, median days after exposure (IQR), 14 (6-30) days). Follow-up tests were performed in 39 contacts at 8 weeks after the first assessment (second assessment). If there were discrepancies between the first and second assessment, a third test was performed after 8

weeks, if possible. This study was approved by the institutional review board (IRB) and informed consent was obtained. QFT and QFT-Plus were performed according to the manufacturer's instructions. For QFT, the results are considered positive when TB Antigen minus Nil IFN-© concentration was ≥ 0.35 IU/mL and ≥25% of Nil value. The QFT-Plus assay was interpreted as positive when either TB antigen tube (TB1 or TB2) minus Nil IFN-© concentration was ≥ 0.35 IU/mL and ≥25% of Nil value.

Results

The agreement between QFT and QFT-Plus was strong (110 coupled tests, kappa, 0.857 (95% confidence interval, 0.746 – 0.967), overall concordance rate, 94.5%). Positive rates of QFT and QFT-Plus at the first assessment were 24.6% (17/69) and 26.1% (18/69), respectively. The prophylactic treatment was performed in 8 subjects between the first and second assessments. Positive rate QFT and QFT-Plus at the second assessment were 23.1% (9/39) and 28.2% (11/39), respectively. The QFT-Plus TB2 minus Nil values were significantly higher in the second assessment than thoss in the first assessment (P = 0.032 by Wilcoxon Signed Rank test). QFT TB and QFT-Plus TB1 values were not significantly differenet between the first and second assessments.

Among 39 subjects with available serial test results, conversion rates (negative to positive) of QFT and QFT-Plus were 3.5% (1/29) and 10.7% (3/28), respectively. Reversion rates (positive to negative) of QFT and QFT-Plus were 20.0% (2/10) and 27.3% (3/11), respectively.

Conclusions

Although the number of our data is limited, we showed serial results of QFT and QFT-Plus in contacts to active tuberculosis patients. QFT-Plus TB2 seems to be converted earlier. Follow-up tests would be helpful before starting prophylactic treatment because reversion and delayed conversion are considerable. Moreover, it should be assessed at sufficient time to allow for maturation of the immune response.

doi: 10.1016/j.cca.2022.04.809

W072

Producing ©-aminobutyric acid (GABA) of rhizopus oryzae MHU 002, isolated from rotten fruits, during tempeh fermentation

N.L. Tao [a], C. Hu [b], Y. Chen [a]

[a] *Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan*
[b] *Department of Food Science and Nutrition, Meiho University, Pingtung, Taiwan*

Background-aim

Rhizopus oryzae is once of popular fungi that used in tempeh fermentation in Indonesia. This fungus is found in rotting fruits, rotting vegetation, and soil. According to the U.S Food and Drug administration (FDA), R.oryzae is considered Generally Recognized as Safe (GRAS) that is safe to used for human consumption.

Methods

In this study, tempeh fermented by R.oryzae MHU 002 was extracted and measured value of ©-Aminobutyric acid (GABA) and isoflavones by high performance liquid chromatography (HPLC).

Results

Result show that tempeh fermented by MHU 002 strain have GABA level significantly higher than tempeh fermented by R.oryzae BCRC 31837 (purchased from Bioresource Collection and Research Centre (BCRC), Taiwan). R.oryzae MHU 002 extraction was also found to be effective against pathogenic bacteria as Escherichia coli, Staphylococcus aureus and Bacillus cereus.

Conclusions

This study demonstrated that tempe fermented by R.oryzae MHU 002 can be used as functional food with natural antioxidants (isoflavones) and high level of GABA.

doi: 10.1016/j.cca.2022.04.810

W073

Carnobacterium divergens bacteremia: A case report and literature review

I.H. Jeong, G.D. Ahn, S.D. Shin, K.H. Kim, N. Kim, J.Y. Han, G.S. Woo

Department of Laboratory Medicine, Dong-A University Hospital, Busan, South Korea

Background-aim

Carnobacterium species are heterofermentative lactic acid bacteria usually isolated from the environment or food such as fish, meat, and dairy products. Lactobacilli are generally known to live in human intestines and female vagina but Carnobacteria are not known members of the human gastrointestinal microbial community. Currently 11 species are known in the Carnobacterium genus, but among them, only Carnobacterium divergens and Carnobacterium maltaromaticum are frequently isolated in food or fish. These two species are known to inhibit growth of Listeria monocytogenes in fish and meat products by production of antimicrobial peptides. Up to date, there are only four published reports of isolation of such pathogen in human world-wide. No reports in Korea have been published until now. Herein, we report a case of C. divergens bacteremia in an adult CNS lymphoma patient who was receiving chemotherapy in our hospital.

Methods

For antimicrobial therapy and treatment of fever, four blood bottles, two from peripheral blood and two from central venous catheter, were drawn on the day of admission, 5th, 6th and 8th day of admission. They were inoculated into two aerobic and two anaerobic blood culture bottles and incubated in BacT/ALERT 3D blood culture instrument (bioMérieux, Marcy-L'Etoile, France). Bacteria isolation was done with MALDI-ToF/MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; bioMérieux, Marcy-l'Étoile, France). For confirmation, 16S rRNA gene sequencing using the MiSeq Microbial Identification System (Macrogen, Seoul, South Korea) was performed. Antimicrobial susceptibility test was performed using VITEK 2 system (bioMérieux, Marcy-L'Etoile, France).

Results

A 65-year old Chinese male patient previously diagnosed with central nervous system (CNS) lymphoma was admitted to our hospital

due to mild fever of 37.8°C and severe oral mucositis. Laboratory findings showed pancytopenia with moderate neutropenia (white blood cell 2,240 /uL, segmented neutrophils 41.0%, hemoglobin 9.9 g/dL, platelet 70,000 /uL) and increased CRP (2.91 mg/dL). He had five consecutive admissions for chemotherapy.Due to oral mucositis, total parenteral nutrition was applied to his central venous catheter on the 6th day of his admission. On biochemical studies, the colonies were catalase and oxidase negative, and alpha-hemolytic. Microscopic examination revealed typical Gram-positive rods. Carnobacterium divergens was identified by MALDI-ToF/MS. The sequencing confirmed C. divergens with homology of 99%. The isolate was resistant to oxacillin (minimum inhibitory concentration (MIC) 4 mg/L) but susceptible to vancomycin (MIC 0.5 mg/L) and linezolid (MIC 2 mg/L). Antimicrobial therapy with tabaxin was empirically started on the date of his admission to cover both Gram-positive and negative bacteria, but after isolation of C. divergens, vancomycin was added due to resistance to oxacillin. Two days later, subsequent peripheral and central venous catheter blood cultures showed no growing bacteria. As his fever subsided and oral mucositis was improved, the patient was discharged.

Conclusions

Unlike the previously published cases of Carnobacterium species, this case was identified by MALDI-ToF mass spectrometry, which is now applied widely in clinical microbial laboratories. In comparison to classical methods of bacterial identification such as API or VITEK, mass spectrometry uses proteomic approach that allows rapid and accurate identification of bacteria as well as yeast and fungi. In fact, the conventional bacterial detection method, VITEK 2, identified the same colony as Enterococcus gallinarum. This implies that the conventional method cannot accurately identify such Gram-positive rod.

The most recent case introduced a 57-year old female patient form France who was diagnosed with acute necrotizing esophagitis. She began parenteral nutrition after surgery, and her four sets of blood culture bottles showed positive results. Unclear results were showed on API Coryne and API Listeria systems (bioMérieux) but the physicians presumed the isolate was Listeria monocytogenes. Following 16S rRNA sequencing confirmed the pathogen was C. divergens.

The second case from Austria presented a 43-year male patient with history of contact with seafood. He was diagnosed with meningitis, and bacterial growth was detected in one of his culture bottles. They also suspected the isolate to be L. monocytogenes. However, gene sequencing discovered that the pathogen was C. divergens. The specimens of the other two cases of Carnobacteria were necrotic tissue or pus. They both had history of either contact with aqueous environment, feeding with parenteral nutrition or both.

It is well known that the mass spectrometry has higher diagnostic sensitivity and specificity than the conventional microbial identification methods but there are three other factors that also support the causative relationship of the patients' bacteremia and such pathogen in our case report. First, the patients' fever subsided after adding vancomycin which covers methicillin/oxacillin resistant Gram-positive bacteria. Subsequent culture from central catheter showed negative results. Second, although the patient history of contact with seafood or other special environment was not definitely confirmed, he was on parenteral nutrition, like the other two published reports. Third, his previous three sets of blood cultures showed no growth, but only after he started nutrition on feeding tube, bacterial growth was observed. However, since the bacteria were present in only one of the four bottles, the possibility of contamination cannot be completely ruled out. We assume the origin of infection was bacterial contamination of the parenteral nutrition bag or colonization of the feeding tube.

doi: 10.1016/j.cca.2022.04.811

W074

Prevalence of acute gastroenteritis (age) pathogens infection among pre-school children in Malaysia

Y.L. Low, M. Mohd. Hareeff

Pantai Premier Pathology Sdn Bhd

Background-aim

Acute gastroenteritis (AGE) is a common illness among infants and children worldwide. The disease is caused by viral, bacterial or parasitic infection in the digestive tract1. Common virus causing AGE includes norovirus and rotavirus. Campylobacter, Escherichia coli (E. coli), Salmonella and Shigella are common bacteria that causing AGE. Parasites like Cryptosporidium enteritis, Entamoeba histolytica, and Giardia lambliacan also enter the body through food or water and settle in the digestive tract. Although it can affect individuals of any age, it presents a significant health risk to those at extremes of age, the very young and the elders2. Using the laboratory data collected in 2019, we aim to determine the prevalence of various viral, bacterial or parasitic infection in causing acute gastroenteritis at young population below 8 years old.

Methods

This is a retrospective study of 2080 children aged 7 years and below, with suspected acute viral and bacterial gastroenteritis in 2019. The stool samples were collected at 8 different branches (HPAK, AS, BG, CH, GMH, Ipoh, RCL and SP) within Peninsular Malaysia. Cary blair transport media was used as transport media to prevent replication of organisms while maintaining viability. A total of 22 pathogens (13 bacteria, 4 parasites and 5 viruses) were identified using FDA-approved BioFire FilmArray GIP. BioFire FilmArray GIP is a multiplex assay that simultaneously detects 22 pathogens including Campylobacter (jejuni, coli, and upsaliesis), Clostridium difficile (Toxin A/B), Plesiomonas shigelloides, Salmonella, Yersinia enterocolitica, Vibrio (parahaemolyticus, vulnificus, cholera), Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia, Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, and Sapovirus (I,II,IV, and V).

Result

Of 2080 samples, pathogens were identified in 1763 patients, with 59.5%, 40.0% and 0.5% caused by bacterial, virus and parasite, respectively. E coli was the most common bacteria that causing AGE, with prevalence of 35.1% for enteropathogenic E. coli (EPEC), 13.9% for enteroaggregative E. coli (EAEC), and 4.9% for enterotoxigenic E. coli (ETEC). Shiga-like toxin E coli, E coli 0157 and Shigella/enteroinvasive E coli infection was less common, with the prevalence of 0.8%, 0.5% and 0.4%, respectively. This was followed by Clostridium difficile toxin A/B, Salmonella, Campylobacter, Plesiomonas shigelloides with the prevalence of 18.7%, 16.0%, 11.8%, and 3.6%, respectively.

As for virus, Norovirus GI/GII was the most common pathogen that causing AGE. It's prevalence of 32.3%, however, was slightly lower compared to enteropathogenic E coli. This is followed by Rotavirus A, Adenovirus F 40/41, Sapovirus, Astrovirus with the prevalence of 20.2%, 7.8%, 7.5% and 3.5%, respectively. Parasitic infection was rare in this country.

Mixed infection was common in AGE. 45% of the positive samples were caused by single infection. 35%, 15% of positive samples were infected with 2 and 3 type of pathogens, respectively. With the highly sensitive multiplex PCR method, the highest number of pathogens that can be detected in one sample can goes up to 6.

Younger infant were more susceptible to AGE whereby most of the AGE cases falls in age 1, followed by 2 and infant below 1 year old.

In AGE, symptoms do not reliably in identifying the causing agent. Hence, microbiological diagnostic in identifying the infectious agent is important for better patient management. The diagnostic landscape for AGE has changed dramatically with the availability of highly sensitive multiplexed PCR methodology, bringing new insights in AGE epidemiology.

Bacterial supersede viral becoming the main causative infectious agent in causing AGE in pre-school children in Malaysia. Treatment of bacterial gastroenteritis is primarily supportive and directed toward maintaining hydration and electrolyte balance. Antibiotic therapy is rarely indicated and should be deferred until culture results are available.

E coli is an anaerobic gram-negative bacillus, is a major component of the normal intestinal flora and is ubiquitous in the human environment. E coli become pathogenic when they acquired certain additional genetic material . Enteroaggregative E coli (EAEC) are quite heterogeneous category of an emerging enteric pathogen associated with cases of acute or persistent diarrhea worldwide in children and adults. Diarrheagenic E coli recognized to date include enterotoxigenic E coli (ETEC) which are characterized by producing heat-labile or heat-stable or both enterotoxins, enterohaemorrhagic E coli (EHEC) which are characterized by attaching and effacing –(A/E) lesions and shiga-like toxin or verotoxins, enteropathogenic E cli (EPEC) which elicit characteristic attaching and effacing lesions on the intestinal mucosa, enteroinvasive E coli, which has the ability to invade epithelial cells similar to Shigella and is characterized by the presence of a large invasiveness plasmid, diffusely adherent E coli (DAEC) demonstrate a characteristic 'stacked-brick' aggregative adherence when cultured with Hep-2 cells.

2 primary mechanisms responsible for acute gastroenteritis are (1) damage to the villous brush border of the intestine, causing malabsorption of intestinal contents and leading to an osmotic diarrhea and (2) the release of toxins that bind to specific enterocyte receptors and cause the release of chloride ions into the intestinal lumen, leading to secretory diarrhea.

Enteropathogenis E coli can be transmitted person to person, usually by the oral/fecal route and even indirectly by contaminated food (contaminated/undercooked meat) or water (swimming, drinking, consuming ice and eating any food washed with or exposed to contaminated water). Casual contact will not usually transmit E coli person to person. However, some strains especially enteropathogenic strains, can be contagious.

Limited literature available on the prevalence of these bacterial infection in AGE in Malaysia. Most of the study focus on viral infection in causing AGE. Norovirus, is a leading cause of foodborne illness. Norovirus causes inflammation of the stomach, intestines and causing symptoms like stomach pain, nausea, diarrhea and vomiting. Most people with norovirus get better within 1-3 days. However, it can be serious for young children and elderly. Rotavirus infection, getting lesser and more controlled over the years, may due to availability of Rotavirus vaccine. Hand washing and cleaning surfaces are the best way to prevent catching the rotavirus.

Study showed that 45% of AGE were single infection. It is still unsure whether co-infection of more than one pathogen is the emerging trending or it just merely the consequences of highly sensitive PCR method. The impact can be significant as it may lead to unnecessary treatment, adverse drug effects and selection of antimicrobial resistance, and unnecessary public health investigation.

Present study has several limitations. Firstly, this is a retrospective observational study that may subjected problems such as selection bias. Finding may be less reliable as we are relying to the samples that sent to our laboratory. Secondly, BioFire FimArray GIP was performed at different laboratories across Peninsular Malaysia and may have introduced variability into the results. Thirdly, it is still not sure if all identified pathogens caused the diarrhoea as some of the bacteria may be normal inhabitants of the human gastrointestinal tract. For example, some people carry the bacterium C. difficile in their intestines but never become sick, though rarely may still spread the infection.

Conclusions

Despite these limitation, restrospective data on laboratory investigation have proved to be invaluable tool in estimating the prevalence of various pathogens in causing AGE, indirectly demonstrate the burden of AGE in the community. Prevalence of the each pathogen infection can be further refined in the future with proper study design or national data on AGE –associated hospitalization data.

doi: 10.1016/j.cca.2022.04.812

W075

Alternative candida lifestyles: Biofilm formation and antifungal resistance

S.K. Mishra, K. Parajuli, A. Sapkota

Tribhuvan University Teaching Hospital

Background-aim

Candida species, a normal commensal flora of human body, may be associated with superficial and deep-seated fungal infections in immunocompromised people. Candida albicans is the predominant etiologic agent of candidiasis; however, Non-albicans Candida species that tend to be less susceptible to the commonly used antifungal drugs are emerging. One of major virulence factors of Candida is biofilm formation, as cells within the biofilms are protected from host immune responses and the action of antifungals. Therefore, this study was designed with a major objective to determine the prevalence of biofilm formation in Candida spp.

Methods

A total of 84 consecutive Candida species were isolated from different clinical specimens over 7 months period at Tribhuvan University Teaching Hospital, Kathmandu. Candida species were inoculated in Sabouraud Dextrose Agar,and tests for the determination of their antifungal sensitivity profile and biofilm formation were performed with the use of standard methods.

Results

In-vitro antifungal susceptibility tests revealed that resistance to azoles was more in Non-albicans Candida (27%) as compared to Candida albicans (15 %).Tissue culture plate method detected biofilm formation in 18% of the Candida isolates. Majority of biofilm producers were Non-albicans Candida (77%). Resistance to azoles was higher among biofilm producers (39%) as compared to non biofilm producers (17%)

Conclusions

Since resistance to commonly used antifungal agents among Candida species is increasing, susceptibility testing of Candida species to antifungals on regular basis is crucial. Moreover, as biofilm-associated infections are more resistant to commonly used antifungals, detection of biofilm is also of utmost importance. This study holds further signifi-

cance in being the first report of biofilm formation in Candida spp. from Nepal.

doi: 10.1016/j.cca.2022.04.813

W076

The effects of pneumococcal conjugate vaccine (PCV13) on Russian children: A single center experience

N. Alyabieva [d], E. Brzhozovskaya [e], O. Ponomarenko [d], O. Shamina [e], A. Mirzaeva [b], A. Lazareva [d], T. Kulichenko [a], N. Mayanskiy [c]

[a] *Emergency department, Morozov Children's City Clinical Hospital, Moscow, Russia*
[b] *Emergency department, National Medical Research Center for Children's Health, Moscow, Russia*
[c] *Laboratory departmant, Russian Children Clinical Hospital, Pirogov Russian National Research Medical University, Moscow, Russia*
[d] *Microbiology laboratory, National Medical Research Center for Children's Health, Moscow, Russia*
[e] *Russian Children Clinical Hospital, Pirogov Russian National Research Medical University, Moscow, Russia*

Background-aim

To monitor the phenotypes and genotypes changes of S. pneumoniae isolates from children, we examined antibiotic susceptibility and serotype (S) distribution of pneumococcal strains, which were isolated before and after introduction the of 13-valent pneumococcal conjugate vaccine (PCV13) in Russia in 2014.

Methods

Pneumococci were isolated from children under 5 years between 2010-2018 years at the pediatric Center in Moscow. The serotypes and antibiotic resistance of these isolates were determined using capsular swelling and E-test method respectively. The Results were interpreted according to EUCAST 2019.

Results

In total, 708 nasopharyngeal pneumococcal strains were isolated, which belonged to 33 serotypes, 11 (1.4%) isolates were non-typeable. The most prevalent serotypes were 19F (19.5%), 6B (13.3%), 23F (11.3%), 14 (10.5%), 15B/C (7.1%) and 6A (7.1%).

The potential coverage of PCV13 was 77.7% in 2010-2015 with a significant decline to 52% in 2018, which was accompanied by an elevation in S15B/C, S11A and S23A prevalence (16%, 9.3% and 8% respectively in 2018).

Penicillin non-susceptible pneumococci comprised 6.2%. Among 248 (35%) erythromycin-resistant isolates, 83.1% strains carried the erm(B)/erm(B) + mef(A/E) genes, 14.5% strains carried only mef(A/E) gene. No mef(A/E)/erm(B) gene was found in 2.4% of erythromycin-resistant isolates.

Multidrug resistance was found in 28.8% of isolates and most often involved nonsusceptibility to erythromycin, clindamycin, trimethoprim/sulfamethoxazole and tetracycline; 38% of MDR isolates were PCV13 serotypes.

Oxacillin, erythromycin, and clindamycin resistance rate increased by 15-20 percent from 2010 to 2016, approaching 40-45% prevalence in 2016. Next 2 years the resistance rate in PCV13 serotypes started to decrease, while in non-PCV13 ones continued increasing from 11% to 25% in 2018.

Conclusions

These results emphasize the need for careful monitoring of the constantly changing pneumococcal population. The effects of PCV13 use on carriage were evident, with the significant decline of PCV13 serotypes. Emerging serotypes may be considered as important for the new vaccines development.

doi: 10.1016/j.cca.2022.04.814

W077 Correlation between thrombocytopenia and serum aminotransferases level in dengue fever patient

S. Shrestha

Norvic International hospital, Kathmandu, Nepal

Background-aim

To analyse the correlation between platelet count and serum aminotransferase level in dengue fever patient.

Methods

Descriptive analytical method.

Results

251 number of patient with a history of fever,152 males and 99 females were found to be dengue fever positive in Norvic International hospital kathmandu.The patient symptoms varied from mild fever to fever with warning signs.Dengue fever positive cases were identified based on Immunochromatography method to detect NS1 antigen on the serum of the patient.Based on the laboratory data,121(48%) patient had leucopenia and 100(39%) patient had thrombocytopenia.63(25%) patient had both leucopenia and thrombocytopenia.49(19%) patient had elevated serum aminotransferase(SGPT) level.41(16%) patient had both elevated aminotransferase level and thrombocytopenia.AST:Platelet ratio index was also calculated.

Conclusions

This study has found positive correlation between the platelet count and serum aminotransferase level in patient with dengue fever.Both this factors predicted the severity of the dengue fever illness.

doi: 10.1016/j.cca.2022.04.815

W078

Clinically relevant antibiotic resistant enterobacteriaceae from hospital wastewater in metro manila, Philippines

C.P. Tolenada [a], G. Dayrit [b]

[a] *Trinity University of Asia, The Family Clinic Inc*
[b] *Trinity University of Asia, The Family Clinic, Inc., University of the Philippines, Manila*

Background-aim

Antibiotic resistant Enterobacteriaceae pose a grave public health threat for hospitalized patients. In the absence of efficient sanitation and sewage disposal, the risks for transmission and spread of hospital organisms into the community are high. The aim of this study was to detect the clinically relevant antibiotic resistant Enterobacteriaceae in different hospital wastewater treatment plants in Metro Manila, Philippines.

Methods

MacConkey agar supplemented with 4mg/L of cefotaxime was used to select third-generation cephalosporin-resistant Enterobacteriaceae. The Vitek 2 compact system confirmed the antibiotic resistance of the bacterial species commonly pathogenic to human and animal namely: Enterobacter cloacae complex, Klebsiella pneumoniae, and Escherichia coli.

Result

Out of 16 bacterial isolates from influent tank of 11 hospitals 100% (16/16), 100% (16/16), 94% (15/16), 94% (15/16), 88% (14/16), 50% (8/16), 31% (5/16),31% (5/16), 31% (5/16), 0.06% (1/16) were resistant to Ampicillin (Penicillins) Cefuroxime (2nd generation cephalosporin family), Ceftazidime, ceftriaxone (3rd generation cephalosporin family), Cefepime(4th generation cephalosporin family), Aztreonam (Monobactam Family) ertapenem, imipenem,meropenem (Carbapenem family), and Colistin, respectively. After the treatment process, 4 bacterial isolates were recovered from the effluent tank of 4 hospitals 100% (4/4), 100% (4/4), 75% (3/4), 75% (3/4), 50% (2/4), 25% (1/4), 25% (1/4) were resistant to Ampicillin (Penicillins) Cefuroxime (2nd generation cephalosporin family), Ceftazidime, ceftriaxone (3rd generation cephalosporin family), Cefepime(4th generation cephalosporin family), ertapenem and meropenem (Carbapenem family).

Conclusions

This study demonstrated the presence of antibiotic resistant Enterobacteriaceae in influent and effluent hospital and tanks. Antibiotic resistant bacteria that could not be completely treated in the system might be a source of spread in the environment. Therefore, proper handling and treatment of wastewater is highly recommended.

doi: 10.1016/j.cca.2022.04.816

W079

Molecular investigaton of carbapenem resistant pseudomonas aeruginosa isolated from tertiary care hospital of Nepal

S. Kafle

Central department of biotechnology, tribhuvan university

Background-aim

Background: P. aeruginosa are categorized as second most critical group of pathogen by WHO. These opportunistic pathogens mainly affect patients with compromised host defense mechanisms. Infections caused by P. aeruginosa can be life threatening. Several resistance mechanisms make them able to resist multiple classes of antibiotics including ®-lactams. Carbapenem are potent beta lactam antibiotics and last resort of drug. However, resistance to this drug also has been reported lately. Loss of OprD porin have significant role against carbapenem. Similarly, ® – lactamase (MBL and ESBL) have potential to hydrolyze carbapenem of which gene blaNDM has great concern. In addition, AmpC beta lactamase with combination to other mechanism also showed resistance to carbapenem. Due to such mechanism attributed by P. aeruginosa against carbapenem it has become very difficult to combat them, leaving fewer options for antibiotic therapy.

Aims: This study aims to provide knowledge on carbapenem resistance mechanism including loss of porin, overexpression of AmpC beta -lactamase and carbapenemase at molecular level.

Methods

In this study, 95 isolates of P.aeruginosa were collected. Antimicrobial susceptibility profile based on the disk-diffusion tests was performed to detect multidrug resistance isolates. Carbapenem resistance isolates were selected and classified into IMPR, MRPR, IMPRMRPR types. Phenotypic detection for MBL-producer, ESBL-producer and AmpC producer were performed. Molecular identification of carbapenem resistant gene of P. aeruginosa was carried out by PCR and followed by sequencing.

Result

Among 95 isolates, total 73 (76.84%) were found to be Multidrug resistance P. aeruginosa and out of 73 MDR, 61 (83.56%) were carbapenem resistant isolates. Phenotypic test revealed that 55 (90.1%) MBL-producer and 45(61.64%)were ESBL producer according to the standard microbiological method CLSI. The PCR analysis result showed that most of the carbapenem resistant isolates were found to have (42.62%) ampC gene while (31.14%) were found to lack OprD gene. Among 61 carbapenem resistant isolates, 7 (11.47%) and 6 (9.8%) were found to have detection to blaNDM gene. The sequence of these genes showed mutation that could potentially lead to stronger carbapenemase activity.

Conclusions

Our results confirmed that multiple resistance mechanism such as OprD loss, carbapenemase and AmpC beta lactamase production conferred resistance to the carbapenem in P. aeruginosa, isolated from hospital settings.

doi: 10.1016/j.cca.2022.04.817

W080

Nanoparticle-enhanced digitof for detection of e. coli directly from urine

S. Kc [b], T. Venema [a], R. Parchen [a], L. Coin [b], M. Blaskovich [b]

[a] *BiosparQ BV, Leiden, The Netherlands*
[b] *Institute for Molecular Bioscience, The University of Queensland, Australia*

Background-aim

Time is a critical factor for diagnosis of bacterial infections. Owing to the need for culturing, current technologies however take 24-48 hrs to produce a complete report. The aim current project is to directly detect

bacterial infections of the urinary tract from urine samples, avoiding time-consuming culture techniques. The vast majority of urinary tract infections (UTI), 95%, is caused by a limited number, 10, of bacterial species, in which UroPathogenic Escherichia coli (UPEC) is dominant; hence, early and direct detection of this limited number of organisms and especially E. coli plays a crucial role in treatment and management of UTIs.

Methods

Direct detection of E. coli from urine samples was achieved by utilizing the DigiTOF technique. DigiTOF is a new MALDI mass spectrophotometry technology that is able to perform a full MALDI analysis on cells individually presented to the mass spectrometer. To prepare these cells, the urine sample is first filtered to remove salts and bind the bacteria onto the filter membrane. The bacteria are then eluted into a sample buffer, where antibody conjugated nanoparticles sequester and concentrate the bacteria from the sample buffer. As the nanoparticles do not interfere with the MALDI process, the nanoparticle-captured bacteria can be reconstituted directly in a MALDI buffer and subjected to the DigiTOF analysis.

Result

E. coli specific spectra were generated from as low as 10^3 CFU/mL of bacterial input (Fig1) in synthetic urine samples. The clinically relevant load of bacteria in urine is 10^5 CFU/mL, hence the sensitivity of the method developed covers the clinical range. The developed method was further tested in clinical samples (pooled urine), again with successful detection of E. coli from as low as 10^3 CFU/mL.

Conclusions

The current project has demonstrated proof-of-concept for the direct detection of bacteria from clinical samples without the need for culture steps. This concept has potential to be extended into other clinical samples such as blood or CSF and significantly reduce the time for diagnosis.

doi: 10.1016/j.cca.2022.04.818

W081

Antimicrobial susceptibility test reporting and interpretation practices survey

Y.P. Dewi [a], A. Dahesihdewi [b]

[a] *Dr. Sardjito General Hospital Yogyakarta Indonesia*
[b] *Infectious and Tropical Diseases Task Force of Indonesian Association of Clinical Pathology and Laboratory Medicine*

Background-aim

The final step of reporting results is crucial in the process of antimicrobial susceptibility testing (AST). Expert rules can assist Laboratory professionals in the interpretation of AST. Expert rules in AST is a new paradigm practice in Indonesia but to the best of our knowledge no large study has assessed how and to what extent this rules is implemented.

Aims of this survey were to evaluate how and to what extent Expert rules in AST was implemented. The data gained would be used to map out AST reporting practice as a baseline data to improve and standardize AST reporting in Indonesia.

Methods

We developed a survey to collect information from Indonesian Association of Clinical Pathology and Laboratory Medicine community assessing current practices and technologies and determining how AST result is interpreted and reported especially the expert rules usage.

Results

32 (78%) surveys were returned from 41 laboratories targeted. Of these responses submitted, 27 (84%) were from Public institutions and 20 (63%) were from type B - hospital. All laboratories used CLSI as standard reference.

28 (88%) laboratories had AST automated instrument systems. Of these, 17 (61%) Vitek 2 (bioMérieux) was the most popular instrument. 27 (96%) users were aware of expert system incorporated in their instruments but only 23 (81%) applied it.

31 (97%) responders had heard about expert rules. Of these, usage of Interpretive, Intrinsic resistances, exceptional phenotype rules were 26 (84%), 23 (74%) and 11 (35%) respectively. Based on expert rules used, mostly editing the results 24 (77%) was action they took on reporting.

Only 13 (41%) laboratories had microbiology Laboratory Information Systems (LIS) and 10 (77%) of them had incorporated expert rules into their LIS.

Conclusions

Expert rules was already implemented in Indonesia especially in laboratories that had automated AST instrument systems although it applied with a heterogeneity of practices. Development of expert rules based on guideline and local data could favor its dissemination and standardization as one important element of antibiotic stewardship program.

doi: 10.1016/j.cca.2022.04.819

W082

Detection of outer membrane porins genes in carbapenem-resistant klebsiella pneumoniae from Moscow

O. Shamina [c], O. Kryzhanovskaya [b], A. Lazareva [b], N. Alyabieva [b], N. Mayanskiy [a], E. Brzhozovskaya [c]

[a] *Laboratory diagnostics center, Russian Children Clinical Hospital, Pirogov Russian National Research Medical University, Moscow, Russia*
[b] *Microbiology department, National Medical Research Center for Children's Health, Moscow, Russia*
[c] *Russian Children Clinical Hospital, Pirogov Russian National Research Medical University, Moscow, Russia*

Background-aim

Klebsiella pneumoniae produces two outer membrane porins, OmpK35 and OmpK36, which allow the passage of small hydrophilic molecules such as iron, nutrients, and antibiotics ones through the outer cell membrane. Loss of outer membrane porins can be responsible for antibiotic resistance, in particular to carbapenems. Herein, we described alterations of membrane porins in K. pneumoniae clinical isolates.

Methods

This study included carbapenem-resistant K. pneumoniae (CRKP) isolates (resistant to meropenem or/and imipenem according to

EUCAST) collected from patients of intensive care units of three hospitals in Moscow in 2012-2017. Alterations in the ompK35 and ompK36 genes were detected using the Sanger sequencing. Insertion sequences (ISs) were analyzed using the IS-finder website. Sequence type (ST) was determined using multilocus sequence typing (MLST).

Results

In total, 159 CRKP isolates were collected. MLST analysis revealed 18 STs. Five leading STs included ST307 (n = 46, 29%), ST395 (n = 40, 25%), ST377 (n = 17, 10%), ST48 (n = 17, 11%), and ST23 (n = 16, 10%) that collectively comprised an 86% proportion of the collection.

The loss of ompK36 and/or ompK35 genes was found in 37% (58/159) isolates. Deletion of both genes was detected in two ST13 isolates; the ompK35 gene was not detected in two isolates of ST307 and ST395; the ompK36 gene was not detected in the majority of isolates (54/58; 93%) of seven different STs: 307, 395, 48, 147, 37, 336 and 86.

In three CRKP isolates of ST307 (3/159, 2%) in ompK36 disruption of the coding sequence occurred via the presence of the miniature inverted-repeat transposable element (MITE) Kpn1, inserted after nucleotide 97 (2/3) and 955 (1/3).

Conclusions

Overall, in our study 35% CRKP isolates lacked the ompK36 gene. The presence of both porins (OmpK35 and OmpK36) in CRKP isolates can be associated with other alterations like point or promoter region mutations. Thereby, further investigations are necessary for the isolates involved into this study.

doi: 10.1016/j.cca.2022.04.820

W083

Understanding a patient population of syphilis serology tests in Korea

E.H. Lee, S.G. Lee, Y. Oh, R. Choi

GC Laboratories, Yongin-Si

Background-aim

Diagnosis of syphilis, a sexually transmitted infection, can be made according to clinical information and various combinations of results of both non-treponemal and treponemal assays. Because understanding a patient population is helpful for test utilization in the clinical laboratory, we aimed to investigate the results of syphilis serology tests requested from local clinics in Korea.

Methods

We retrospectively evaluated the syphilis serology test results of serum Rapid Plasma Reagin (RPR), Treponema pallidum latex agglutination (TPLA), RPR titer, and fluorescent treponemal antibody absorption (FTA-ABS) IgG and IgM tests performed in Korean adults from the laboratory information system of Green Cross Laboratories. We investigated the patterns of syphilis serology test results and possible interpretation according to combinations of syphilis serology based on a traditional testing algorithm.

Results

During the one-year study period, 33,746 RPR tests were performed in Korean adults (7,622 men and 26,124 women) with a median age of 35.1 years (interquartile range 30.8-43.4 years). Among these RPR tests, 316 (0.9%) showed RPR + results and were performed simultaneous to TPLA test. Among these 316 patients, 186 (58.9%) had RPR + /TPLA + results. Among 130 patients with RPR + /TPLA- results, FTA-ABS IgG and IgM were only tested in 15 (11.5%): 14 were IgG-/IgM-, and one who underwent only the IgM test showed a negative result (probably a biological false positive of RPR). Among 186 RPR + /TPLA + patients, 34 (2.1%) had RPR titer ≥ 16:1, and 10 were FTA-ABS IgM + (including weak reactive results probably due to current infection or rare biological false-positive). Among 146 patients with RPR + /TPLA + /RPR titer ≤ 1:8, 30 were tested for FTA-ABS IgG and/or IgM: the two FTA-ABS IgM + (including weak reactive) patients had an RPR titer of 1:4.

Conclusions

This study may help to understand patient populations and test utilization for syphilis serology test interpretation in clinical laboratories in Korea. Further studies are needed to investigate the clinical impact of test utilization of syphilis serology in Korea.

doi: 10.1016/j.cca.2022.04.821

W084

Assessment of a novel matrix-assisted desorption/ionization time-of-flight mass spectrometry platform, asta microidsys, for identification of various acinetobacter species, compared with bruker maldi biotyper

W. Kim [c], W. Kim [c], H. Lee [c], G. Park [b], S. Kang [b], K. Youngjin [b], J. Shin [a], E. Won [a], S. Lee [d], C. Kim [d], J. Sookjin [b]

[a] *Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, Korea*
[b] *Department of Laboratory Medicine, Chosun University College of Medicine, Gwangju, Korea*
[c] *Department of Laboratory Medicine, Chosun University Hospital, Gwangju, Korea*
[d] *Department of Premedical Science, Chosun University, Gwangju, Korea*

Background-aim

Acinetobacter infections have been posing a growing threat to global public health because of increasing incidence and the capability of developing multidrug resistance. However, conventional automated phenotypic methods were not very successful in accurate identification of these bacteria to the species level, thereby necessitating implementation of labor-intensive, time-consuming molecular techniques such as gene sequencing. Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for diagnosis of microbial infections has been proven to be a rapid, reliable alternative to molecular-based studies and it is becoming more commonly used in clinical laboratories. There are a number of commercially available MALDI-TOF MS platforms, including Bruker MALDI Biotyper (Bruker Daltonics, Germany), VITEK MS (bioMérieux, France), and newly developed ASTA MicroIDSys (ASTA, South Korea). The purpose of our study was to compare the performance of ASTA MicroIDSys with Bruker MALDI Biotyper in identifying various Acinetobacter species.

Methods

A total of 207 specimens, consisting of 185 clinical isolates and 22 KCTC Acinetobacter reference strains, which were previously identified by rpoB gene sequencing and found to be distributed across 24 species, were tested upon the two MALDI-TOF systems. Their results were compared to each other, with rpoB gene sequencing being the reference method.

Results

Overall, correct identification rates to the species level by ASTA MicroIDSys and Bruker MALDI Biotyper were 76.8% (159/207) and 91.3% (189/207), respectively ($p < 0.001$). Bruker MALDI Biotyper generated significantly fewer misidentifications (1.2%, 2/172) than ASTA MicroIDSys (19.8%, 34/172) when identifying bacteria belonging to Acinetobacter baumannii complex at the species level ($p < 0.001$). Similar results were obtained for identification at the ABC group level (3.49% for ASTA MicroIDSys, 0.00% for Bruker MALDI Biotyper. $p < 0.05$). On the other hand, when identifying non-ABC Acinetobacter isolates, correct identification rates from ASTA MicroIDSys were comparable to those from Bruker MALDI Biotyper (60.0%, and 54.3%, respectively).

Conclusions

Bruker MALDI Biotyper demonstrated superior performance over that of ASTA MicroIDSys especially in discrimination of the bacterial strains of Acinetobacter baumannii complex.

doi: 10.1016/j.cca.2022.04.822

W085

Evaluation of matrix-assisted laser desorption/ionization-time of flight mass spectrometry, asta microidsys for the identification of anaerobic bacteria: Comparison with bruker biotyper

Y.J. Ko [b], J. Kook [c], Y.K. Lim [c], S. Lee [b], C. Kim [d], J.H. Shin [a], S.H. Kim [a], S. Kang [b], G. Park [b], S. Jang [b]

[a] *Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, South Korea*
[b] *Department of Laboratory Medicine, College of Medicine, Chosun University, Gwangju*
[c] *Korean Collection for Oral Microbiology and Department of Oral Biochemistry, College of Dentistry, Chosun University, Gwangju*
[d] *Premedical Science, College of Medicine, Chosun University, Gwangju*

Background-aim

Anaerobic bacteria are the predominant components of human microbiota and some of them also can be pathogens of life-threatening infections. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) data libraries, based on 16S rRNA gene sequencing enables rapid and accurate identification of anaerobic bacteria. This study aimed to compare the identification performance of two MALDI-TOF MS systems in human origin anaerobic bacteria: Bruker (Bruker Daltonik, Bremen, Germany) and New ASTA MicroIDSys (ASTA, Suwon, South Korea).

Methods

In June 2019, 178 strains of previously-stored in Korean Collection for Oral Microbiology (KCOM) were identified by using ASTA MicroIDSys of Chosun University Hospital and Bruker biotyper of Chonnam National University Hospital. Anaerobic bacteria previously-stored in KCOM are mainly clinical strains commissioned from Chosun University Dental Hospital, and some strains are provided by KCTC. All strains were collected after confirmation by 16S rRNA gene sequencing. Statistical analysis was performed by proportional analysis.

Result

A total of 174 strains of Prevotella sp., Actinomyces sp., Veilonella sp., Porphyromonas sp., Propionibacterium sp., Atopobium sp., Parvimonas micra, Bacteroides sp. and others were identified. The overall identification accuracy was 97.1% of MicroID and 85.6% of biotyper for genus identification ($P = 0.0001$). The overall identification accuracy was 92.5% of MicroID and 73.0% for biotyper for species identification ($P < 0.0001$).

Conclusions

Both systems are suitable for the rapid identification of anaerobic bacteria in clinical microbiology laboratories.

doi: 10.1016/j.cca.2022.04.823

W086

Positive result rate of anti-treponema pallidum atibodies before and after reagent renewal

L. Gogeliene [a], D. Vitkus [b,c], D. Petronyte [a]

[a] *CENTER OF LABORATORY MEDICINE OF VILNIUS UNIVERSITY HOSPITAL SANTAROS CLINICS, LITHUANIA*
[b] *CENTER OF LABORATORY MEDICINE OF VILNIUS UNIVERSITY HOSPITAL SANTAROS CLINICS, LITHUANIA*
[c] *VILNIUS UNIVERSITY, FACULTY OF MEDICINE, INSTITUTE OF BIOMEDICAL SCIENCE, DEPARTMENT OF PHYSIOLOGY, BIOCHEMISTRY, MICROBIOLOGY AND LABORATORY MEDICINE*

Background-aim

There is a mandatory serological testing for anti-Treponema pallidum (Anti-TP) antibodies for all blood donations in Lithuania. Positive blood samples are then tested additionally with the Westernblot (WB) qualitative method to determine whether they are true positive or not. From 2015 to 2019 the ARCHITECT Syphilis TP assay reagents were renewed twice. Aim was to compare how reagent renewal between 2015 and 2019 affected the false positive result rate for Anti-TP antibodies.

Methods

Blood samples were collected into BD Vacutainer® SST™ II Advance plastic tubes (BD, USA). Anti-TP antibodies were measured by chemiluminescent microparticle immunoassay (CMIA) technology on Architect ci8200 system using ARCHITECT Syphilis TP assay (Abbott, USA). The presence or absence of Anti-TP antibodies in the specimen was determined by comparing the chemiluminescent signal of the reaction to

the cutoff signal assigned from the last calibration. If the chemiluminescent signal of the specimen was greater than or equal to the cutoff signal, the specimen was considered reactive for Anti-TP. Anti-TP positive samples were tested for the human antibodies of the immunoglobulin class IgG and IgM against TP antigens in the serum by the EUROLINE WB (Germany) test kit. The test kit contains test strips with electrophoretically separated antigens of TP. Additionally, each test strip has a membrane chip coated with cardiolipin antigen of 3 concentrations. If Anti-TP antibodies are present in the sample, specific antibodies of the IgG and/or IgM will bind to the antigens.

Results

During 2015 21191 blood samples were tested of which 42 were positive by ARCHITECT Syphilis TP assay. Further testing with WB test kit of 32 of these samples confirmed 1 sample IgG positive while 10 were borderline. 2 samples were IgM positive. During 2019 24209 blood samples were tested of which 19 were positive by ARCHITECT Syphilis TP assay. Further testing of 18 of these samples by WB confirmed 4 samples IgG positive while 7 were borderline. 1 sample was IgM positive and 2 were borderline.

Conclusions

Study results clearly show significant improvement of ARCHITECT Syphilis TP reagent performance characteristics as number of false positive results decreased by more than 50% during a 5 year period.

doi: 10.1016/j.cca.2022.04.824

W087

Seroprevalence of influenza a virus (H1N1) IGM in patients attending CMC-TH and its association with liver function test

A. Shrestha

MEDICAL LABORATORY,CHITWAN MEDICAL COLLEGE,CHITWAN

Background-aim

Influenza is an acute infection of the respiratory tract which spreads rapidly around the world in seasonal epidemics. Influenza A/H1N1 virus usually affects the respiratory tract. Although the liver may not be the primary target organ of viral infection, it can be collaterally damaged.

Methods

This study was conducted from March 2018 to August 2018 in CMC-TH during which suspected patients were examined by ELISA for influenza A (H1N1) IgM antibody. Clinical features were collected and recorded for positive cases. Liver function tests were examined and recorded for all the samples and data was analysed using SPSS version 20.

Results

Among 188 total samples processed 42 (22.34%) were tested positive for Influenza A (H1N1) IgM antibody. Females were infected more than males and seropositivity was highest among the age group 18-49 years (54.76%). Fever was the most common clinical presentation followed by a sore throat. The highest number of positive cases were observed in the month of August while the lowest positive cases were observed in the month of May. Determination of association between influenza A(H1N1) IgM ELISA result and ALT (Alanine transaminase) status by Pearson chi-squared test at 5% level of significance show a significant association between both ALT status, AST (Aspartate transaminase) status and Influenza infection.

Conclusions

The results of this study suggest that influenza positive patients have a significant elevation of liver enzymes alanine transaminase and aspartate transaminase.

doi: 10.1016/j.cca.2022.04.825

W088

Bacteremia in febrile neutropenic patients: Isolates and their antibiotic susceptibility profile

E.M. El Bouchtili, M. Bouthaina, F. Aziz, I. El Mazgueldi, A. Maleb

Microbiology department, Mohammed VI teaching hospital

Background-aim

Patients with febrile neutropenia are more likely to develop life-threatening bacterial infections due to lack of the inflammatory response [1]. The use of empirical broad-spectrum antimicrobial therapy at the onset of fever, taking into account locally prevalent pathogens and their susceptibility profile, is important for appropriate treatment of the infection that improves survival in these patients [2].

The objective of this study is to report the organisms responsible for bacteremia in patients admitted to the Mohammed VI University Hospital Department with febrile neutropenia, as well as their antimicrobial susceptibility profile.

Methods

All subjects with febrile neutropenia admitted to the Mohammed VI teaching hospital in Oujda between June 2016 and January 2020 with a positive blood culture were analyzed. The samples were processed in accordance with the recommendations of the medical microbiology reference system (REMIC).

Out of 874 blood cultures taken during the study period, 236 were positive, i.e. 27%. The male sex was most at risk. Pediatrics, neonatology and internal resuscitation medicine were at the top of the list of services concerned.

Result

501 bacterial strains have been identified. Gram-positive Cocci were responsible for 64.5% of the bacteremia and Gram-negative bacilli were responsible for 23.9% of the bacteremia. The most frequently isolated species were Coagulase-negative Staphylococcus (43.7%), Klebsiella pneumoniae (9.5%), Staphylococcus aureus (8.78%), Staphylococcus epidermidis (8.58%) and Acinetobacter baumannii (6.38%).

Details of the various organisms and their antimicrobial susceptibility are given in [Table 1] and [Table 2].

Table 1 : The susceptibility of Gram-positive Cocci (GPC) to different antibiotics.

Nb Ampicilline Oxacilline Rifampicine Norfloxacine Gentamycine Vancomycine Linézolide

Staphylococcus aureus 44 36 (81.1 %) 10 (22.7 %) 4 (0.9 %) 3 (6.8%) 5 (11.36 %) 0 0

Coagulase-negative staphylococcus 219 127 (57.9 %) 136 (62.1 %) 38 (17.3 %) 77 (35.1 %) 103 (47 %) 2 (0.9 %) 0

Enterococcus spp 30 11 (36.6 %) 1 (3.3 %) 20 (66.6 %) 0 8 (26.6 %) 0 0

Streptococcus pneumoniae 3 0 1 (33.3%) 0 0 0 0 0

Table 2 : the susceptibility of Gram-negative Bacilli (BGN) to different antibiotics

Nb Pipéracilline - tazobactam Amoxicilline - acide clavulanique C3G Gentamycine Amikacine Imipinème Norfloxacine Colisitne

E. coli 23 15 (65.2%) 16 (69.56%) 16 (69.5%) 9 (39.1%) 0 0 10 (43.47%) 0

Enterobacter spp 22 20 (90.9 %) 22 (100 %) 20 (90.9 %) 16 (72.7%) 4 (18.1 %) 1 (0.45%) 10 (45.4%) 0

kliebsella spp 48 38 (79.1%) 39 (81.2 %) 35 (73.9 %) 27 (56.25 %) 3 (6.25 %) 6 (12.5 %) 13 (27 %) 0

pseudomonas spp 28 7 (25 %) 0 0 2 (7.14%) 1 (3.57%) 1 (3.75%) 0 0

Conclusions

In compliance with the results of the EORTC (International antimicrobial therapy cooperative group) we report gram-positive as well as gram-negative pathogens. Staphylococcus coagulase-negative (CONS) is the most common isolated organism, which is also reported by Elmaataoui et al [3]. The analysis of resistance profiles of all species, except enterococci, has shown the emergence of essentially multi-resistant phenotypes.

For this, the establishment of a monitoring programme became necessary to define the characteristics of bacteremia in each structure, and thus provide the basis for appropriate empirical treatment of febrile neutropenic subjects.

doi: 10.1016/j.cca.2022.04.826

W089

Emerging threat of biofilm producing MDR acinetobactercalcoaceticusbaumanniicomplex clinical isolates causing healthcare associated infections in a teaching hospital in Nepal

S. Shreekhanda Shrestha [a], S.K. Mishra [b]

[a] *Department of Clinical Microbiology, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal*
[b] *University of New South Wales, Sydney, Australia*

Background-aim

Acinetobactercalcoaceticusbaumanniicomplex(Acbcomplex), a species complex, present ubiquitously in the environment, has emerged as an extremely daunting pathogen with its infection control and prevention being a major public health problem. Acbcomplex associated infections especially in the hospital settings are difficult to treat, primarily attributed towards its colonization ability, increased resistance to numerous antibiotics and biofilm formation. The infections includes ventilator associated pneumonia, wound infection, urinary tract infection, blood stream infection and meningitis. This study is an attempt to characterize the antibiogram pattern, biofilm production in Acbcomplex isolated from clinical specimen of hospitalized patients in a teaching hospital at Kathmandu, Nepal.

Methods

This laboratory based cross sectional study was carried out in the clinical microbiology laboratory of Tribhuvan University Teaching Hospital, Kathmandu from June 2019 to December 2019. A total of 81 Acbcomplex clinical isolates were obtained from various clinical specimen and processed for antibiogram test, biofilm formation. The antibiogram profile and detection of ®-lactamases(ESBL, MBL) was performed phenotypically following the updated guidelines of CLSI M-100 29th Edition and biofilm formation was detected using by the standard Tissue culture plate method. The data obtained were analyzed according to statistical method using SPSS version 17.

Results

Among the 81 clinical isolates of Acbcomplex, Piperacillin showed highest resistance(90.12%) followed by Ceftazidime(85.18%),Gentamicin(86.4%), Imipenem(80.24%), Meropenem(80.24%), whereas Polymyxin B(100%), Colistin sulphate(100%), Tetracycline(87.1%) were highly susceptible followed by Ampicillin-Sulbactam(63.4%) Cefoperazone-Sulbactam(55.9%). Further on, 29.62% isolates were ESBL producers, 67.9% were MBL producers and 86.41 % were Biofilm producers. There was a significant association between biofilm production and MBL ($P < 0.05$). Biofilm producers were more resistant to most commercial antimicrobials ($p < 0.05$) than biofilm nonproducers.

Conclusions

A large number of MDR Acbcomplex has been noted in this study. This situation is further magnified by the emergence of ESBL and MBL production aided by biofilm production in Acbcomplex. Hence, sensible use of antimicrobial agents is necessary in clinical settings with the continuous surveillance of drug resistivity in laboratories.

doi: 10.1016/j.cca.2022.04.827

W090

Effect of carbapenemase, qace, qace⊗1, and cepa genes on chlorhexidine sensitivity in clinical isolates of gram-negative bacteria with multiple resistance

N. Esaulenko [a], O. Kameneva [b], O. Tkachenko [a], K. Komur [a], S. Kazakov [c,a]

[a] *Burdenko main military clinical hospital of Ministry of defense, Moscow, Russia*
[b] *Children's city hospital N° 22, Saint Petersburg, Russia*
[c] *Russian medical Academy of continuing professional education of Ministry of health, Moscow, Russia*

Background-aim

To conduct a comparative analysis of the sensitivity to chlorhexidine of multiple-resistant gram-negative bacteria, pathogens of infectious conditions in patients of various medical organizations, and to study the relationship between the presence of resistance genes and the minimum inhibitory concentration (MIC) of chlorhexidine.

Methods

The study included 138 strains of multiple-resistant gram-negative bacteria isolated in the period from 2018 to 2019 from various clinical

material, including 51 (37.0%) of those with genes of the most common metal-®-lactamases (VIM, IMP, NDM) and serine carbapenemases (KPC, OXA-48).

Results

Among 138 gram-negative bacteria tested, 7 (5.1%) isolates of the K. pneumoniae species were simultaneously identified with the blaNDM and blaOXA-48 genes. However, chlorhexidine resistance genes (qacE, qacE⊗1, cepA) were more often detected in associations of 2 or 3 genes in bacteria of different species – 21 (15.2%) than associations of carbapenemase genes. Efflux system genes were not detected in isolates of K. ozaenae, M. morganii, and S. marcescens. at the same time, all P. mirabilis isolates had qacE, qacE⊗1, or cepA genes, but the small number of tested strains does not allow us to speak about a General species-specific pattern of distribution of these genes. Strains with the cepA gene were widely distributed (41.7–100.0%), although for E. cloacae and P. aeruginosa, the proportion of such strains was 20.0% and 14.8%, respectively. The frequency of detection of qacE and qacE⊗1 genes was lower in enterobacteria than in non-fermenting gram-negative bacteria and did not exceed 40.0% in individual species, except for species represented by single isolates (C. braakii, C. freundii, P. rettgeri).

The results obtained indicate a high resistance of gram-negative bacteria to chlorhexidine (MIC_{90} from 16 mg / l to 256 mg/l), which corresponds to the results of other authors. The lowest level of resistance to chlorhexidine was found among E. coli strains (MIC_{90} 16 mg / l), the other strains were characterized by high resistance: MIC_{90} P. aeruginosa and A. baumannii 128 mg/l, K. pneumoniae, E. cloacae and P. mirabilis – 256 mg/l. The highest frequency of detection of carbapenemase genes was observed in K. pneumoniae strains – 56.0% and P. aeruginosa-48.1%. CepA genes were widely distributed (enterobacteria-47.8%, A. baumannii - 42.9%), qacE and qacE⊗1 genes were detected more often in non-fermenting gram-negative bacteria than in enterobacteria.

Conclusions

The distribution of cepA, qacE, and qacE⊗1 genes among multiple-resistant gram-negative bacteria in K. pneumoniae, E. coli, and A. baumannii isolates was revealed. We found no significant correlation between the presence or absence of these genes and the MIC of chlorhexidine in gram-negative bacteria. The formation and spread of resistance of bacteria to chlorhexidine has a multifactorial nature, including the defect of qac genes, and also depends on the methodology of research.

doi: 10.1016/j.cca.2022.04.828

W091

A seroprevalence of hepatitis b virus among patients attending tertiary care hospital at Bhairahawa

S. Lal Karn

Department of Microbiology, Universal College of Medical Sciences, Bhairahawa, Nepal

Background-aim

Viral Hepatitis, an acute inflammation of the liver, has emerged as a major public health problem occurring endemically throughout the world.

Prevalence of Hepatitis B varies from country to country and depends upon a complex mix of behavioral, environmental and host factors.

Aims & Objective

The aim of this study was to determine the Seroprevalence of Hepatitis B viral infections among patients attending tertiary care hospital at Bhairahawa, Nepal and also to investigate the sero-epidemiologic feature of HBV in this region.

Methods

Samples of blood were collected from the patients. Serum was separated by centrifugation. Screening of HBsAg was done by Hepacard and positive HBsAg test samples were confirmed by Enzyme linked immune sorbent assay (ELISA), HEPALISA.

Results

Out of 4041 serum samples, 29 samples were positive both by rapid diagnostic kit (RDT) and HEPALISA method. Male positivity were observed in 17(1.20%) and female positivity were observed in 12 (0.45%) cases. Prevalence of HBV infections was 0.71%. Highest incidence of HBV infections was observed in patients of age group 30-39 years contributing 27.5% of all positive cases.

Conclusions

Since the present study also highlighted the prevalence of HBsAg sero positivity as highest among the young adults, so the most promising way of decreasing HBV infection and its further complications is to implement routine vaccination of high risk young adults especially those attending sexually transmitted diseases centers and drug treatment and rehabilitation centers.

doi: 10.1016/j.cca.2022.04.829

W092

Sorting of peripheral blood cells for testing for the content of HCV RNA in patients with chronic hepatitis C

A. Ivanov [a], I. Nikitin [a], A. Krivoruchko [a], M. Yaremenko [b]

[a] *Department of clinical biochemistry and laboratory diagnostics, Military Medical Academy n.a. S.M. Kirov, Saint-Petersburg*
[b] *Department of infectious diseases, Military Medical Academy n.a. S.M. Kirov, Saint-Petersburg*

Background-aim

There are many facts accumulated about the presence of hepatitis C virus (HCV) not only in hepatocytes, but also in peripheral blood cells (PBCs). This can cause not only chronicity of the process, but also the ineffectiveness of treatment with antiviral drugs. Purpose of the study - using a cell sorter, isolate neutrophil, monocyte, and lymphocyte populations to test for HCV RNA.

Methods

The peripheral blood of 28 patients with diagnosed chronic hepatitis C and the absence of a virological response at week 24 of the disease after antiviral therapy (AVT) with pegylated interferon ⟨-2a was studied. In all patients, viral RNA was detected in blood plasma. For cell sorting, samples (PBCs) were stained with CD45 labeled monoclonal antibodies

labeled with PC7 (Beckman Coulter, USA). Stained cells were lysed and washed 2 times with phosphate-buffered saline. The procedure of gating populations of granulocytes, monocytes, lymphocytes and their further sorting into sterile tubes was performed on a MoFlo Astrios EQ cell sorter (Beckman Coulter, USA). At the end of the sorting process, the purity of the selected populations was checked. Then, the sorted populations were analyzed for the presence of HCV RNA by RNA reverse transcription and PCR amplification of cDNA with hybridization-fluorescence detection in real time (AmpliSens HCV-monitor-FL, FGUN TsNIIE, Moscow).

Result

In the examined patients, the level of viral load in the blood plasma was 11 000-10 000 000 IU / ml (2 563 444 ± 1 263 215 IU / ml). Low viral load (<800,000 IU / ml) in plasma was found in 10 (36%) people with the HCV genotype 3a and 2 (11,000-490,000 IU / ml). The remaining 18 patients (64%) had the HCV 1b genotype and high viremia> 800,000 IU / ml (860,000-10,000,000 IU / ml) in plasma. Using a MoFlo Astrios EQ cell sorter, at least 50,000 monocytes, 100,000 granulocytes, 100,000 lymphocytes in each sample were sorted. PCR analysis showed that 18 (64%) patients had HCV RNA in their blood cells. In 8 (28%) patients, viral RNA was detected in granulocytes (viral load <300 IU / ml), in 14 (50%) - in lymphocytes (viral load 310 and 32,000 IU / ml) and in 3 (11%) - in monocytes (<300 IU / ml). In 10 (36%) people, there was no viral RNA in peripheral blood cells. Plasma viremia did not correlate with the level of viral load in blood cells.

Conclusions

In most patients with HCV, HCV RNA is present in the blood cells. Using new cell sorting technologies, it is advisable to test blood cell populations for the presence of HCV RNA before and after AVT in order to control it and predict the development of a relapse of the disease.

doi: 10.1016/j.cca.2022.04.830

W093

Specificity, sensitivity, positive and negative predictive values of nitrite and leukocyte esterase in predicting urinary tract infections at FV hospital

L.T. Loan, F. Maviza

FV Hospital

Background-aim

To determine the reliability of urine dipstick nitrite and leucocyte esterase results in predictive urine culture results.

Methods

We retrospectively reviewed data between 01st January 2018 to 31st December 2019 from 3,597 patient samples with orders for both urine dipstick and urine culture. Dipstick urinalysis for nitrite and leucocyte esterase was performed using Combur-Test® strips (Roche, Mannheim, Germany). The sensitivity, specificity, positive and negative predictive values were calculated and compared using Excel 2016 and Microsoft Power BI.

Result

13.2% of the patients (475 out of 3,597) were aged between 0 to 16 years old; 57.9% (2,084) patients were aged between 16 to 65 years old and 28.9% (1,038) patients were over 65 years old. The ratio of culture positives in each age group was 30% (0 to 16 years old), 31.5% (16 to 65 years old) and 51.2% (65 years and above). Nitrite had sensitivity and specificity of 7% and 99% respectively with positive and negative predictive values of 62% and 83% respectively. Leucocyte esterase had sensitivity and specificity of 67% and 75% respectively with positive and negative predictive values of 55% and 83% respectively. Combined positive nitrite and positive leucocyte esterase had sensitivity of 48%, specificity of 97% and positive predictive value of 87% with a negative predictive value of 83%.

Conclusions

The incidence of urinary tract infections in patient over 65 years old was higher than in the other stratified age groups. Leucocyte esterase is highly sensitive as an indicator for urinary tract infections and positive nitrite result is highly specific in predicting urinary tract infections. A combination of positive nitrite and positive leucocyte esterase demonstrated a very high positive predictive value for urinary tract infections compared to either test individually.

doi: 10.1016/j.cca.2022.04.831

W094

Fecal carriage of multidrug resistance escherichia coli in healthy children: A community based study

A. Sharma

School of Health and Allied Sciences, Pokhara University

Background-aim

Escherichia coli is enteric Gram negative bacilli, earlier recognized as non-invasive commensal. Invasive strains of E.coli have been identified and are associated with variety of human infections. Increasing drug resistance among the E coli has been reported. Multi drug resistance is an ability of organism to resist at least one agent in three or more antimicrobial categories.

Objectives: The aim of this study was to detect fecal carriage of MDR E.coli isolates among healthy school children.

Methods

A total of 139 stool samples were collected from Rastriya Seconday School, Purano Tudikhel, Pokhara-1. Isolation, identification and AST pattern of E. coli isolates were done by standard microbiological techniques. Further ESBL test was performed by Combined Disc Test and data analysis was done by using Microsoft Excel.

Result

A total of 106 E. coli were isolated from the stool samples. MDR E. coli were found to be 8 (7.55%) and 7 E. coli isolates were resistant to Ceftazidime which were further tested for ESBL and 5 isolates were found to be ESBL producer. The resistance of E. coli to Ampicillin, Nitrofurantoin, Ceftriaxone, Ceftazidime, Ciprofloxacin and Gentamicin was

27.36%, 43%, 10.38%, 6.60%, 6.60% and 2.83% respectively. Only one isolate was resistant to all antibiotics used except Imepenem.

Conclusions

The incidence of antimicrobial resistant organisms and multidrug resistance is increasing rapidly especially among gram negative bacteria. E. coli population susceptible to all antibiotics used was a much more diverse group than the resistant and ESBL producing E. coli.

doi: 10.1016/j.cca.2022.04.832

W095

Clinicomycological study of dermatophytosis in Tribhuvan University Teaching Hospital

N.B. Khadka [b], S.K. Mishra [b], S. Parajuli [a], N.P. Shah [b]

[a] *Department of Dermatology, Maharajgunj Medical Campus, Institue of Medicine, Tribhuvan University Teaching Hospital*
[b] *Department of Microbiology, Maharajgunj Medical Campus, Institue of Medicine, Tribhuvan University Teaching Hospital*

Background-aim

Dermatophytosis is a common superficial fungal infection of keratinized tissues caused by dermatophytes. Dermatophytes spread via direct contact from other infected people, animals or soil as well as indirectly from fomites. The characteristic feature includes an inflammation at the edge of skin lesion, noted by redness, sometimes blister formation. It may be seen in both developed and developing countries.

Aim

The aim of this study is to find out the clinicomycological profile of dermatophytosis and to isolate various causative agents at Tribhuvan University Teaching Hospital.

Methods

This was a prospective laboratory-based study that was conducted over a period of six months from April to September 2018. A total of 80 samples were collected at the Department of Dermatology and the specimens were processed at the Department of Microbiology of Tribhuvan University Teaching Hospital for direct microscopic examination (KOH mount) and fungal culture following standard protocol. Specimens from each patient were inoculated into three sets of culture media-Sabouraud dextrose agar, Sabouraud dextrose agar with chloramphenicol & cycloheximide, and dermatophyte test medium. The growth was observed for up to 4 weeks. Microsoft Excel was used for statistical analysis.

Result

Among the total 80 specimens from the patients suspected of dermatophytosis, Tinea corporis 25(31.25%) was most common clinical types followed by Tinea pedis 22(27.5%), Tinea unguium 11(13.75%), Tinea cruris 9(11.25%), Tinea manuum 5(6.25%), Tinea capitis 4 (5.0%) and Tinea faciei 4(5.0%) respectively. Direct microscopy KOH mount was positive in 32 (40.0%) and culture was positive in 34 (42.5%). Trichophyton mentagrophytes and Trichophyton rubrum were the most common fungal isolates which were 12(35.29%) followed by Trichophyton interdigitale, Trichophyton verrucosum, Trichophyton concentricum, Trichophyton equinum, Microsporum fulvum, and Microsporum gypseum respectively. Dermatophytosis were more common in male 42(52.5%) and less common in female 38(47.5%). The male to female ratio was 1.1:1, and the most common age group was 31-40 years (28.75%).

Conclusions

Tinea corporis was the most common clinical type followed by tinea pedis and tinea unguium respectively. Trichophyton mentagrophytes and Trichophyton rubrum were the commonest dermatophytes. KOH mount and culture reports were found to be complementary to each other for the diagnosis of dermatophytosis. Culture should be followed by an antifungal sensitivity test whenever possible.

doi: 10.1016/j.cca.2022.04.833

W096

Prevalence of dengue disease among the suspected Nepalese individuals in a tertiary care setting. A hospital based study

R.B. Khadka

Bachelor In Medical Lab Technology, Health Science, Pokhara University / Crimson College Of Technology, Butwal

Background-aim

Dengue an arboviral disease is one of the foremost important arthropod borne manifestation which is highly prevalent in tropical and subtropical regions of the world. It is estimated that annually around fifty million populations are infected with dengue globally. Clinical manifestations of dengue includes from mild fever to severe life threatening complications like dengue hemorrhagic fever and dengue shock syndrome.

The objective of this study was to determine the prevalence of dengue disease among the suspected individuals in a tertiary care setting.

Methods

A retrospective study was done from March 2019 to May 2019 among 821 participations in Crimson Hospital, Butwal, Nepal. Blood samples were collected for the screening of dengue. A World Health Organization Good Manufacturing Practices (GMP) certified rapid solid phase immune-chromatographic method was used for qualitative detection of Dengue Non – Structural Protein 1antigen (NS1), Anti IgM and Anti IgG antibody following standard protocol. Collected data were tabulated in MS-Excel and analyzed by using Statistical Package for the Social Sciences software version 20.

Result

Out of 821 study participants 389 (47.4%) were Positive for NS1test and was more prevalent among the age group of 21 to 30 years, whereas

175 (21.3%) and 11(1.3%) were positive for IgM and IgG antibodies respectively.

Conclusions

Our study revealed the high prevalence of dengue cases, so the methods for early diagnosis, steps for awareness of prevention, better case management and faster public health response is must to reduce the disease burden.

doi: 10.1016/j.cca.2022.04.834

W097

Usefulness of urine biomarkers for the prediction of in-hospital mortality in the COVID-19 patients

D. Morell-Garcia [a], D. Ramos-Chavarino [c], J.M. Bauça [c], P. Argente Del Castillo [c], M.A. Ballesteros-Vizoso [c], B. García [c], L. Valiña [c], C. Gómez-Cobo [c], L. García De Guadiana Romualdo [b], I. Llompart [c], A.M. García-Raja [c]

[a] *Health Research Institute of Balearic Islands, Palma de Mallorca, Spain*
[b] *Laboratory Medicine Department, Santa Lucía University Hospital, Cartagena, Spain*
[c] *Laboratory Medicine Department, Son Espases University Hospital, Palma de Mallorca, Spain*

Background-aim

Risk factors associated with severity and mortality attributable to COVID-19 have been reported in different cohorts, highlighting the occurrence of acute kidney injury (AKI) in 25% of them. Among other, SARS-CoV-2 targets renal tubular cells and can cause acute renal damage. The aim of the present study was to evaluate the usefulness of urinary parameters in predicting mortality in hospitalized patients with COVID-19.

Methods

A retrospective observational study, in a tertiary care hospital, between March 1st and April 19th, 2020 was done. We recruited adult patients admitted consecutively and positive for SARS-CoV-2. Urinary and serum biomarkers were correlated with in-hospital mortality and evaluated using a logistic regression model and receiver-operating characteristics (ROC) curves.

Result

A total of 199 COVID-19 hospitalized patients were included. Twenty patients died during hospital admission, which represents 10.1% of the number of hospitalized patients. The ROC curve analyses performed on the different clinical variables and biomarkers revealed that the highest area under the curve (AUC) was reached by a model including age above 65 years, presence of blood in urine higher than 0.06 mg/dL of hemoglobin and lactate dehydrogenase levels in serum higher than 400 U/L, with an AUC of 0.923 (95% CI 0.866-0.979; $p < 0.001$). Median time from urinalysis result to death was 8.04 days (IQR: 19.02).

Conclusions

For hospitalized patients with COVID-19, renal involvement and early alterations of urinary and serum parameters on admission are useful as prognostic factors of in-hospital mortality.

doi: 10.1016/j.cca.2022.04.835

W098

Bacterial etiologies, their antimicrobial susceptibility pattern and associated factors among meningitis suspected patients at debre markos comprehensive specialized hospital, Northwest Ethiopia

Z. Hibistu [b], A. Mullu [a], A. Sileshi [b], A. Mihret [a], H.M. Mengist [b]

[a] *Armauer Hansen Research Institute (AHRI), P.O.Box 1005, Addis Ababa, Ethiopia*
[b] *Department of Medical Laboratory Science, College of Health Sciences, Debre Markos University, P.O.Box 269, Debre Maros, Ethiopia*

Background-aim

Bacterial meningitis a medical emergency that requires quick and aggressive therapy. It is still a public health threat with considerable mortality and morbidity worldwide, especially in low-income countries in particular to the meningitis belt of Africa where Ethiopia is not an exception. This study aimed to assess bacterial etiologies of meningitis, their antimicrobial susceptibility pattern, and associated factors among meningitis suspected patients at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia.

Methods

A cross-sectional study was conducted among 152 meningitis suspected patients by using consecutive convenient sampling technique from 1 March to 30 May, 2021. Socio-demographic and clinical data were collected using structured questionnaires. Cerebrospinal fluid was collected aseptically and gram stain, culture and biochemical tests were performed to identify the bacterial etiologies. Antimicrobial susceptibility test was conducted using disc diffusion method on modified Meuller Hinton agar. Data were entered into database (EpiData version 3.1) and exported to SPSS version 23 software for analysis.

Result

A total of 152 bacterial meningitis suspects were enrolled in the study. Half (50%, 76/152) of the participants were males. Overall prevalence of BM was 17 (11.2%) (95% CI = 5.9-16.4) whereas Gram-positive bacteria were responsible for 5.92% (9/152). The most frequent bacterial isolates identified were Staphylococcus aureus and Klebsiella pneumonia each accounting for 29.4% (5/17). All Gram-positive and negative isolates were susceptible to imipenem. About 41.2% (7/17) of bacteria isolates were multi-drug resistant where 6 out of 7 were Gram-negative bacteria. No socio-demographic characteristics were significantly associated with BM ($P \leq 0.05$). Stiff neck [AOR, 95% CI, 47.529 (3.2-10.92), $P = 0.023$] and tonsillectomy [AOR, 95% CI, 137.015 (6.25-12.34), $P = 0.02$] were significantly associated factors for bacterial meningitis.

Conclusions

The prevalence of bacterial meningitis is significantly high in the study area. Specifically, Klebsiella pneumoniae and Staphylococcus aureus were the leading causes of BM among meningitis suspects. A high prevalence of multi-drug resistant bacteria was also observed. An increasing rate of multi-drug resistant bacteria is a striking alarm for policymakers to revise the drug regulatory policy in the country.

doi: 10.1016/j.cca.2022.04.836

W099

Urinary infection with acinetobacter baumannii: Experience of the microbiology laboratory of Chu Mohammed VI of Oujda

I. Elmezgueldi, I. Naji, B. Mouhoub, A. Naili, A. Azghar, F. Aziz, A. Maleb

Faculty of Medecine and Pharmacy of Oujda

Background-aim

Acinetobacter baumannii appears to be one of the most problematic pathogens in health care institutions. Its ability to survive for a long time in hospitals associated with the emergence of resistance potentiates its capacity of nosocomial propagation.

Methods

This is a retrospective descriptive study of 20 strains of Acinetobacter Baumannii, responsible for urinary tract infection over a period from May 2016 to April 2019 at the Laboratory of the CHU Mohamed VI Oujda. Urine samples were obtained from hospitalized and outpatients. Urinary tract infection was investigated by the Cytobacteriological Examination of Urines. Subsequently, Acinetobacter baumannii strains were identified by the BD PHOENIX system. Finally, the antibiogram was performed by the diffusion method on Mueller Hinton medium according to EUCAST recommendations.

Result

During this study 20 strains of uropathogenic Acinetobacter baumannii were isolated, with an overall isolation frequency of 1.8%. 1.40% of patients received antibiotic therapy, 30% were immunocompromised (n = 6), Regarding the mode of collection, 40% were collected by midstream, 35% by indwelling catheter, 20% by collection bag and 5% by pyelostomy. Most of the patients came from the intensive care unit (45%), followed by surgical departments (20%) and outpatients (20%). Antibiotic resistance of the isolated Acinetobacter baumannii strains showed resistance rates to imipenem of 70%, and only 15% remained sensitive to imipenem only.

Conclusions

A. Baumannii is a very occasional cause of urinary tract infection with 1.6% of urinary tract infections acquired in intensive care. Classically, this bacterium is found in the presence of a urinary catheter. Here again, colonization is frequent and differentiation from infection is often difficult.

A. Baumannii is capable of evolving towards multi- or even total resistance. Multi-resistance to antibiotics as well as the ability to adhere to biotic and abiotic surfaces favors the dissemination of A. baumannii in the hospital environment and makes the treatment of this opportunistic pathogen difficult.

doi: 10.1016/j.cca.2022.04.837

W100

The clinical significance of composition and functional diversity of the vaginal microbiome in recurrent vaginitis

M.J. Kim [d], S. Lee [b], M.Y. Kwon [a], M. Kim [c]

[a] *Department of Clinical Medicine Research, Bucheon St. Mary's Hospital, The Catholic University of Korea, Bucheon, Repulic of Korea*
[b] *Department of Laboratory Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Incheon, Republic of Korea*
[c] *Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[d] *Department of Obstetrics and Gynecology, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Bucheon, Republic of Korea*

Background-aim

The vaginal microbiome protects the female genital tract from various diseases, such as vaginitis, which is the inflammation of the vagina with abnormal discharge, itching, and pain. To evaluate the clinical relationship between the vaginal microbiome and the pathophysiology of recurrent vaginitis (RV), we investigated the microbiome taxonomic profile (MTP) in the vaginal samples of Korean female patients with RV.

Methods

Forty women of reproductive age diagnosed with RV were enrolled. The vaginal MTP of patients was analyzed using 16S ribosomal RNA gene sequencing, and the results were compared with that of healthy women (n = 100). Further, the association of the vaginal community state type (CST) with the clinical characteristics was analyzed.

Result

The species abundance of MTP was significantly lower in patients with RV than in healthy women ($p < 0.05$), whereas species evenness and diversity were significantly higher in patients with RV than in healthy individuals ($p < 0.05$). The proportion of the most common vaginal Lactobacillus spp. was significantly lower in the MTP of patients with RV than healthy women ($p < 0.01$). The beta diversity distance was also significantly different between patients with RV patients and healthy individuals ($p = 0.001$). Based on the CST, the MTP of 40 RV samples was categorized as follows: 21 (52.5%) for CST IV, 8 (20.0%) for CST III, 5 (12.5%) for CST I, 2 (5.0%) for CST II, 1 for (2.5%) for CST V, and 3 (7.5%) for mixed CST. Patients with underlying uterine diseases (uterine leiomyoma, adenomyosis, and endometrial polyps) (n = 17) showed higher species richness and diversity than those without (n = 23) ($p < 0.05$).

Conclusions

Changes in the species abundance and microbial diversity in the vagina were strongly associated with recurrent vaginitis. A low proportion of Lactobacillus spp. was found in patients with RV than in healthy women. The abundance and diversity of bacterial taxa were significantly higher in patients with underlying gynecologic disease than in those without. Our study offers an insight into the nature of the vaginal micro-

biome and proposes that surveying the vaginal microbiome is valuable for detecting and treating gynecologic diseases in the future.

doi: 10.1016/j.cca.2022.04.838

W101

Stable preservation of SARS-COV-2 RNA from gargle samples on FTA cards

G. Kriegshäuser [a], A. Berndt [b], D. Alpar [b], C. Oberkanins [b]

[a] *IHR Labor Medical Diagnostic Laboratories, Vienna, Austria*
[b] *ViennaLab Diagnostics, Vienna, Austria*

Background-aim

RT-PCR of SARS-CoV-2 RNA isolated from swabs and gargle samples has become a gold standard to confirm COVID-19 diagnosis. Viral transport media are commonly used to collect and store such specimen, but render them fragile and potentially hazardous. Flinders Technology Associates (FTA) cards were shown to be a reliable option for safe transport and storage of viral RNA pathogens. This study aimed at investigating the stability of SARS-CoV-2 RNA from gargle samples on FTA cards.

Methods

17 confirmed SARS-CoV-2 positive (Ct 18 to 36) and 3 negative gargle samples were spotted onto FTA Classic Cards (Whatman). After drying, eight 3 mm discs were punched out from each card and eluted into TE buffer. Remaining sampling areas were stored either at -20°C or at room temperature for up to 3 weeks. RNA was extracted using the QIAamp Viral RNA Mini Kit. The SARS-CoV-2 RealFast Assay (ViennaLab Diagnostics) covering two viral sequences (N and RdRP genes) plus the ACTB human control gene was applied for PCR. This assay is capable of detecting 10 viral RNA copies per reaction, and has a demonstrated diagnostic specificity and sensitivity of 99% and 100%, respectively.

Result

The RNA status of all 20 specimen was confirmed from gargle samples in parallel to spotting onto FTA cards. After drying and elution, PCR was repeated from FTA discs. A median Ct shift of 4.1 (N gene) and 3.7 (RdRP gene) was observed for discs in comparison to the original liquid samples. PCR testing from FTA cards kept at -20°C for one week showed a median Ct shift of 5.5 (N) and 5.2 (RdRP) against the original gargle samples. Comparable results were obtained from cards kept at room temperature for one week (N: 5.6; RdRP: 5.3). Tests were repeated after 3 weeks and revealed no further loss of detectable RNA (-20°C: N 5.0 / RdRP 4.5; RT: N 5.0 /RdRP 5.3). All gargle samples with Ct < 34 in liquid state maintained PCR positivity from FTA cards.

Conclusions

Our data suggest that FTA cards provide a reliable matrix to preserve SARS-CoV-2 RNA for storage and transportation also at elevated temperatures. The Ct shift observed upon testing from FTA cards can, to a large extent, be attributed to sample input, which in our case was approx. 12-fold higher from liquid gargle samples.

doi: 10.1016/j.cca.2022.04.839

W102

Correlation between the atellica SARS-COV-2 IGG assay results and the presence of SARS-COV-2 neutralizing antibodies

J. Bedini [b], X. Filella [a], S. Hidalgo [a], C. Gonzalez-Escribano [b]

[a] *Biochemistry and Molecular Genetics Department, Hospital Clinic, Barcelona, Spain*
[b] *Core Laboratory, Hospital Clinic, Barcelona, Spain*

Background-aim

Antibodies against SARS-CoV-2 detected by routine immunoassays ensure the existence of antibodies binding the virus, but not necessarily the elimination of the infection. Instead, neutralizing antibodies protect against SARS-CoV-2. Virus neutralization test remains the gold standard, but other neutralization assays have been developed to estimate the neutralizing potential against SARS-CoV-2, among them the FDA approved cPass SARS-CoV-2 Neutralization Antibody Detection (cPass) assay.

We studied the correlation between the results obtained with SARS-CoV-2 IgG (Siemens Healthineers) and cPass (GenScript) assays in 218 patients.

Methods

SARS-CoV-2 IgG is a 2-step sandwich immunoassay automated on Atellica analyzer. It is an assay based on indirect chemiluminescent technology used for the qualitative and quantitative detection of IgG antibodies (sCOVG) against SARS-CoV-2.

cPass assay is a blocking ELISA detection assay using the HRP-conjugated recombinant receptor binding domain (RBD) from the viral spike protein and the human Angiotensin-Converting Enzyme 2 (ACE2). The interaction between HRP-RBD and ACE2 will be blocked if neutralizing antibodies against SARS-CoV-2 RBD are present in the sample. Results higher than 30% indicate the presence of neutralizing antibodies.

Result

We observed that the presence of SARS-CoV-2 neutralizing antibodies correlated with sCOVG results, observing that neutralizing antibodies using the manufacturer cutoff (>30%) were present in 5% of samples with sCOVG <1, 83% of samples with sCOVG between 1 and 2, 96% of samples with sCOVG between 2,01 and 10, and 100% of samples with sCOVG >10.

On the other hand we studied the agreement between the titers obtained for both tests using Passing Bablok regression analysis, obtaining a regression coefficient of 0.881.

Finally, we compared the accuracy of the results of the sCOVG and the cPass tests. The area under the curve obtained in the comparison between both tests was 0,969. Furthermore, we observed a concordance between both tests considering the respective cut-off points (≥1 for sCOVG and ≥ 30 for cPass assay) in 96% of patients included in our study.

Conclusions

We concluded that the Atellica SARS-CoV-2 IgG assay correlates well with the detection of neutralizing antibodies.

doi: 10.1016/j.cca.2022.04.840

W103

The serological profile of toxoplasmosis in pregnant women: Experience of the central laboratory of Mohammed VI University Hospital of Oujda

I. Naji, I. Elmezgueldi, A. Naili, E. Sebbar, A. Hami, M. Choukri

Faculty of Medecine and Pharmacy of Oujda

Background-aim

Toxoplasmosis is a parasitic infection caused by the protozoan Toxoplasma gondii. The infection is asymptomatic in the majority of cases for immunocompetent subjects, presenting a serious risk only for pregnant women, HIV-positive persons and subjects with a weakened immune system. The objective of our study is to evaluate the serological profile of toxoplasmosis in pregnant women, for examinations received at the Central Laboratory of Mohammed VI University Hospital of Oujda.

Methods

This is a retrospective study, conducted over a period of six years (2016-2021), including all requests for toxoplasmosis serology performed in pregnant women as part of the pregnancy assessment. Serology was performed by quantification of IgG and IgM antibodies by the microparticle chemiluminescence immunoassay method on the ABBOTT Architect 8200ci automated system.

Result

The study included 5239 pregnant women with an average age of 27.54 years. Serological tests showed seropositivity in 41.7% of women, seronegativity in 57.1% and seroconversion in 0.2%. The analysis of the different profiles shows a predominance of non-immune pregnant women.

Conclusions

Toxoplasmosis is a disease that can cause significant sequelae in the fetus when contracted during pregnancy. The results of our study show a high rate of non-immunized women, which highlights the importance of implementing preventive measures against toxoplasmosis as well as monthly serological surveillance in non-immunized pregnant women.

doi: 10.1016/j.cca.2022.04.841

W104

Seroprevalence of rubella in pregnant women: Experience of the central laboratory of Mohammed VI University Hospital OF Oujda

I. Naji, I. Elmezgueldi, A. Naili, E. Sebbar, M. Choukri

Faculty of Medecine and Pharmacy of Oujda

Background-aim

Rubella is a benign viral disease of childhood. However, when a woman contracts the disease during pregnancy, the consequences can be dramatic for the fetus, especially if the infection occurs during the first trimester. The objective of our study is to evaluate the seroprevalence of rubella in pregnant women, for examinations received at the Central Laboratory of Mohammed VI University Hospital of Oujda.

Methods

This is a retrospective study, conducted over a period of six years (2016-2021), including all requests for rubella serology performed in pregnant women as part of the pregnancy assessment. Serology was performed by microparticle chemiluminescence immunoassay method on the ABBOTT Architect 8200ci automated system.

Results

The mean age of the patients in our study was 29 years. A percentage of 15% of these pregnant women were seronegative. 64% of the multiparous women remained seronegative despite the recommendations of vaccination after delivery. No cases of seroconversion were found among all patients during the study period.

Conclusions

Congenital rubella is a serious condition that should be eradicated because there is an effective live attenuated vaccine against the disease. Every woman of childbearing age should be immunized. A woman should be tested for rubella before she becomes pregnant or even before she gets married. Interpretation of the serology becomes more complicated if it is done during pregnancy.

doi: 10.1016/j.cca.2022.04.842

W105

Epidemiological profile of SARM strains at the Chu Mohammed VI in Oujda (2018–2021)

A. Naili

Medecin résident

Background-aim

Methicillin-resistant Staphylococcus aureus (MRSA) posed a major public health problem and was responsible for nosocomial and community infections that were difficult to treat [2-3]. The aim of this work is to determine the prevalence of strains of methicillin-resistant Staphylococcus aureus (MRSA) isolated at Mohammed VI University Hospital in Oujda, their resistance profile to other antibiotics as well as the solutions put in place to fight against the spread of these germs.

Methods

This is a retrospective study on brain abscesses at Mohammed VI Oujda University Hospital, during the period from June 2018 to September 2021.The microbiological study of cerebral pus was carried out in accordance with the recommendations of the medical microbiology standard (REMIC) and the antibiotic sensitivity study was carried out in accordance with EUCAST recommendations.

Result

A total of 485 strains of S. aureus isolated from various biological products of patients, ten percent of which were resistant to methicillin

(MRSA strains). These MRSA were isolated from blood cultures in 44.9% of cases, deep or superficial pus in 36% of cases and urine in 8.1% of cases. The samples came from the pediatric (18.37%), neonatology (8.6%), resuscitation (10.2%) and surgery (16.4%) departments while 18.37% of the samples; (n = 9) were from outpatients. A varying proportion of MRSA strains expressed resistance to other families of antibiotics: aminoglycosides (KTG phenotype) 37%, Erythromycin 31%, rifampicin 12%, Sulfamethoxazole – trimethoprim 27%, fluoroquinolones 16%, and glycopeptides 6% The evolution of this resistance over the years was as follows 2018 (33.33%), 2019 (12.87%), 2020 (11.26%) and (4.85%) in 2021.

Conclusions

It is certain that the application of preventive measures will stabilize or reduce the spread of multi-resistant bacteria (BMR), but monitoring tools (local and national network), still absent in Morocco, must also be set up, in order to assess the impact of preventive actions.

doi: 10.1016/j.cca.2022.04.843

W106

Control of the eradication of helicobacter pylori at the level of the Mohammed VI University Hospital of Ouja about of 548 cases

A. Naili

Medecin résident

Background-aim

Helicobacter pylori infection is a real problem of public health. Despite its high prevalence in populations (71% in Morocco), this infection is usually successfully treated [1-2]. The objective of our study is to report the rate eradication of Helicobacter-pylori infection diagnosed in patients of the CHU Mohammed-VI of Oujda as well as the different therapeutic protocols adopted by medical doctors.

Methods

This is a retrospective study, based on the treatment computerization of nearly 548 urea analysis requests c13 over a period of 48 months (from November 2016 to July 2019).

- The resident doctor oversees: Patient reception and questioning "Survey". Accompaniment of the patient on all stages of the respiratory test (Protocol). Performing the breath test on the automaton HELIFAN PLUS*

Result

- We received 548 patients with a ratio of 0.46 and an average age of 47.5 years.
- The overall eradication rate was 77.5%.
- 61% of patients could not document their antibiotic therapy while the rest received a of the following four eradication protocols

[Figure A]:

- Protocol A (IPP + Amoxicillin for 14 days) in 14.7% of patients with a rate of 80% success.
- Protocol B (IPP + Amoxicillin for 5 days then PPI + clarithromycin + Metronidazole for 5 days), in 12.3% of patients with an 85% success rate.
- Protocol C (IPP + Amoxicillin + Clarithromycin + Metronidazole for 10 days), in 58.6% of patients with a rate of 83% success.
- Protocol D (IPP + Bismuth + Metronidazole + tetracycline for 10 days), in 4% of patients with a rate of 100% success.

Conclusions

- The system we use is efficient in HP eradication control, but in the event failure, only the culture and study of sensitivity to antibiotics can prove possible resistance.
- A national plan should be put in place to reduce the increasing prevalence of this infection in our country by controlling and rationalizing prescriptions and emphasizing information and patient involvement in the protocol eradication.

doi: 10.1016/j.cca.2022.04.844

W107

Staphylococcus aureus bacteremia at Mohammed VI University Hospital in Oujda: Epidemiological profile and antibiotic resistance

N. Benhamza, S. Farih, Y. Sbibih, A. Saddari, H. Zarroui, A. Maleb

Laboratory of Microbiology, Mohamed VI teaching hospital/ Faculty of Medicine and Pharmacy (University Mohammed the first), Oujda, Morocco

Background-aim

The objective of our study was to describe the epidemiological profile of blood culture isolates positive for Staphylococcus aureus at the Mohammed VI University Hospital of Oujda (Morocco) as well as the resistance profile of the strains isolated to antibiotics.

Methods

This is a retrospective and descriptive study, lasting 15 months, from July 2016 to September 2017, carried out in the microbiology laboratory of the Mohammed VI University Hospital in Oujda. Blood cultures were treated in accordance with the recommendations of the medical microbiology standard REMIC (medical microbiology standard) and EUCAST (European Committee on Antimicrobial Susceptibility Testing).

Result

We collected 2314 requests for blood cultures, including 130 (12%) positive for S. aureus. Most of the requests came from the medical services (57%; n = 74), followed by the intensive care units (34%; n = 44). 60% (n = 78) of bacteremia occurred in the context of wearing an intravascular device (IVD). 88% (n = 115) of S. aureus strains resistant to unprotected penicillins, 17% (n = 22) were resistant to methicillin (MRSA), 10% (n = 13) were resistant to gentamicin and no strains was not resistant to glycopeptides. The prognosis of S. aureus

bacteremia is often unfavorable with high mortality rates. Our results are close to those of other national studies.

Conclusions

The main risk factor for acquiring S. aureus bacteremia in our study would be the wearing of IVDs. The correct management of IVDs (placement, duration, HDM, skin antisepsis, etc.) should make it possible to reduce the occurrence of S. aureus bacteremia in our establishment.

doi: 10.1016/j.cca.2022.04.845

W108

Urinary tract infections in intensive care at Mohammed VI University Hospital in Oujda: Epidemiological profile and antibiotic resistance

N. Benhamza, Y. Sbibih, A. Saddari, H. Zarroui, S. Farih, A. Maleb

Laboratory of Microbiology, Mohamed VI teaching hospital/ Faculty of Medicine and Pharmacy (University Mohammed the first), Oujda, Morocco

Background-aim

The objective of this work is to establish the epidemiological profile of urinary tract infection in patients admitted to intensive care and to study the resistance of the isolates to antibiotic.

Methods

This is a 37-month retrospective study from March 22, 2016 to April 11, 2019 concerning requests for cytobacteriological examination of urine (ECBU) at the level of the microbiology laboratory of Mohammed University Hospital. VI of Oujda. The urine was treated in accordance with the recommendations of the medical microbiology standard REMIC (medical microbiology standard) and EUCAST (European Committee on Antimicrobial Susceptibility Testing).

Result

We collected 945 ECBU requests from the intensive care units representing 4% of all ECBUs requested by the CHU (n = 23,217). ITU was found in 7.3% (n = 69). Among UTIs, Male represented 58.7% (n = 37). Enterobacteriaceae was the most isolated family of germs (60.9%) (n = 42) with E. coli as the leader (36.2%; n = 25). The resistance of these Enterobacteriaceae to penicillins and sulfonamides were 80% (n = 20), 40% (n = 10), respectively.

Our results agree with those of studies carried out at the national level.

Conclusions

The frequency of urinary tract infections in intensive care and their consequences remain worrying and justify optimal management.

The management of these infections leads to adapt the antibiotic therapy to the data of the antibiogram and the corresponding field.

doi: 10.1016/j.cca.2022.04.846

W109

Prevalence of hepatitis B virus and hepatitis C virus co-infection in both apparently healthy adults and suspected viral hepatitis patients of the chemical pathology laboratory, University College Hospital, Ibadan

T.D. Ogunleye, O.O. Popoola, O.I. Famuyiwa

Chemical Pathology Department, University College Hospital, Ibadan

Background-aim

Hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection poses a high risk of progressive decline in liver functions if not carefully and aggressively treated. This could eventually lead to several clinical outcomes associated with chronic liver disease. This study was carried out to determine the prevalence of HBV and HCV co-infection within a period of fifteen months at the Chemical Pathology Laboratory of the University College Hospital, South western part of Nigeria.

Methods

Venous blood samples were obtained in K3 EDTA bottles and spun at 4000prm for about 10mins, and the plasma separated was transferred into plain bottles for analysis on Abbott i1000sr auto analyzer.

Both HBV and HCV were assayed on Abbott i1000sr ARCHITECT system using a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus (Anti-HCV) in human plasma. Levels 1 and 2 qual¬ity control materials specific for HBsAg and Anti-HCV respectively produced by Abbott was always included in routine analysis. Any result less than 0.9 Col was considered non reactive while results between 0.9 to 1.0 were considered borderline results which were re¬peated for confirmation. Any result greater than 1.0 was considered reactive.

The laboratory records of both Hepatitis B virus (HBV) and hepatitis C virus (HCV) in apparently healthy adults and suspected viral hepatitis patients from October, 2020 to December, 2021 was compiled.

Result

A total number of 103 apparently healthy adults and suspected viral hepatitis patients who were requested to simultaneously get tested for both HBsAg and Anti-HCV were investigated. 59 (57.3%) of the population were male while 44 (42.7%) of the popula¬tion were female. Only three 3 (2.9%) of the population tested positive to HCV and two out of the three simultaneously tested negative to HBV. However 19 (18.4%) of the population tested positive to HBV and 18 out of the 19 simultaneously tested negative to HCV. This suggests that Hepatitis B viral infection is higher than Hepatitis C viral infection. This study shows that only one (0.97%) out of the 103 population studied had HBV and HCV co-infection.

Conclusions

A decline in the level of hepatitis B virus infection whose prevalence is highest could be achieved through public enlightenment campaign, massive immunization of children and adults who are at risk. Effective diagnosis, aggressive treatment and follow-up should be provided for those with co-infection of HBV and HCV.

doi: 10.1016/j.cca.2022.04.847

W110

Escherichia coli urinary tract infections at Mohammed VI University Hospital in Oujda: Epidemiological profile and antibiotic resistance

N. Benhamza, A. Saddari, Y. Sbibih, H. Zarroui, S. Farih, A. Maleb

Laboratory of Microbiology, Mohamed VI teaching hospital/Faculty of Medicine and Pharmacy (University Mohammed the first), Oujda, Morocco

Background-aim

Urinary tract infection (UTI) is one of the most common bacterial infections and is a major public health problem.

The objective of this work is to propose, based on updated data, the microbial ecology of E.Coli urinary tract infection at the MED IV University Hospital of OUJDA, and to monitor its profile of sensitivity to antibiotics.

Methods

This is a retrospective study with a descriptive aim, which was carried out on 1129 urine cytobacteriological examinations positive for E. Coli from hospitalized and outpatients, treated in the microbiology laboratory of the CHU MED IV of OUJDA, over a period of 36 months from 04/05/2016 to 13/04/2019.

Cyto-bacteriological examinations are carried out by conventional or automated techniques. The culture is carried out on agar medium. The antibiogram is done by automated technique (The Phoenix®) and the interpretive reading of the antibiogram according to the recommendations of the EUCAST (European Committee on Antimicrobial Susceptibility Testing). Statistical analysis of the results is done in Excel.

Result

Out of all the ECBUs examined, 1129 met the inclusion criteria, 48.79% of the requests came from the emergencies (n = 551) distributed in specialized emergencies with 27.45% (n = 310) and in pediatric emergencies with 21.34% (n = 241), UTIs are mainly of a community nature, given that 92% of infections are not associated with care, samples from the jet medium and adhesive collection bag represent 86.26% (n = 974), the female sex represents 71.21% (n = 804) of patients with UTI, with an F / M sex ratio of 2.47.

The age groups affected by ECBU applications revealed the presence of two predominant populations:

A pediatric population (<14 years) which represents 25.95% (n = 293), and a senile population aged 55 to 79 years which represents 34.01% (n = 384).

And in combination of the two factors we found that For the female sex there are 2 peaks of Age, the first between 3 years and 12 years which represents 17.71% (n = 200) and the second between 51 and 69 which represents 17 , 80% (n = 201).

For the male sex there are 2 peaks of age, particularly between 1 and 6 years which represents 2.8% (n = 32), and the second between 50 and 72 which represents 11.6% (n = 131).

Reading and interpretation of antibiograms showed low sensitivity for: amoxicillin with 55.53%; amoxicillin + clavulanic acid with 35.96%; ampicillin with 55.8% sulfamethoxazole-trimethoprim with 25.86%; tazobactam-piperacillin with 21.78%, piperacillin with 42.42%, Ciprofloxacin with 16.03%, and ticarcillin with 42.95%.

Conclusions

E. Coli is a worrying problem, hence the need for rigorous application of hygiene rules and rational prescription of antibiotics.

Knowledge of the bacteriological profiles and the use of the targeted antibiogram will allow management better suited to each hospital context.

doi: 10.1016/j.cca.2022.04.848

W111

Assessment of synergistic effect of various antimicrobial combinations on extensively drug-resistant (XDR) acinetobacter baumannii clinical isolates

Y.J. Ko [a], K. Oh [a], C. Kim [b], G. Park [a], S. Kang [a], S. Jang [a]

[a] *Department of Laboratory Medicine, Chosun University, College of Medicine, Gwangju, South Korea*
[b] *Department of Premedical Science, Chosun University, College of Medicine, Gwangju, South Korea*

Background-aim

The high rate of carbapenem-resistant Acinetobacter baumannii is a major obstacle to nosocomial infections in South Korea. There were few treatment options for this high rate of multidrug-resistant and extensively drug-resistant (XDR) A. baumannii infection. We evaluated the synergistic activity for combinations of colistin and antibiotics or peptides against XDR A. baumannii using a time-kill assay to find combinations with high effectiveness.

Methods

Time-kill assays were performed for 7 XDR clinical isolates of A. baumannii by using colistin, doripenem, fusidic acid, linezolid, minocycline, tigecycline, vancomycin, and Alyteserin E4K peptide (Anygen, Gwangju, South Korea) combinations. Concentrations representative of clinically achievable levels for each antibiotic (colistin: 2 g/mL, doripenem: 8 g/mL, fusidic acid: 1 g/mL, linezolid: 8 g/mL; minocycline: 4 g/mL, tigecycline: 2 g/mL, and vancomycin: 32 g/mL) and 1/4 MIC for Alyteserin E4K were used in this study.

Result

The colistin-minocycline and colistin-Alyteserin E4K combination displayed the highest rate of synergy by criteria 1 (57.1%) and criteria 2 (33.3%), respectively. The colistin-Alyteserin E4K combination showed the highest rate of bactericidal activity (33.3%) in clinical isolates of A. baumannii. The colistin-minocycline and colistin-vancomycin combination showed a low rate of synergy by criteria 2 (14.3%) and bactericidal activity (14.3%). The colistin-tigecycline combination showed a higher rate of synergy by criteria 1 (42.9%), compared with the colistin-vancomycin combination by criteria 1 (28.6%). No antagonism was observed with any colistin-antibiotic combination.

Conclusions

The colistin-Alyteserin E4K combination was found to have in-vitro synergy and bactericidal activity among clinical isolates of XDR A. baumannii. These findings could serve as the basis for clinical studies.

doi: 10.1016/j.cca.2022.04.849

Clinica Chimica Acta 530 (2022) S325–S331

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Miscellaneous

M203

Strengthening community-based health insurance (CBHI) towards a value-based laboratory medicine

K. Sarong

Institute of Clinical Laboratory Sciences, Silliman University

Background-aim

Establishment of community-based health insurance (CBHI) has been considered as an intermediate stage, safeguarding from households' direct payment for healthcare services to forms of prepayment, in transition to universal health coverage (UHC) (WHO, 2015). Although universal health coverage has been championed by health policymakers around the world (Etienne & Wong, 2015) and the Philippine Health Agenda 2016-2022 and the Universal Health Care Act came into being under the recent government administration (DOH, 2017), a reality lags far behind ensuring UHC in low- and middle-income countries (LMICs) like the Philippines and the utilization of services. Thus, this study aims to determine whether social capital, which includes groups and networks, trust and solidarity, information and communication, social cohesion and inclusion, empowerment and political action of CBHI members in Negros Oriental, is related to the socioeconomic-demographic profile (age, sex, religion, number household members), CBHI services, and health quality.

Methods

A total of 239 respondents participated in answering the written questionnaires and data gathered were subjected to statistical analysis to determine the relationships among the variables.

Results

Results revealed that there is a significant relationship between socioeconomic-demographic profile to social capital (but not all variables, namely, number of household members, religion and educational attainment with p values of 0.049, 0.012, 0.046, 0.000, respectively), while no significant relationships were observed for socioeconomic-demographic profile (number of household members, religion, and educational attainment) to CBHI services, social capital to CBHI services (p values = 0.603, 0.299) and health quality of members to CBHI services (p value = 0.639).

Conclusions

It can be concluded that CBHI may not be suitable in all situations, but can still play an important role to health care programs and public health. Thus, heightened awareness, proper education, and expanded healthcare services through integrative medicine are highly recommended.

doi: 10.1016/j.cca.2022.04.853

M204

Publication outcomes of the abstracts presented at the annual conferences of Pakistan Society of Chemical Pathologist (PSCP) and reasons for non-publication

H. Majid [c], i. Siddiqui [c], M. Aamir [a], L. Jafri [c], A.H. Khan [c], A. Ijaz [b]

[a] *Department and Pathology, Armed forces Institute of Pathology, Rawalpindi*
[b] *Department and Pathology, Bahria International Hospital, Rawalpindi*
[c] *Department of Pathology and laboratory Medicine, Aga Khan University*

Background-aim

Annual conference of PSCP is the largest national event fully dedicated to the analytical, clinical, epidemiological and translational areas of research in Chemical Pathology in Pakistan. Since 2012 PSCP annual conferences are jointly conducted with PAP annual conference. Researchers are invited to submit scientific abstracts via email at least 2-3 months prior to the conference. Abstracts received are then assessed for relevance and scientific value by an abstract review committee, which selects abstracts to be presented as oral or poster presentations. The aim was to determine the publication rate of abstracts presented at the PSCP annual conference and assess the reasons for non-publication.

Methods

All abstracts accepted for presentation at the PSCP conferences from 2012-2018 were identified from the abstract books, previous conference program details and/or members of the scientific committee.

Two members of PSCP conducted a manual search of the online databases for each abstract using a ywords and author names. And authors of those abstracts, not found on the aforementioned search engines were contacted to gather information about the full manuscript published.

To determine the barriers and challenged in publishing abstracts presented at conferences a focus group discussion (FGD) with three young researchers and junior Pathologists and a survey with authors of abstracts who have not published their work.

Results

The average rate of full manuscript publication was 24.2% for the abstracts presented at joint PSCP and PAP conferences, ranging from

11.1 – 35.1%, more for oral (27%) compared to poster presentation (18.8%). Most of the manuscripts were published after 3 years of abstract presentation.

A survey questionnaire to identify factors related to non-publication of abstracts was sent to presenters who have not yet published there research, the response rate was 30%. The main factor identified was lack of time, followed by limited writing and paper submissions skills, lack of funding for journal publication fee and article not submitted due to statistically low or non-significant results.

Conclusions

Almost 25% of abstracts were published. The main factor of not publishing a research paper identified was lack of time and limited writing skills. This data reflects that there is need to arrange workshops/symposia for presenter to improve their writing and time management skills and improve the quantity and quality of abstracts/full manuscripts published.

doi: 10.1016/j.cca.2022.04.854

M205

Verifications of a new heparin tube with an antiglycolysis for glucose and biochemical testing

K. Moonla [b], R. Wiriyaprasit [b], N. Apiratmateekul [a], W. Treebuphachatsakul [a]

[a] *Medical Device Research Laboratory, Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University Phitsanulok, Thailand*
[b] *We Med Lab Center, Muang, Phitsanulok, Thailand*

Background-aim

Heparin is commonly used as an anticoagulant for blood specimen collection for biochemical tests. Innomed ® tube is a novel blood collecting tube that coated inside with lithium heparin (LH) and anti-glycolysis, D-mannose, as additives at the optimal concentration. The objectives of this study were to verify Innomed ® tube (LH/D-mannose) on the efficiency of preserving glucose levels and to determine the influence of biochemical tests.

Methods

Blood samples from 40 subjects (20 healthy adults and 20 diabetic patients) were collected in different tubes: Innomed ® tube with LH/D-mannose, NaF/EDTA commercial tube (reference tube for glucose) and serum tube without additive (reference tube for biochemical analytes). Plasma glucose concentrations obtained from Innomed ® tube with LH/D-mannose and reference tube were determined by hexokinase method and compared at base line to 8 hours to investigate the efficiency of preserving glucose. Fourteen biochemical tests obtained from Innomed ® tube (LH/D-mannose) were compared to reference serum tube by using bias (%) within desirable bias ($bias_d$) as acceptance criteria.

Results

Plasma glucose concentrations obtained from Innomed ® tube were not significantly differ at base line ($p = 0.958$) and 8 hours ($p = 0.618$) from those from reference tubes. No hemolysis occurred in plasma samples of Innomed ® tube. Fourteen biochemical tests: cholesterol, triglyceride, HDL,LDL, uric acid, BUN, creatinine, AST, ALT, ALP, total protein, albumin, total bilirubin and direct bilirubin obtained from Innomed ® tube were not clinical difference from those obtained from reference serum tubes with biases less than $bias_d$.

Conclusions

Innomed ® tube (LH/D-mannose) could be used for blood specimen collection for plasma glucose and other 14 biochemical tests. Innomed ® tube (LH/D-mannose) could effectively preserve glucose at least 8 hours. Innomed ® tube (LH/D-mannose) may useful for glucose and other biochemical tests within one single tube in urgent case and patients with difficult blood collection.

doi: 10.1016/j.cca.2022.04.855

M206

High resolution HPLC as a tool for screening hemoglobin variants. Managing extra information technology offers in a clinical laboratory

C. Minea, M. Gómez Chinchón, L. Montero Herrero, T. Pérez Gaya, A. Porres Cubero

Analisis Clínicos, Hospital Fundación Jiménez Díaz, Madrid

Background-aim

HPLC is a very known method that can separate normal and hemoglobins variants as A, A2, F, S, C, D. Also, HPLC is still in many places a method of choice for the study of beta thalassemia.

With the advantage of an automated system, HPLC is used nowadays in many hospitals for the determination of HbA1c.

The HPLC method with high resolution mode permits not only the determination of the percentage of the HbA1c but also provides information about variants of hemoglobin such as S, C, D, E, fetal. These variants of hemoglobin may be informed with the result of the HbA1c. Sometimes the variant hemoglobin is detected by the presence of “unknown” peak or by a P3 peak greater than 10%. If the presence of a suspicious peak is detected in the HA1c analysis of hemoglobin variant the study should be completed with other methods such as electrophoresis.

Methods

We wanted to see how many different hemoglobin variants we can find using HPLC high resolution System D-100 from BIORAD®, with the aim of implementing a different approach in the way we use all this extra data that D100 offers.

Retrospectively we review all the HbA1c determination from the month of October 2019 and we had a total of 21514 petition. We discarded all the petition where HbA1c had already a result greater than 7% in the previous three months period or less than 7% in the previous six months period (487 determinations). We selected all the hemoglobin variants that were identified.

Results

From a total of 21027 determinations we identify 59 cases of hemoglobin variants. From this 59 cases HbS represented 62.7% of the total, HbC a 15.25%, HbE a 11.8%, HbD a 5.2% and fetal Hb a 5.2%.

In 13 cases we were unable to inform the results because of the presence of a pick previous to A0 hemoglobin that interfere with the result of HbA1c and we recommended following up with fructosamine as our protocol stipulates and completed the study with electrophoresis by System D-10 from BIORAD® in 7 cases. We confirm three cases of beta- delta thalassemia and one case of sickle cell anemia; in the rest of the cases electrophoresis did not found any abnormal Hb or found Hb without clinical relevancy.

Conclusions

The use of automated high resolution HPLC system is a rapid way for HbA1c determination in Clinical Laboratory. The additional information that this kind of method provides may be explored as a screening method for hemoglobin variants. We have seen in our study that the frequency of the Hb variants is the same as described in literature.

However, dealing with such information may required new decisions or protocols in the hospital such as:

1. Confirming the hemoglobin variant
2. Evaluating haw this extra information is received by the specialists interested in HbA1c results
3. The possibility of offering genetic counseling for those patients where their offspring may be clinically affected in the eventuality of both partners having the same hemoglobin variant.

doi: 10.1016/j.cca.2022.04.856

M207

Simultaneous measurements of nicotine and its metabolites in urine by LC-MS/MS in the general Korean population

J. Oh [a], M. Chun [a], J.H. Hwang [b], J. Lee [b], J.H. Jee [b], S. Lee [a]

[a] *Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea*
[b] *Health Promotion Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea*

Background-aim

Measuring nicotine metabolites is the most commonly used objective method for identifying nicotine exposure. However, the concentrations and relative ratios of nicotine metabolites are different among individuals, so it is necessary to measure various kinds of metabolites. In addition, detection of minor tobacco alkaloids such as anabasine can provide information about smoking status or compliance with nicotine replacement therapy. In this study, we determined 5 urinary biomarkers of smoke exposure using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the general Korean population.

Methods

Cotinine, nicotine, 3-OH cotinine, nornicotine, and anabasine were measured in urine samples from 824 subjects (652 male and 172 female, median age 53 years) who visited health promotion center. Smoking history was obtained from medical chart review. Each 30 μl of urine sample was diluted with 90 μL of acetonitrile containing 5 deuterated internal standards. LC-MS/MS analysis was performed by a Waters XEVO TQ-S tandem quadrupole MS (Waters Corporation, USA) in multiple reaction monitoring mode. The method was validated by evaluating sensitivity, selectivity, precision, accuracy, linearity, extraction recovery, matrix effect, and carry-over.

Results

In current smokers, all 5 markers were detected in 105 subjects (86.8%) and 4 markers were detected in 116 subjects (95.9%). Cotinine, nicotine, 3-OH cotinine, nornicotine, and anabasine were detected in 99.2%, 97.5%, 98.3%, 96.7%, and 88.4% of current smokers, respectively. No analytes were observed in 94.5% of 597 non-smokers and two or less analytes were detected in 99.7%. Cotinine, nicotine, 3-OH cotinine, nornicotine, and anabasine were not detected in 98.5%, 98.0%, 98.5%, 99.8%, and 96.0% of non-smokers, respectively. Positive correlations among nicotine metabolites (cotinine with nicotine, $r = 0.378$; cotinine with 3-OH cotinine, $r = 0.612$; cotinine with nornicotine, $r = 0.502$; nicotine with nornicotine, $r = 0.769$; and $P < 0.05$, respectively.) were noted.

Conclusions

To our knowledge, this is the first study that simultaneously investigated multiple urinary biomarkers of smoke exposure in Korea. Our results showed significant potential for sensitive and accurate detection of multiple markers in urine to evaluate smoke exposure.

doi: 10.1016/j.cca.2022.04.857

M208

Pakistan inherited metabolic disorders network (PAK-IMD-NET): Breaking silos and bringing synergies

H. Majid [a], L. Jafri [a], A. Ijaz [b], A.H. Khan [a]

[a] *Department of Pathology and laboratory Medicine, Aga Khan University*
[b] *Department of Pathology, Bahria International Hospital, Rawalpindi*

Background-aim

The Pak-IMD-Net is a working group of PSCP for strengthening the diagnostics, education, and research in the area of inherited metabolic disorders (IMDs). Aim of Pak-IMD-Net was capacity building of laboratorians by enhancing and updating their knowledge regarding screening, diagnosis, management, and prevention of IMDs through workshops and courses.

Methods

This working group was approved in November 2018 and officially launched in on March 1st, 2019 and PSCP members were offered membership of Pak-IMD-Net. This project had three major activities knowledge dissemination, developing national guidelines, encouraging pathologists to engage in collaborative scientific research and to support the diagnostics and newborn screening activities, in the field of IMDs across Pakistan.

Results

As of January 2020, Pak-IMD-Net has established a network of seventeen members in ten different institutes across Pakistan and one corporate member. From this platform s symposium on 3-methylcrotonylglycineuria, two hands-on workshops on Aminoaci-

dopathies and Organic acidurias were conducted. 'Rare-links' a quarterly CME seminar was initiated with first on urea cycle disorders with an international speaker. More than 100 participants from different institutes and cities of Pakistan attended 'Rare-links' and there were pathologists, pediatricians, pediatric neurologists, medical students, trainees and technologists from various hospitals across Pakistan. Capacity building of one junior pathologist was accomplished by facilitating training at an international biochemical genetics lab and two educational grants were submitted. Members represented at international conferences and a manuscript on developing a nation-wide newborn screening program is under review.

Conclusions

Pak-IMD-Net has made important steps towards improving and sharing knowledge on IMDs through the educational activities conducted and stepwise extension of the network. The group has a significant role to improve diagnostics and care of patients with IMDs in Pakistan.

doi: 10.1016/j.cca.2022.04.858

M209

Usefulness of reference change values for delta check limits

S. Lee [a], J. Son [a], H. Kwon [a], K.H. Park [a], G.G. Yu [a], E. Han [a], D.W. Jekarl [b], Y. Kim [c]

[a] *Department of Laboratory Medicine, The Catholic University of Korea, Incheon St. Mary's Hospital*
[b] *Department of Laboratory Medicine, The Catholic University of Korea, Seoul St. Mary's Hospital*
[c] *Department of Laboratory Medicine, The Catholic University of Korea, St. Vincent. Hospital*

Background-aim

Within-subject biological variation (CV_I) is used for the reference change value (RCV) delta check limit. In this study, the usefulness of CV_I and RCV delta check limits, with reference to the EFLM biological variation database, were investigated by comparing the population distribution-based delta check limits.

Methods

For the six tests including AST, ALT, ©-GTP, Glucose, Creatinine and Hemoglobin, $RCV_{95\%}$, $RCV_{99\%}$ and $RCV_{99.9\%}$, delta limits were obtained. The nonparametric 95% range and 99% range delta limits were obtained from the population distribution of delta percentage difference of health examination group (Jan 2014 to Dec 2018), and the outpatient and inpatient groups (Jan to Dec 2018). Excess rates (%) in total and all three subgroups were examined according to the different delta check limits. In addition, we analyzed the correlation between median CV_I estimates and population-delta check limits for six tests.

Results

The delta percentage difference of the six tests showed a non-normal distribution, and the median was statistically significant different among the health examination group, the outpatient group and the inpatient group (all, $P < 0.001$). In total six tests, the overall excess rates (%) decreased in the order of $RCV_{95\%}$, $RCV_{99\%}$ and $RCV_{99.9\%}$, 95% range, and 99% range delta limits, and the proportion of the health examination group was gradually decreased and the proportion of inpatients increased. A good correlation was observed between median CV_I estimates (ranged from 3.5 to 10.1% for six tests) and population-delta check limits ($r = 0.98$).

Conclusions

The RCV delta check limit needs to be applied differently depending on the health and disease group. CV_I can be useful for estimating population-delta check limits.

doi: 10.1016/j.cca.2022.04.859

M210

Hematological and biochemical differences in school going children with sickling disorder in steady state

R. Pandit [b], B.K. Yadav [a]

[a] *Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Nepal*
[b] *National Public Health Laboratory, Ministry of Health and Population, Kathmandu, Nepal*

Background-aim

Sickling disorders refer to the clinical condition that range from asymptomatic sickle cell trait to the severe and sometimes life threatening sickle cell anemia. Chronic oxidative stress, abnormal lipid homeostasis and unceasing state of hemolysis in sickling disorder patients may lead to abnormal hematological and biochemical parameters. In this study, we tried to assess hematological and biochemical differences in sickling disorder patients with the help of laboratory parameters.

Methods

A cross-sectional study was carried out in 60 sickling disorder patients in steady state, aged 10-18 years. Age and sex matched 50 healthy volunteers were taken as control. The samples were tested for complete blood count, lipid profile (triglyceride, total, HDL and LDL-cholesterol) and liver function test parameters (total and direct Bilirubin, ALT, AST and ALP). The data was analyzed using SPSS; student's t-test was used to compare the mean and, p-value less than 0.05 was considered statistically significant.

Results

Total cholesterol [118.9 ± 25.6 ($p = 0.01$)] and high-density lipoprotein cholesterol (HDL-C) [37.83 ± 9.3 ($p = 0.001$)] were significantly lower in cases compared to control. The levels of Triglyceride [95.6 ± 37.8 ($p = 0.02$)], total bilirubin [1.02 ± 1.01 ($p = 0.001$)], direct bilirubin [0.18 ± 0.15 ($p = 0.05$)] and alkaline phosphatase (ALP) [321.2 ± 157.4 ($p = 0.000$)] were significantly higher in cases than those of controls. We also obtained significantly lower values ($p = 0.000$) for hemoglobin, PCV, MCV, MCHC and Platelets in cases compared to control.

Conclusions

Sickling disorder patients had dyslipidemia and abnormal liver function test parameters which indicates the high risk for cardiovascular and

liver disease. Those patients also had increased risk for organ damage due to decreases oxygen supply resulting from oxidative stress.

doi: 10.1016/j.cca.2022.04.860

M211

Serum procalcitonin in the pediatric emergency room

C. Minea [a], J. Aguilera Correa [b], M. Martin Garcia [b], S.V. Llanos [b], A. Macias Valcayo [b], R. Fernandez Roblas [b]

[a] *Analisis Clínicos, Hospital Fundación Jiménez Díaz, Madrid*
[b] *Microbiology Departement, Hospital Fundación Jiménez Díaz, Madrid*

Background-aim

Procalcitonin(PCT) is a serum biomarker used in the management of sepsis. PCT is produced as a consequence of the endotoxin production or mediators released in response to bacterial infections. The use of PCT in the clinical practice may help distinguish bacterial infection from other causes of infection or inflammation. Also ware described correlation between serum PCT concentration and gram negative (GN) and GP bacteria.

T was petitioned between 2009 and 2018 in the Pediatric Emergency Room. We also collected the diagnostics at discharged and the microbiologic identification of the microorganism by cultures and PCR.

Methods

We have collected in a retrospective observational study the data from 204 pediatric patients till 16 years of age, where PCT was petitioned between 2009 and 2018 in the Pediatric Emergency Room. We also collected the diagnostics at discharged and the microbiologic identification of the microorganism by cultures and PCR.

Results

From the 203 patients 49% had a microbiologic diagnostic.

56 % of the patients were male and 73% had less than one month years old. 60% of the infection we found to be caused by viruses and the most frequent diagnostic was respiratory tract infection (37%). We found that PCT had higher concentration in bacterial infection than in viral infection with a mean of PCT of 2.30ng/mL and 0.99ng/mL (normal value of PCT in our laboratory: < 0.5ng/mL) respectively and this difference was statistically significant (p: 0.02). We also found higher PCT concentration in patients with GP bacteria but that had not a statistically significance.

Conclusions

There are many studies about PCT and the use varies among hospital and experts. In our retrospective study we ware able to see significant differences of PCT concentration between virus and bacterial infection bus we did not find statistical differences between GP and GN bacteria as described in some studies in the literature.

doi: 10.1016/j.cca.2022.04.861

M212

Molecular &; biochemical investigations of inborn errors of metabolism & altered redox homeostasis – An underrecognised cause with emerging importance

S. Mukherjee

All India Institute of Medical Sciences, Bhopal

Background-aim

India, like other developing countries, is facing an accelerating demographic switch to non- communicable diseases.Inborn errors of metabolism (IEM) constitute a diverse heterogeneous group of disorders with protean clinical manifestation presenting mainly in the paediatric population Congenital malformations and genetic disorders are important causes of morbidity and mortality worldwide and Indian scenario is not much different. Inborn errors of metabolism (IEMs) are a group of monogenic disorders characterized by dysregulation of the metabolic networks that underlie development and homeostasis.

Methods

HPLC, GC -MS and other Biochemical methods.

Results

Emerging evidence points to oxidative stress and mitochondrial dysfunction as major contributors to the multiorgan alterations observed in several IEMs. The accumulation of toxic metabolites in organic acidurias, respiratory chain, and fatty acid oxidation disorders inhibits mitochondrial enzymes and processes resulting in elevated levels of reactive oxygen species (ROS). In other IEMs, as in homocystinuria, different sources of ROS have been proposed. In patients' samples, as well as in cellular and animal models, several studies have identified significant increases in ROS levels along with decreases in antioxidant defences, correlating with oxidative damage to proteins, lipids, and DNA. Elevated ROS disturb redox signaling pathways regulating biological processes such as cell growth, differentiation, or cell death; however, there are few studies investigating these processes in IEMs.

Conclusions

In this review, we describe the published data on mitochondrial dysfunction, oxidative stress, and impaired redox signaling in branched-chain amino acid disorders, other organic acidurias, and homocystinuria, along with recent studies exploring the efficiency of antioxidants and mitochondria-targeted therapies as therapeutic compounds in these diseases.

doi: 10.1016/j.cca.2022.04.862

M213

The therapeutic potentials of moringa oleifera in the treatment of lead-induced toxicity may be mediated through inhibition of oxidative stress

C.E. Onah [c], S.C. Meludu [b], C. Dioka [a]

[a] *Department of Chemical Pathology, Faculty of Medicine, Nnamdi Azikiwe University, Awka*

[b] *Department of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Awka*
[c] *Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Akwa*

Background-aim

Lead poisoning is a global public health problem that has been associated with poor treatment outcomes. We therefore evaluated the ability of Moringa oleifera (M. oleifera) to reduce blood lead level (BLL) and lead-induced oxidative stress in relation to dimercaptosuccinic acid (DMSA) in albino wister rats.

Methods

Thirty rats were allocated into five groups consisted of 6 rats each. Control group (A) received normal rat chow and water ad libitum for 12 weeks. Group (B-E) initially received 100 mg/kg body weight lead acetate per oral for 6 weeks. Thereafter, groups B, C, D and E received DMSA and different doses of M. oleifera and their combination for another 6 weeks. Blood samples were collected prior to treatment, 6 weeks, and 12 weeks post-treatment for the analysis of BLL, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione-s-transferase (GST).

Results

BLL and MDA increased significantly ($p < 0.05$) while serum SOD, CAT, and GST activities decreased significantly ($p < 0.05$) from their pre-treatment levels after 6 weeks of lead acetate administration. However, 400 mg/kg body weight M. oleifera administration after 12 weeks post-treatment significantly ($p < 0.05$) decreased 6 weeks BLL by (40.5%); MDA (52%); and significantly ($p < 0.05$) increased the activities of serum SOD by (35%); CAT (26.3%); and GST (53%).

Conclusions

M. oleifera was observed to not only effectively reduce blood lead levels but also ameliorate lead induced oxidative stress through enhanced antioxidant activities. M. oleifera may therefore, serve as an alternative therapeutic approach to lead poisoning especially in resource limited settings.

doi: 10.1016/j.cca.2022.04.863

M214

Red blood cells folate vs serum folate: A review of the tests utility

Z. Asiri [a], Z. Alshehry [b]

[a] *Clinical Biochemistry Resident, King Fahad Medical City (KFMC)*
[b] *Consultant, Head of Biochemistry Laboratory, Director of the Saudi Board for Clinical Biochemistry program (SBCB) at (KFMC)*

Background-aim

Folic acid test measures the amount of folic acid in the blood. Deficiency in folate can lead to premature infants, hemolytic anemias, malabsorption syndrome and alcoholics. Furthermore, research showed a link between folate deficiency and pregnancy complications especially neural tube defect (NTD). Here at the Biochemistry section in King Fahad Medical City, folate values can be measured using either a serum folate or a red cell folate assay. However, red cell folate is more time-consuming and a costly test. This report aims to assess whether the red cell assay demonstrated greater performance characteristics to justify these disadvantages. In order to help communicate our recommendation that serum folate is a suitable substitute for red blood cell folate levels and that the latter should be discontinued.

Methods

A retrospective study was conduct at the Biochemistry Section, results were collected for both serum folate and RBC folate from 1st January 2019 up till October 2019. Additionally, normal samples were freshly collected, and later tested on different time interval using the RBC folate assay to investigate tests accuracy, and precision. Moreover, to be able to assess the testing process and phases. Finally, a review was conducted on both serum and RBC folate assays records of validation data, QC data, and proficiency testing (PT).

Results

By observing the actual process of the RBC assay the assay consumes around 2.5 hours from the operating staff. Also, the data analysis revealed that physicians order serum folate around (7x) times more often than RBC folate (3660 serum folate samples) and (478 RBC folate samples). Additionally, 82 results (17%) of RBC folate samples were abnormal, and only 237 results (6.4%) in the case of serum folate samples. Also, in patients with high folate in serum only 11 cases (6%) were followed by RBC assay, and in the cases of high folate in RBC folate, 65 cases (35.5%) of the cases were tested again for serum folate. Correspondingly, in the cases of low folate in serum folate, 1 case (2%) were tested once more for RBC folate. Similarly, in the cases of low RBC folate, 1 case (5.8%) were tested for serum folate.

Conclusions

The disadvantages seen in the RBC folate assay make it hard for the Biochemistry section to adhere to quality standards and protocols. Consequently, as seen from several articles and other laboratories experience with the matter that serum folate is a suitable substitute for RBC folate and some argue even further that folate testing itself is outdated. However, we are aware that discontinuing a test require further investigations and involvements with physicians, admins, and other stakeholders, yet this initial investigation provide a solid base for us to raise the question of the utility and cost-effectiveness of the RBC folate assay.

doi: 10.1016/j.cca.2022.04.864

M215

Usefulness of various albumin adjusted ischemia modified albumin as novel biomarker for early prediction of renal damage in diabetes

C. Yadav [a], A. Ahmad [b], P. Manjrekar [c]

[a] *Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India*
[b] *Dept. of Biochemistry, G.S Medical College & Hospital, Pilkhuwa, U.P, India*
[c] *Dept. of Biochemistry, Kasturba Medical College, Manipal Academy of Higher Education, Mangalore, Karnataka, India*

Background-aim

Urine albumin creatinine ratio (UACR) has been the standard for detecting albuminuria in diabetes patients but recent evidence of "non-albuminuric renal impairment" encouraged the search for novel markers. Increased serum ischemia modified albumin (IMA) levels are detected in diabetic nephropathy cases but its estimation by conventional albumin cobalt assay is confounded by serum albumin levels. Hence, this study was taken up to estimate serum IMA and albumin adjusted IMA's to assess their utility in diagnosing early the renal damage in diabetes.

Methods

This cross sectional study in 30 controls and 60 diabetes patients (classified as normo-, micro- and macro-albuminuric groups having UACR of <30, 30-300 and >300mg/g of creatinine respectively) estimated serum IMA using ELISA. Albumin adjusted IMA's were calculated, namely, Adjusted IMA { (individual serum albumin concentration/median albumin concentration of population) $\times$ IMA value} ; IMA index { serum albumin $\times 23 +$ IMA–100} and IMA ratio { IMA/serum albumin} . SPSS ver. 20 employed ANOVA for intergroup comparison of means, Pearsonn's correlation coefficient for correlation analysis and ROC curve to assess the diagnostic performance.

Results

Serum IMA, adjusted IMA, IMA index and IMA ratio significantly increased in diabetics showing an increasing trend across the normo-, micro- and macro-albuminuric groups. IMA correlated inversely with serum albumin in micro- and macro-albuminurics whereas IMA ratio correlated strongly in all the groups. ROC curve showed highest sensitivity and specificity for IMA (97.5% and 78% at 99ng/ml cut-off) and IMA ratio (97.5% and 76% at 24.5 cut-off); AUC 0.93 for both.

Conclusions

Similar increasing trend of IMA and albumin adjusted IMA's from normo- to micro- to macro-albuminuria is suggestive of no effect of serum albumin concentration on IMA estimation within the normal reference range of serum albumin. Albumin adjusted IMA ratio showed greatest diagnostic performance for early detection of renal damage in diabetes in comparison to IMA and other albumin adjusted IMA's.

doi: 10.1016/j.cca.2022.04.865

Clinica Chimica Acta 530 (2022) S332–S347

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Molecular Diagnostics, Genetic Testing

W112

Study of vitamin-D and angiotensin II receptor gene polymorphisms in newly diagnosed hypertension

M. Prasad Naidu [b], D. Rajarajeswari [b], K. Ramalingam [b], R. Viswakuamar [b], A. A.khan [a], S. Vishwakarma [a]

[a] *CLRD, Deccan College of Medical Sciences, Kanchanbagh, Hyderabad, Telangana 500058, India*
[b] *Dept. of Biochemistry, Narayana Medical College, Nellore-524003*

Background-aim

Essential hypertension is a typical example of a complex, multifactorial and polygenic trait. There are several causal genes, which together contribute to between 30% and 50% of the variation in BP among humans. liganded Vitamin-D Receptor suppresses renin expression by binding to the transcription factor cAMP-response element-binding protein (CREB). by a vit D receptor mechanism. The vit D receptor gene serves as a good candidate gene for susceptibility to several diseases. Angiotensin II is mediated through angiotensin II type 1 (AT1) receptor. A silent polymorphism with adenine changed to cytosine at position 1166 (A1166C).In this context, this study assessed the association between these levels and VDR and AT1R gene polymorphism in subjects with essential hypertension.

Methods

Both male and female hypertensive patients aged between 25-60 years (SBP ≥140mmHg and/ or DBP ≥ 90mmHg) were included. Quantification of VDR and Angiotensin II receptor gene polymorphism by using the RFLP (PCR) method.VDR gene was amplified using a forward primer (5'- AGCTGGCCC TGGCACTGA CTCTGCTCT -3') and reverse primer (5'- ATGGAAACACCTTGCTTCTTCTCC CTC - 3') at 60C annealing.

Result

In the present study In VDR Fok 1 gene constitution of an organism is referred to as its genotypes, such as the letters F and f. This F refers to dominant (wild/normal) genotype and f refers to recessive (variant/ lethal) genotype. VDR Fok 1 gene for ff genotype was found to be associated with 8.06 folds increased risk for EHTN compared to the FF genotype. The data represent VDR f allele (variant) genotype frequency distribution when compared to control more number in EHTN. This f allele lethal genotype may high risk for EHTN. The codominant, recessive and over dominant models showed statistical significance in EHTN when compared to controls. The present study AT1 receptor gene constitution of an organism is referred to as it genotype, such as the letters A and C. This A refers to dominant (wild/normal) genotype and C refers to recessive (variant / lethal) genotype. In our study allel frequency distribution CC genotype was found to be associated with folds increased risk for Essential hypertensives compared to AA genotype. The present data represent codominant, dominant and recessive models showed statistical significance in EHTN when compared to controls. The clarification of EHTN etiologies at the molecular level and the identification of genetic variation that confer disease susceptibility are likely to contribute both to disease presentation and development of new medicine.

Conclusions

The VDR and Ang II genetic variants were found to have an association with the severity of the clinical features of EHTN. Further clinical trials are required to delineate the effects of vit D supplementation in EHTN patients. However, some studies on vit D supplements for the treatment of EHTN in a specific population will not only be a significant breakthrough that dramatically improves clinical practice for EHTN intervention but also substantially decreases the public health burden of the management of EHTN.

doi: 10.1016/j.cca.2022.04.867

W113

Clinical performance evaluation of a rapid, low throughput test for KRAS, BRAF and EGFR mutation analysis

A.G. Muriira, R. Mukadam

Pathologists Lancet Kenya

Background-aim

Genomics plays an important role in cancer diagnostics and treatment. Over 500 genes are known to play a part in cancer development and progression of which EGFR, KRAS and BRAF oncogenes are becoming increasingly popular in cancer investigations.Different molecular methods exist for testing oncogenes in tissue blocks but testing is limited by sensitivity and speed of analysis. The aim of this study was to establish the clinical performance of the novel fully automated polymerase chain reaction based Idylla mutation tests on formalin fixed paraffin embedded tissue blocks.

Methods

This was a retrospective study carried out with known archived formalin fixed paraffin embedded tissue blocks and Horizon EQA tissue sections. Results were compared between the EQA samples and known archived samples previously run on Cobas 4800 to determine correla-

tion. 10 samples for each test I.e. KRAS, BRAF and EGFR were tested on the Idylla instrument. The Idylla instrument is a fully automated system that covers the entire process from sample to result with fully integrated sample preparation followed by real time amplification and detection of the target sequences. The test cartridges are ready to use, single use only and contain the necessary reagents and endogenous control gene that serves as a sample processing control to check for adequate execution of the entire process from sample to result. A reduced number of samples was used in this study due to cost implications.

Result

Data from the thirty samples was used to show overall agreement between the two instruments.

Five Horizon samples and five archived samples were tested for KRAS mutations in exons 2,3 and 4 of the KRAS oncogenes where the tests consists of five allele specific multiplex PCR reactions designed to amplify KRAS gene sequences with mutations in codons 12,13,59,61,117 and 146. The five Horizon EQA samples shown 100% correlation with all having mutations detected in KRAS codon 13.The other five archived samples; 3 had no mutation detected (wild type) and 2 other had mutations detected in KRAS codon 12(mutant).

Two Horizon samples and eight archived samples were used in verification of EGFR mutation assay intended for the qualitative detection of exon 18(G719A/C/S), exon 21(L858R, L861Q), exon 20 (T790M, S768I) mutations, exon 19 deletions and exon 20 insertions in the EGFR oncogene. The two Horizon samples shown 100% correlation with no mutations detected and the eight archived samples; three had no mutation detected, four had mutations detected on L858R on exon 21 and 1one had mutations on L858R and S7681I on exon 20.

One Horizon sample and nine archived samples were used in verification of BRAF mutation assay intended for qualitative detection of V600E/E2/D and V600K/R/M mutations in codon 600 of the BRAF gene. One horizon sample shown 100% correlation with mutation detected. Five of the archived samples had no mutations detected in BRAF codon 600 while four had mutations detected in BRAF codon 600.

Conclusions

The Idylla instrument was acceptable for analysing KRAS, BRAF and EGFR mutation tests based on the 100% confidence achieved as there were no discordant results that were witnessed. The Idylla KRAS, BRAF and EGFR mutation assays allows for fast and reliable analysis of results with a turnaround time of 2 hours.

doi: 10.1016/j.cca.2022.04.868

W114

DNA methylation analysis using a methylation-sensitive restriction enzyme

A. Kikuchi [a], A. Naruse [a], T. Sawamura [a], K. Takagi [b]

[a] *Daiyukai Research Institute for Medical Science, Daiyukai Health System, Ichinomiya*
[b] *Urology, Daiyukai Daiichi Hospital, Ichinomiya*

Background-aim

Bisulfite conversion is widely used to analyze the methylation sequencing and methylation-specific PCR of DNA methylation. Although it converts unmethylated cytosine to uracil, it decreases the Tm value of the target sequence, which may complicate its design by primer or probe. It has been demonstrated that bisulfite conversion causes genomic DNA (gDNA) fragmentation and that some unmethylated cytosine is not converted. In this study, we treated the promoter regions of the NID2, MLH1, and POU4F2 genes with methylation-sensitive restriction enzymes and then performed methylation analyses by real-time quantitative PCR using a hydrolysis probe.

Methods

We used a methylated control gDNA: EpiScope Methylated HeLa gDNA (Takara Bio), EpiTect Control DNA and Control DNA Set (Qiagen), and 27 cases of transurethral resection of bladder tumor (TURBT) tissues. All gDNA extractions were performed with the HighPure PCR Template Preparation Kit (Roche). The extracted gDNA were digested with CpoI, Psp1406I, and HapII (Takara Bio) and analyzed by real-time quantitative PCR. We located the primer and probe in the promoter region of each gene along with a hydrolysis probe system using the Universal ProbeLibrary Probe (Roche). The DNA methylation rate was calculated from the Cp value for each sample based on the Cp value obtained from the methylated control gDNA, which were compared with those obtained by the bisulfite sequence PCR (BSP).

Result

Both the primer and the probe showed excellent reactivity with control gDNA. The methylation rate was 81.5% and 92.6% for NID2 and POU4F2, respectively. No methylation was observed in MLH1, and the obtained results correlated well with those of the BSP.

Conclusions

Our results show that this method can be used in TURBT samples to avoid various problems during bisulfite conversion in methylation analysis. Furthermore, our results are similar to those obtained using the BSP method. The quantitative estimation of methylation was also thought possible.

doi: 10.1016/j.cca.2022.04.869

W115

Functional evaluation of genetic variants in notch signaling responsible for down-sloping hearing loss in Korean deafness patients

J.H. Rim, J. Jung, J.Y. Choi, H.Y. Gee

Yonsei University College of Medicine

Background-aim

Hearing loss is the most common sensory disorder that affects approximately one in every 500 newborns worldwide. While diverse signaling pathways are essential for fine tuning, Notch signaling is known to be crucial for cochlear hair cell differentiation during development. Herein, we aim to elucidate functional evaluation of genetic variants found in adult-onset hearing loss patients with down-sloping audiogram among Yonsei University Hearing Loss cohort (YUHL).

Methods

A total of 3 variants in DLL1, an important ligand for Notch signaling, were detected in whole exome sequencing analysis for YUHL patients with ski-slope audiogram defined as normal ABR thresholds

in low frequencies (500 Hz and 1 kHz) but abruptly high ABR thresholds in high frequencies. Functional studies including biotinylation western blot, immunofluorescence staining, and co-culture assay were performed for a total of 4 variants (including recently reported pathogenic mutation associated with neurodevelopmental disorders).

Result

Among a total of 27 candidate genes selected based on population database frequency, in-silico results, and inheritance mode in YUHL cohort patients, three missense variants in DLL1 were co-segregated within the families. Biotinylation western blot showed gain of function in two missense variants. While one previously reported pathogenic mutation for neurodevelopmental disorders showed lack of membrane trafficking by immunofluorescence staining, three DLL1 variants in YUHL patients did not have trafficking defects. Co-culture assay with Notch1 over-expressed HEK293T cells revealed higher levels of NICD (Notch intracellular domain) and Hes1 expression for all three variants, indicating gain of function effects in these variants compared to wild type.

Conclusions

We comprehensively studied deleterious functional effects of missense variants found in the Notch signaling component from exome sequencing analysis for Korean down-sloping hearing loss patients. DLL1 variants associated with down-sloping hearing loss might induce activation of Notch signaling by gain of function in adult cochlea which might have already accumulated acoustic trauma and ageing effects with adult-onset phenotypes.

doi: 10.1016/j.cca.2022.04.870

W116

Cepheid genexpert® for determining the epidemiology of RPO-B gene mutation in M. Tuberculosis: An experience from rural district, Nepal

S. Adhikari

Pyuthan District Hospital, Pyuthan

Background-aim

Mycobacterium Tuberculosis ranks among top ten causes of deaths in Nepal despite successfully implementing National Tuberculosis Program. Many cases of tuberculosis (TB) are still missing and its incidence is increasing each year. According to the National Tuberculosis Prevalence Survey (TBPS) 2018-19, out of 69000 cases around 54% cases remained undiagnosed and untreated in Nepal. The survey also suggests use of molecular technique based rapid, point of care testing system for early diagnosis of TB. Cepheid GeneXpert® is most commonly used for tuberculosis diagnosis in Nepal. It is a nested real-time PCR based semi-quantitative in-vitro technique. It delivers report within 2-3 hours. It is detects mutations in the beta-subunit of RNA polymerase (rpoB) gene that leads to rifampicin-resistance (RR) in Mycobacterium tuberculosis complex.

The study was done to determine the usefulness of GeneXpert® system in rapid diagnosis of TB, determine multi-drug-resistance and at the same time know the epidemiology of rpoB gene mutation in rural districts.This will help the policy makers in taking prompt action to meet the goals of End TB Strategy 2030.

Methods

Analysis of data obtained from laboratory register for demographic details of the patients and from the database of the Cepheid GeneXpert® machine for muation in rpoB gene of each rifampicin resistant isolates.

Result

Present study describes the findings in a rural laboratory setting in Pyuthan hospital, Nepal by using Xpert MTB/RIF assay. Of the 2733 samples tested, 297 were positive for tuberculosis. 285 patients were rifampicin sensitive, 02 were rifampicin indeterminant while 10 (3.36%) patients were rifampicin resistant. Among positive ones, 205 (69.03%) were male and 92(30.97%) female. All rifampicin-resistant patients were male (100%)., 155 (52.18%) of TB positive patients were of 55 years and above age. Among positive male patients, 108 (52.68%) were of age 55 or above years which was followed by 64 (31.22%) patients of age group 35-54 years, 32 (15.6%) were of age group 15-34 years and one patient was below the age of 15 years. Among positive female patients 47 (51.087 %) were of age group 55 years and above, followed by 26 (28.261%) patients of age group 15-34 years, 17 (18.478%) were of age group 35-54 years and 2 (2.174%) were of age group 0-14 years. In ethnicity-based classification among positive patients, 142 (47.81%) were Janajati followed by 75 (25.25%) Dalits, Chhetri 68 (22.9%) and Brahmin 12 (4.04%). Among rifampicin-resistant patients, 5 (50%) had shown mutation located in the region of Probe E of the rpoB gene followed by 2 (20%) in Probe D region, 2 (20%) in Probe C and 1 (10%) in probe B region. No mutation was observed in the region of Probe A. The epidemiology of mutation was similar to that in India.

Conclusions

Determining RR-TB is of great significance in determining Multi Drug Resistant Tuberculosis (MDR-TB) as 90% of all RR-TB patients are also resistant to Isoniazid. The Cepheid GeneXpert® system will be a promising tool to rapidly diagnose TB, MDR-TB and at the same time help find out the molecular epidemiology of Rifampin-Resistance associated mutations in resource-limited rural areas and laboratory with limited infrastructure. Knowledge of molecular epidemiology will help in understanding the source of infection and drug-resistance and take prompt measures to reach the goals of End TB Strategy 2030.

doi: 10.1016/j.cca.2022.04.871

W117

Mutations of resistance to tyrosine kinase inhibitors in chronic myeloid leukemia

I. Kihel [b], M. Nachi [a], M.A. Bekadja [c]

[a] *Biochemistry, medecin department, university of ORAN 1*
[b] *Biochemistry, pharmaceutical department, university of ORAN 1*
[c] *Hematology, medecin department, university of ORAN 1*

Background-aim

Despite the high efficacy of tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia(CML), the phenomenon of resistance appears.BCR-ABL tyrosine kinase domain mutations represent the most studied ITK resistance mechanism the aim of this work is the research and the identification of the resistance mutations which are

important in the identification of the primary or secondary failure to the treatment with Imatinib or other inhibitor.

Methods

On blood patients with therapeutic failure and who's molecular monitoring was carried out at university establishment hospital of Oran-Algeria.

The search for DTK mutations was carried out by direct bidirectional sequencing of the rearranged allele of the ABL gene, after a PCR nested in the areas of interest.

Result

Out of 18 patients treated with ITK and who's molecular follow-up showed failure after 6, 12 or 18 months of treatment, 4 carried mutations in DTK resistance; 3 of them had CML type b3a2 and one type e19a2. The sequencing revealed 5 types of substitution mutations: "Q252H which substitutes Glutamine for Histidine, T315I which substitutes threonine for Isoleucine, M244V which substitutes Methionine for Valine. the Mutation V299L which substitutes Valine for Leucine and the mutation E355G which substitutes glutamate for glycine "which could be expressed alone or combined in the same patient.

Conclusions

The main cause of resistance to Imatinib is mutations in the BCR-ABL gene and clonal evolution.

The identification of these mutations in resistance to tyrosine kinase inhibitors allows individualization of therapy for a better prognosis.

doi: 10.1016/j.cca.2022.04.872

W118

Analysis of gender-specific associations between aldehyde dehydrogenase 2 (ALDH2) RS671 genetic polymorphisms and serum uric acid levels in Han Chinese using big data mining

J. Wu, C. Ma, J. Yi, Y. Chen, A. Ye, L. Kong, L. Qiu, T. Xu

Peking Union Medical College Hospital, China

Background-aim

Serum uric acid (SUA) is influenced by lifestyle and genetics, and unbalanced SUA levels are linked to various common disorders. While the aldehyde dehydrogenase 2 (ALDH2) rs671 polymorphism appears to be associated with SUA levels, the evidence remains inconclusive. The aim of this study was to examine the distribution of the ALDH2 rs671 polymorphism among Han Chinese in Beijing and determine the association between this polymorphism and SUA.

Methods

A total of 6461 subjects, who underwent health checkups in Peking Union Medical College Hospital, were recruited retrospectively. A physical examination was performed and fasting blood was collected for biochemical tests, including blood lipids, uric acid, etc. Real-time PCR was applied to determine the ALDH2 rs671 polymorphism. The distribution of the ALDH2 genotype and the relationship between genotype and the levels of serum lipids and uric acid were analyzed.

Result

The ALDH2 rs671 genotype frequencies were 68.1%(G/G), 29.3% (G/A), and 2.6%(A/A). There was no significant difference in allele distribution between males and females. In males, different ALDH2 genotypes exhibited significant differences in several biochemical analytes, including body mass index (BMI), blood glucose (Glu), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), glutamyl transpeptidase (GGT), and creatinine (Cr) ($P < 0.05$). However, no such differences were found in females. SUA levels in G/A and A/A-carrying males were significantly lower than those of G/G-carrying males. The association between the ALDH2 polymorphism and UA was still significant after further adjustment for factors including BMI, GLU, TC, HDL-C, Cr, and GGT.

Conclusions

The ALDH2 polymorphism is related to SUA in Beijing males, and A allele-carrying males have lower SUA levels.

doi: 10.1016/j.cca.2022.04.873

W119

pilot assessment of the diagnostic accuracy of cepheid genexpert HIV-1 qual for early infant diagnosis

R. Khunga [b], J. Bitilinyu-Bangoh [a], R. Ortuño [c], A. Shigayeva [c], C. Metcalf [d], M. Rumaney [d], H. Bygrave [d], Z. Ndlovu [d]

[a] *Laboratory Department, Queen Elizabeth Central Hospital, Blantyre*
[b] *Laboratory Department, Thyolo District Hospital, Thyolo*
[c] *MSF Malawi Mission, Blantyre*
[d] *Southern Africa Medical Unit (SAMU), MSF, Cape Town*

Background-aim

The World Health Organization (WHO) recommends testing of HIV exposed infants 4 to 6 weeks after birth using an HIV polymerase chain reaction (PCR) test for early infant diagnosis (EID). In Malawi, access to EID remains low, with only 30% of exposed infants less than 2 months old receiving HIV PCR results. Cepheid recently released GeneXpert (Xpert) HIV-1 Qual, a simplified HIV PCR test that can be performed near the point of care. The purpose of this pilot study was to assess of the diagnostic accuracy of Xpert HIV-1 Qual for diagnosis of HIV infection among infants and children. In Malawi, PCR testing is only available in central laboratories, leading to long turn-around times (TATs) to as long at 29 days from sample collection to result printing.

Methods

The pilot was conducted at Queen Elizabeth Hospital laboratory in Blantyre, Malawi between December 2015 and January 2016. Consecutive dried blood spot (DBS) samples from HIV-exposed infants and children aged 6 weeks to 18 months, were tested with Xpert HIV-1 Qual in parallel with the Abbott RealTime HIV-1 Qualitative assay, the reference test, within 2 months of sample collection. The sample size was determined by the number of Xpert HIV-1 Qual cartridges available.

Result

Paired testing was done on 378 samples, of which 17 (4.5%) were positive on the Abbott assay. Xpert HIV-1 Qual detected all 17 HIV pos-

itive on Abbott: sensitivity 100% (95% CI; 80.5% to 100%). Xpert HIV-1 Qual detected 358/361 HIV negative cases on Abbott: specificity 99.2% (95% CI; 97.6% to 99.8%). Assuming the sensitivity and specificity values found in our study, the negative predictive value (NPV) would be 100% assuming an HIV prevalence between 1% and 10%, and the positive predictive value (PPV) be 55.8% at an HIV prevalence of 1%, increasing to 93.3% at an HIV prevalence of 10%.

Conclusions

Assuming the sensitivity and specificity values found in our study, the negative predictive value (NPV) would be 100% assuming an HIV prevalence between 1% and 10%, and the positive predictive value (PPV) be 55.8% at an HIV prevalence of 1%, increasing to 93.3% at an HIV prevalence of 10%.

doi: 10.1016/j.cca.2022.04.874

W120

Genotypic and phenotypic characteristics of hereditary leiomyomatosis and renal cell cancer syndrome in Korean patients

I. Park [d], B. Keam [e], M. Kim [e], S. Yoon [b], J.L. Lee [b], K. Park [c], J.Y. Seo [a]

[a] *Department of Laboratory Medicine, Gil Medical Center, Gachon University College of Medicine, Incheon, Korea*
[b] *Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea*
[c] *Division of Medical Oncology and Hematology, Department of Internal Medicine, Pusan National University Yangsan Hospital, Yangsan, Korea*
[d] *Division of Medical Oncology, Department of Internal Medicine, Gil Medical Center, Gachon University College of Medicine, Incheon, Korea*
[e] *Division of Medical Oncology, Department of Internal Medicine, Seoul National University Hospital, Seoul, Korea*

Background-aim

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant cancer predisposition syndrome characterized by the development of cutaneous leiomyomas, early-onset uterine leiomyomas, and type 2 papillary renal cell cancer (RCC), which is caused by germline fumarate hydratase (FH) deficiency. Here, we aimed to investigate the genotypic and phenotypic features of Korean patients with HLRCC.

Methods

We performed a direct sequencing analysis of FH in 13 patients with suspected HLRCC and their family members. Chromosomal microarray test was performed in female patients with negative sequencing results but highly suspected HLRCC. In addition, we analyzed the clinical features and evaluated the genotype–phenotype correlations.

Result

We identified six different pathogenic or likely pathogenic FH variants from six of 13 patients (46.2%). The variants were composed of two nonsense variants, two splicing variants, one frameshift variants, and one missense variant. Of the six variants, three (50%) were novel variants. Type 2 papillary RCC and early-onset uterine leiomyoma were frequently observed in families with HLRCC, while cutaneous leiomyoma was less common. No significant genotype–phenotype correlation was observed.

Conclusions

This study shows the genotypic and phenotypic spectrum of Korean patients with HLRCC and highlights the need of a more comprehensive approach for appropriate surveillance and tailored treatment.

doi: 10.1016/j.cca.2022.04.875

W121

Evaluation of mirnas 146A-5P, 210-3P and 222-3P in occupationally lead exposed workers of Jodhpur, India

S. Sharma, P. Mitra, T. Goyal, P. Sharma

All India Institute of Medical Sciences, Jodhpur

Background-aim

Lead (Pb) is an environmental and occupational pollutant. Due to its versatile physico-chemical properties, it has been used in activities in various industries. Accumulation of lead may result in deleterious effects on hemopoietic, nervous, respiratory, digestive, urinary and reproductive system. Recent, studies demonstrated lead exposure is associated with epigenetic alterations including DNA methylation, histone modification and miRNA dysregulation. miRNAs, a form of small non coding RNAs, may bind to 3'-UTR of the target gene resulting in altered gene expression. Very few studies have reported alteration of miRNA expression with lead exposure. In the present study, we aimed to study levels of circulating miRNAs 146a-5p, 210-3p and 222-3p in occupationally exposed workers and correlate them with blood lead levels.

Methods

A total of 110 individuals working in factories (handicraft, welding) for at least more than 2 years were recruited in the study after due informed consent. Blood lead level served as marker for lead exposure, and was estimated by graphite furnace atomic absorption spectrophotometry (Thermo Fischer ICE 3000 series). Circulating miRNA were isolated by Qiagen miRNA isolation kit and subsequently converted to cDNA using miRCURY LNA RT kit. miRNA expression was performed using Qiagen SYBR green miRNA PCR assay.

Result

The study population was divided into two groups on the basis of blood lead levels: low lead level (<3.5 g/dL) and high lead level (>3.5 g/dL). Statistically significant difference was observed between the BLL of the two groups ($p<0.0001$). Among the three miRNAs, two miRNAs miR-146a-5p and miR-222-3p showed an upregulation with a fold change of 2.18 and 8.05 respectively, while miR-210-3p showed a downregulation with a fold change of 0.23, when compared between the two groups.

Conclusions

In conclusion, finding from our study suggests alteration of miRNA in lead exposed individual which may lead to systemic effects of lead toxicity.

doi: 10.1016/j.cca.2022.04.876

W122

MIR-30E-3P is down regulated in treated diabetes mellitus compared to prediabetes and undiagnosed diabetes mellitus

C.J. Weale [d], G.M. Davison [d], G.M. Hon [d], A.P. Kengne [b,c], R.T. Erasmus [a], T.E. Matsha [d]

[a] *Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, National Health Laboratory Service (NHLS) and Stellenbosch University, Cape Town 7505*
[b] *Non-Communicable Diseases Research Unit, South African Medical Research Council, Cape Town 7505*
[c] *Department of Medicine, University of Cape Town, Cape Town 8000*
[d] *SAMRC/CPUT Cardiometabolic Health Research Unit, Department of Biomedical sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville 7535*

Background-aim

In a preliminary experiment using genome wide miRNA profiling, miR-30e-3p was shown to be dysregulated in undiagnosed diabetes mellitus (DM) compared to DM cases on treatment, and individuals with normoglycaemia individuals. In this study, we aimed to investigate the expression of miR-30e-3p in a community-based sample participating in a DM screening study.

Methods

Quantitative reverse-transcription PCR (RT-qPCR) was performed on a Quantum Studio 7 (Life Technologies, USA) in 467 participants (men = 137) partaking in the Vascular and Metabolic Health study. Participants without known DM underwent OGTT. Anthropometric measurements, lipid profile and ultra-sensitive C-reactive protein were measured in all participants.

Result

Women were significantly older than men (mean ± SD 51.5 ± 15.5 and 47.6 ± 15.8, p = 0.016, respectively). 323 participants had normoglycaemia, 65 prediabetes, 22 undiagnosed DM and 57 known DM on treatment, of which 41 (72%) of the known diabetics were poorly controlled (HbA1c ≥7.0%). The expression of 30e-3p in known DM was approximately 1.2-fold decreased compared to individuals with normoglycaemia or undiagnosed DM, and 1.5-fold less compared to individuals with prediabetes. Furthermore, a 1.3-fold upregulation was observed in prediabetes compared to individuals with normoglycaemia.

Conclusions

The results of this study showed that mi-30e-3p expression is significantly decreased in poorly controlled DM and this needs further investigation in a bigger sample size.

doi: 10.1016/j.cca.2022.04.877

W123

Micro RNAS expression profiling in myelodysplastic syndrome – Additional diagnostic method with future?

I. Mandac Rogulj [b], M.M. Kardum Paro [c], M. Martinović [b], G. Kaić [a], Z. Šiftar [c], S. Ostojić Kolonić [b]

[a] *Department of Clinical Cytology and Cytogenetics Merkur University Hospital, Zagreb, Croatia*
[b] *Department of Internal Medicine Merkur University Hospital, Zagreb, Croatia*
[c] *Department of Medical Biochemistry and Laboratory Medicine Merkur University Hospital, Zagreb, Croatia*

Background-aim

Micro Messenger Ribonucleic Acids (miRNAs) as short, non-coding RNA molecules contribute in myelodysplastic syndrome (MDS) pathogenesis, act as regulators of epigenetic mechanisms and represent new molecular targets for early diagnosis of MDS. In 2017 MDS Working Group of the Croatian Cooperative Group for Hematologic Diseases (CROHEM) and the Croatian Society for Haematology of the Croatian Medical Association published guidelines based on clinical and morphological characteristics of hematopoietic cells, supplemented with cytogenetic analysis and bone marrow (BM) flow cytometry immunophenotyping (FCI). The aim was to investigate whether the miRNA expression profiling (miR-125a, miR-99b, miR-125b and let-7a) could serve as an additional diagnostic method for diagnosis of MDS.

Methods

Whole blood (26 healthy volunteers and 44 MDS patients diagnosed in Reference Center for MDS, Croatian Ministry of Health) was drawn into EDTA containing tubes (BD Vacutainer), processed to plasma separation, miRNAs extraction (miRNeasy Serum/Plasma Kit) and reverse transcription (miScript II RT Kit). miRNA expression profiling (miScript SYBR Green PCR Kit/Custom PCR Array) was performed on TaqMan qPCR platform in molecular laboratory accredited according ISO 15189. Data normalization (miR-126-3p) and data analysis (web- based software) were done according to the manufacturer instructions (Qiagen).

Result

Statistically significant differences of miRNAs fold changes between healthy volunteers and MDS patients were observed for let-7a (p = 0,030) and miR-125a (p = 0,021). Both miRNAs were down-regulated in MDS patients (-7.34 vs. -1.03), but only let-7a over 2-fold change of healthy volunteers. miR-99b and miR-125b were up-regulated (4.46 vs. 1.88) in MDS patients, but only miR-99b over 2-fold change of healthy volunteers.

Conclusions

In summary, significant down-regulation of let-7a as well as up-regulation of miR-99b in MDS patients could serve as a new molecular targets of miRNA expression profiling and future diagnostic method for early diagnosis of MDS, but it should extend on additional miRNAs (miR-16, miR-21, miR-144, miR-451, miR-651 or miR-655) able to split the MDS patients into two groups with regards to the risk level. Acknowledgments. This work was supported by Ana Rukavina Foundation, Zagreb, Croatia.

doi: 10.1016/j.cca.2022.04.878

W124

Comparison of two RT PCR methods with two different genes for detection of severe acute respiratory syndrome coronavirus 2 (SARS-COV2)

M. Alabdullatif [b], A. Hazizi [a], A. Alsadoon [e], F. Albloui [a], Y. Hawsawi [d], F. Alseraye [a], W. Tamimi [c]

[a] *Department of Laboratory medicine, Security Forces Hospital, Riyadh, Saudi Arabia*
[b] *Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia*
[c] *King Abdulaziz Medical city, KIng Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*
[d] *King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia*
[e] *Molecular Laboratory, SmartLab, Riyadh, Saudi Arabia*

Background-aim

World Health Organization (WHO) announced that diagnostic testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV2) should be performed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Most of these methods use different gene props and therefore the sensitivity and specificity of each method may different. In this study, we have compared two RT-PCR methods using two different genes for detection SARS-COV2.

Methods

A total of random 40 nasopharyngeal swab samples were collected, transported and received in iced box shipment. All samples were performed on two separate semi-automated PCR systems (Qiagen and Abbott m2000). For Qiagen method, 200uL from each sample were added in 96-well QiAcube plate which loaded in QiAcube HT (SN:019658; Qiagen, Germany) to extract RNA. Following extraction, master mix prepared using SARS-COV2-RT-PCR kit 1.0 (REF: 821005; Altona, Germany) for 44 samples including 40 patient samples, two negative controls using nuclease-free water (with and without internal control), and two positive controls (with and without internal control). Extraction elute of each sample (20uL) added to master mix (10uL) to have a total volume of 30uL which uploaded into Rotor-Gene Q (SN: R0219307; Qiagen, Germany). The primer pair used to amplify S gene and E gene in SARS-COV2. Amplifications were done as follow: reverse transcriptase (20 minutes at 55oC); initial denaturation (2 minutes at 95oC); 45 cycles of denaturation (15 seconds at 95oC), annealing for (45 seconds at 55oC), and extension (15 seconds at 72oC). Results reported as valid for internal control less than 35 cycle threshold (CT).

The Abbott m2000 System uses SARS-CoV-2 assay was a dual target assay for the RdRp and N genes. All 40 samples were extracted using m2000sp (Abbott, United States) as recommend by manufacture using 100uL. An RNA sequence that was unrelated to the SARS-CoV-2 target sequence was introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence was simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. Following extraction, master mix (20uL) added to extraction elute of each sample (30uL) to have a total volume of 50uL which uploaded into m2000rp (Abbott, United States). Amplification were done as follow: reverse transcriptase (25 minutes at 55oC); initial denaturation (5 minutes at 94oC); 40 cycles of denaturation (20 seconds at 94oC), annealing for (55 seconds at 55oC), and extension (15 seconds at 72oC).

Result

All samples had valid extraction process with a CT value of internal control between 26.97 to 28.89. A total of 30 samples displayed positive results and 10 samples exhibited negative results with 100% agreement for both methods. This has resulted with a 100% accuracy between both methods.

Conclusions

Both semi-automated methods from Qiagen and Abbott are comparable and accurate despite different technology and different primer genes.

doi: 10.1016/j.cca.2022.04.879

W125

Evaluation of HIV viral load specimen rejections in the pre-analytical phase at centralized testing site in Lusaka province, Zambia

M.D. Hamomba

University of Lusaka

Background-aim

Clinical laboratory testing is usually divided into three phases namely, pre-analytical, analytical and post analytical. Pre-analytical phase accounts for approximately 60 to 70% of laboratory errors. The pre-analytical phase encompasses steps from test order or requisition by a clinician to specimen collection, transportation, specimen receipt and registration at the testing site. The main aim of this study was to determine or evaluate HIV viral load specimen rejections in the pre-analytical phase of testing at Levy Mwanawasa University teaching Hospital for the past one year, assess reasons for HIV viral load rejections and determine possible factors associated with specimen rejections.

Methods

This was a cross-sectional laboratory-based retrospective quantitative study that was conducted from 1st January, 2019 to 31st July, 2019. 20,626 viral load specimens sent to the Levy Mwanawasa University Teaching Hospital Molecular Biology Laboratory (LMUTHMBL) were extracted from laboratory information system, polymerase chain reaction (PCR) registers and specimen rejection log. The period reviewed in this study was from 1st June, 2018 to 31st May, 2019. Data were analyzed using STATA Version 13 to assess reasons for rejection, explore the prevalence rate for viral load rejections and determine possible association factors of specimen rejections.

Result

A total of 20,626 specimen were received at LMUTHMBL, 158 viral load specimens were rejected, representing 1% of overall rejections for the period under review. Of the 1% rejected, the reasons for rejection were; clotted specimen 68 (42.6%), Duplicate entry into the laboratory information system 20 (12.8%), Hemolysis 30 (19.2%) and Insufficient or inadequate sample volume 38 (24.4%). The results also showed that there was an association between variables such as month, reasons for rejections and sex.

Conclusions

The sample rejection in this study was about 1%. The factors associated with sample rejection were site, month and reason for rejection. These factors should be considered in trying to sort out the problem of sample rejection for viral load testing in Lusaka Province.

doi: 10.1016/j.cca.2022.04.880

W126

Performance evaluation of a targeted next-generation sequencing-based CYP2D6 genotyping method

M.R. Kim [a], W. Choi [b], E.S. Shin [a], J. Hong [b], H. Kim [b], W. Lee [b], S. Chun [b], W. Min [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

CYP2D6 is an important enzyme involved in the metabolism of about 25% of commonly used drugs. Accurate CYP2D6 genotyping can guide appropriate prescription of related drugs. However, it is not easily implemented in clinics because it has only been possible using both Sanger sequencing (SS) and multiplex ligation-dependent probe amplification (MLPA) methods due to complex genetic alterations, including copy number variation (CNV), numerous sequence variants, and its highly polymorphic feature, with over 100 haplotypes. In this study, we evaluated the performance of custom-made targeted next-generation sequencing (NGS)-based CYP2D6 genotyping by comparison with SS and MLPA methods known as the gold standards.

Methods

CYP2D6 genotype was determined in 91 patients based on custom-made targeted NGS panel for hereditary tumor. For comparison with NGS, 1,456 variant positions and CNVs were accessed with SS and MLPA, respectively. Taken together, we determined CYP2D6 genotype and predicted the phenotype using assigned activity scores.

Result

The NGS-based CYP26D genotype showed 95.6% concordant with results from SS and MLPA. Four cases showed discordant genotypes but the predicted phenotype was the same, regardless of the different genotype. Four discordant cases were attributed to discordant CNV detection and results of the 1,456 variant positions were concordant.

Conclusions

We established a NGS-based CYP2D6 genotyping method and it showed good performance, compared with the gold standard methods, suggesting their possible utility in clinical practice.

doi: 10.1016/j.cca.2022.04.881

W127

Integrated approach of acute myeloid leukemia diagnostics using multiplex RT-PCR, FLT3-ITD fragment analysis, conventional karyotyping and targeted NGS assay

Y. Cho, S. Jang, E. Seo, S. Hwang, C. Park

Department of Laboratory Medicine, University of Ulsan, College of Medicine and Asan Medical Center, Seoul, Korea

Background-aim

We investigated the diagnostic utility of integrated analysis of recurrent fusion genes, FLT3-internal tandem duplication (ITD) status, chromosomal abnormalities and mutations in the diagnosis of acute myeloid leukemia (AML).

Methods

A total of 220 samples from AML patients were simultaneously tested using multiplex reverse transcriptase-PCR (mRT-PCR), FLT3-ITD fragment analysis with semiquantitative assessment of FLT3-ITD allelic ratio, G-banding analysis and targeted next generation sequencing (NGS) assay. Targeted NGS assay covering 141 genes were performed on the MiSeqDx platform (Illumina). The lower limit of variant allele frequency was set to 2.0% of mutant allele reads. The diagnosis and risk stratification were based on the 2016 WHO classification and the NCCN guideline for AML version 3.2020, respectively.

Result

Of 177 patients at diagnosis, 95 patients (53.7%) were classified as AML with recurrent genetic abnormalities based on recurrent fusion genes (n = 38, 21.5%) detected in mRT-PCR, recurrent mutations (n = 53, 29.9%) detected in NGS assay, and other recurrent translocations (n = 4, 2.3%) in G-banding analysis. Forty-seven patients (26.6%) were classified as AML with myelodysplasia-related changes, followed by 29 (16.4%) with AML not otherwise specified, 4 (2.3%) with AML associated with Down syndrome and 2 (1.1%) with therapy-related AML. According to the NCCN guideline for AML, all patients were categorized as favorable risk (n = 64, 36.2%), intermediate risk (n = 40, 22.6%), or adverse risk (n = 73, 41.2%). Of 43 patients during relapse or refractory disease, adverse mutations (ASXL1, RUNX1, and TP53) were identified in 14 (32.6%) patients, whereas adverse fusion genes were detected only in 3 patients (7.0%). In a total of 220 patients, we additionally demonstrated that targeted therapy was available in 49 patients (22.3%) with FLT3 mutations, 28 (12.7%) with IDH2 mutations, 17 (7.7%) with IDH1 mutations, 14 (6.4%) with KIT mutations, and 10 patients (4.5%) with acute promyelocytic leukemia, respectively.

Conclusions

We confirmed the diagnostic utility of integrated analysis in terms of classification, prognostication, and screening therapeutic targets in patients with AML. Thus, targeted NGS assay-centered integrated approach should be a routine diagnostic modality for patients with AML.

doi: 10.1016/j.cca.2022.04.882

W128

Discrepancy between molecular genotyping and phenotyping of alpha-1-antitrypsin results due to a novel null mutation

S. Pandey [a], N. Anderson [a], B. Lara [c], D. Parr [c], D. Grammatopoulos D [b]

[a] *1Institute of Precision Diagnostics and Translational Medicine, University Hospital Coventry and Warwickshire, Coventry & Warwickshire Pathology Service*
[b] *Institute of Precision Diagnostics and Translational Medicine, University Hospital Coventry and Warwickshire, Coventry & Warwickshire Pathology Service and Division of Translational Medicine, Warwick Medical School*
[c] *University Hospital Coventry and Warwickshire, Department of Respiratory Medicine*

Background-aim

Alpha -1-antitrypsin (⟨1AT) deficiency is one of the most common autosomal genetic disorders in humans, associated with respiratory and liver disease. The deficiency is most common among Caucasians of Northern European descent; the incidence in the UK is approximately 1:2,000. ⟨1AT is encoded by the SERPINA1 gene, which is highly polymorphic and at least 70 naturally occurring protein variants have been described and characterised by their distinct migration on isoelectric focusing gels. Detection of the two most common ⟨1AT deficient variants PI*S and PI*Z, can be carried out by PCR amplification. This robust molecular genotyping approach has become a major analytical tool in the investigation of patients presenting with low ⟨1AT levels.

Methods

A genomic DNA sample was received by the Molecular Diagnostics laboratory, from patient XY with severe ⟨1AT deficiency (⟨1AT levels <0.2g/L). As part of our routine protocol, the presence of variants PI*S and PI*Z was investigated by PCR amplification with fluorescence-labelled hybridization probes. Genetic analysis was also carried out by Sanger sequencing.

Result

PCR results identified that patient XY was heterozygote for PI*Z at exon 5 of the SERPINA1 gene. This mutation is a change of guanine to adenine at nucleotide 1096 of the cDNA (NM_000295.4:c.1096G > A) generating an amino acid change of Glutamic (Glu) to Lysine (Lys) at codon 366 (p.Glu366Lys). Surprisingly, this result differed from previous ⟨1AT phenotyping results by isoelectric focusing, suggesting that the patient was Pi*ZZ.Further analysis by Sanger sequencing confirmed that the patient was heterozygous for Pi*Z and also identified a deletion of adenine in codon 105 of exon 2 in heterozygosity (NM_000295.4: c.314delA). This deletion produced a frameshift in the sequence, and introduced a premature stop codon in the protein p.(Asn105Ilefs*32). Search in relevant databases (dbSNP, GnomAD) confirmed that this mutation has not been previously described .

Conclusions

This case identified a potential limitation of ⟨1AT phenotypic assays that might result in incorrect patient classification. This might have important consequences in the investigation of other affected family members.

doi: 10.1016/j.cca.2022.04.883

W129

A study on BDNF polymorphism (RS6265) in schizophrenia

V.S.N.K.K. Pilla [a], P. Mitra [a], S. Sharma [a], N. Nebhinani [b], P. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur*
[b] *Department of Psychiatry, All India Institute of Medical Sciences, Jodhpur*

Background-aim

Schizophrenia is a severe psychiatric disorder effecting 1% of the world population. The exact cause of the disease is unknown, but studies shows that disturbances in neuronal development and changes in synaptic connection are important factors in the pathogenesis of schizophrenia. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family which plays a critical role in the development, regeneration, survival, and maintenance of neurons. Among several polymorphisms reported in BDNF, the rs6265 polymorphism is known to be associated with many neuropsychiatric diseases. So, it was hypothesised that this SNP may have an association with schizophrenia. So, this study was aimed to determine the frequency of BDNF (rs6265) polymorphism and to check for plausible association of this polymorphism with schizophrenia.

Methods

In total, 125 schizophrenia patients and 50 healthy controls were recruited after obtaining informed written consent. Cases were diagnosed as per the ICD-10 criteria. Severity was assessed using GAF and PANSS score. BDNF (rs6265) polymorphism was genotyped using tetra primer-amplification refractory mutation system-based PCR.

Result

Single SNP allele and genotype associations were analysed using different models (i.e., additive, dominant, and recessive models). The genotype frequencies i.e. GG, AG and AA among the cases ($N=125$) was 63%, 29.6% and 5.6% respectively while in control ($N=50$) GG, AG and AA frequencies was 58%, 36% and 6% respectively. The allele frequency of the A and G allele in controls was 82% and 18% respectively and where as in cases it was 78% and 22% respectively. Both genotype and Allelic distribution between the cases and the controls were significant with $p< 0.05$. However, on comparing the severity scores between the val/val and met/met genotype only the GAF score were found to be significant with $p=0.02$ and on comparing the GG genotype with the AA + AG PANSS negative scores were found to be statistically significant.

Conclusions

The study supports that there is a significant association of BDNF genetic polymorphism val66met with schizophrenia. Findings also revealed that A allele of BDNF val66met gene has increase genetic risk of schizophrenia.

doi: 10.1016/j.cca.2022.04.884

W130

Association of vitamin D receptor genetic variants with end stage renal disease maintaining haemodialysis

S.K. Shah

Department of Public Health, National Academy for Medical Science, Purbanchal University

Background-aim

Vitamin D receptor (VDR) genotypes differentiation may cause a modification of VDR structure, ultimately leading to changed receptor function, which may alter VDR protein gene expression thereby causing end-stage renal disease (ESRD). VDR gene polymorphism (BsmI and FokI) is known as reliable markers of abnormal vitamin D signaling pathway. VDR polymorphism (Bsml and Fokl) may be associated with ESRD. The aim was to find out the status of Vitamin D and to assess the relationship of VDR gene polymorphism in ESRD patients on maintenance hemodialysis.

Methods

A cross-sectional study involving 207 participants, having 138 ESRD patients from different hospital maintenance hemodialysis and 69 healthy participants from July 2016 to September 2018. In molecular studies, the two major VDR Gene polymorphism (BsmI and FokI) were genotyped by adopting PCR and RFLP techniques.

Result

Enrolled ESRD patients were significantly ($P < 0.05$) low serum 25 (OH) vitamin D level (13.76 ±6.59) as compared to healthy control having serum 25 (OH) vitamin D level (32 ± 10.27). BsmI genotype frequencies as BB (67), Bb (57) and bb (14) in ESRD patient group, whereas BB (36), Bb (25) and bb (8) in the healthy control group ($P = 0.775$). FokI genotype frequencies as FF (74), Ff (56) and ff(8) in ESRD patient group and FF(34), Ff (32) and ff(3) in the healthy control group ($p = 0.700$). No statistically significant difference in Vitamin D polymorphism (BsmI and FokI) genotype frequency could be observed between hemodialysis ESRD patients and healthy controls, which suggests that the pathogenesis of ESRD had no association with Vitamin D receptor BsmI and FokI gene polymorphism.

Conclusions

High prevalence of hypovitaminosis D in ESRD patients as compared to healthy control. Vitamin D polymorphism (BsmI and FokI) was not associated with ESRD among the Nepalese Population.

doi: 10.1016/j.cca.2022.04.885

W131

Detection of compound heterozygotes for GAA expansion & a frataxin gene (FXN) point mutation in patients with friedreich's ataxia

A. Pagdhune [b], A. Dalal [a], M. M [a]

[a] *CDFD HYDERABAD*
[b] *ICMR- NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH*

Background-aim

Introduction: Friedreich ataxia (FRDA) is an autosomal recessively inherited neurodegenerative disease, caused due to expansion of the intronic GAA trinucleotide repeats or mutations in the FXN gene on chromosome 9q13. The size range of the repeat is between 6 and 34 repeats in the wild-type gene. An expansion of the repeat in disease conditions increases the repeat size to 66–1700 or more repeats, resulting in the reduced levels of frataxin protein. Formation of sticky DNA, DNA-RNA hybrid, and epigenetic changes are the proposed mechanisms for disruption of FXN gene expression. Most cases of FRDA are homozygous having expansion of the GAA trinucleotide repeat in the first intron of the FXN gene, whereas few cases can be heterozygous, in which there is GAA expansion in one allele and point mutation or deletion in the other allele of FXN gene.

Aim and objective:

Analysis of Friedrich Ataxia(FA) cases for heterozygous mutations in those cases where heterozygous GAA trinucleotide expansion was detected.

Methods

Molecular analysis was performed for five patients with heterozygosity for the GAA repeat expansion and 5 healthy controls.

Methodology: 1.DNA isolation 2.Quality and Quantity of DNA with Nanodrop 3.Triple primed PCR(TP-PCR) to look GAA expansion and conventional PCR (short PCR) for zygosity (homo or hetero). 4.Genotyping with capillary electrophoresis sequencer & analysis by using Gene Marker software. 5.If homozygous allele expansion on analysis then diagnosis of Freiedrich Ataxia is confirmed. 6.If only one allele shows expansion (Heterozygous) and if typical clinical features of Friedrich Ataxia are present 7 Then proceed for point mutation detection on other allele by conventional Exon Specific PCR.

Result

01 novel point mutation was found in patient 1 out of five patients by TP PCR followed by sangers sequencing of exons 1 to 5b, at position c.64C>T [g.588C>T] [Q22*] in exon 1 of FXN gene.

Conclusions

Our results emphasize the importance of performing molecular genetic analysis for point mutations in FRDA patients.

doi: 10.1016/j.cca.2022.04.886

W132

Rapid screening detection of staphylococcal bacteremia virulence factors using novel CPA amplification with SPR biosensor methodology

Z. Xu

South China University of Technology

Background-aim

As one of the most important nosocomial pathogen, MRSA has been considered to be a leading pathogen responsible for bacteremia, which poses a significant concern in public healthcare worldwide. Consequently, its rapid and accurate detection is necessary and clinically

important for early diagnostic. A novel nucleic acids isothermal amplification assay, Crossing Priming Amplification (CPA) have been used in combination with SPR biosensor, for the detection of bacteremia MRSA strains and its virulence factors.

Methods

Nineteen MRSA isolates have been subjected to genomic DNA extraction, followed by CPA amplification on the SPR platform for femA, mecA and its virulence factors, including sea, seb and pvL. CPA primers were designed and further optimized by Primer Premier 5.0. The isothermal amplification assay was carried out on the SPR sensor with specific chips to detect mentioned genes, in less reagents and shorter time about 15 min than that of conventional CPA reactions.

Result

Results of conventional CPA were measured by gel electrophoresis and typical ladder bands pattern was observed for 19 samples, suggesting all the examined MRSA strains had both femA and mecA genes. According to the CPA-SPR results, 13, 6 and 3 of 19 MRSA strains were positive for sea, seb and pvl, respectively, which is in accordance with conventional method.

Conclusions

In this study, evidence has been provided to support the development and further application of CPA-SPR biosensor for detection of virulence genes in bacteremia MRSA with high sensitivity, easy-operation and cost-effective.

doi: 10.1016/j.cca.2022.04.887

W133

Development of the rapid, thermal and label-free detection of pseudomonas aeruginosa from bacteremia using a surface plasmon resonance DNA-based biosensor

J. Liu

South China University of Technology

Background-aim

Polymerase Spiral Reaction (PSR) of DNA has been reported to be a rapid, sensitive and cost-effective methodology for rapid detection of genes without sequencing. As its principle is concerned, the reaction is conducted under isothermal conditions for approximately 60 min which could be performed in water bath or a heating block, thus shows high potential to be exploited in surface plasmon resonance (SPR) biosensor for real-time detection of various pathogens. In this study, development of a rapid, thermal and label-free detection of P. aeruginosa from bacteremia using a surface plasmon resonance DNA-based biosensor.

Methods

A total of 25 P. aeruginosa were included in this study. Firstly, DNA extraction has been performed by DNA extraction kit (Dongsheng Biotech, Guangzhou, China) according to the instruction. Secondly, specific set of primers for amplification (Ft and Bt) were designed using Primer Primer 5.0. Thirdly, PSR amplification was performed for detection of oprI. For the results determination, meanwhile, agarose gel electrophoresis was conducted, as well as SPR biosensor. At last, both PSR and SPR were fabricated to detect P. aeruginosa.

Result

According to the results, sensitive and specific SPR detection assay has been developed for the rapid detection of P. aeruginosa. Also, amplification curve of PSR-SPR showed similar tendency with that of Q-PCR when DNA samples were positive in the detection loci. For assay of PSR integrated with SPR, results could be achieved in 15 min in the form of sensorgram, in which the vertical and horizontal signal suggested positive and negative results for biosensor.

Conclusions

In conclusion, development of SPR-DNA array for rapid, sensitive and specific detection of P. aeruginosa has been reported and further applied.

doi: 10.1016/j.cca.2022.04.888

W134

High flux screening of 4 most common bacteremia pathogens using isothermal amplification platform

Y. Li

Guangzhou Women and Children's Medical Center

Background-aim

E. coli, P. aeruginosa, S. aureus (especially methicillin-resistant Staphylococcus aureus, MRSA) and Salmonella are the leading pathogens responsible for bacteremia. However, bacterial identification of such pathogens requires up to several days, which poses a major concern for clinicians. In this study, a high flux screening of the above 4 bacteremia pathogens assay has been developed based on the loop-mediated isothermal amplification methodology.

Methods

For E. coli, P. aeruginosa, S. aureus and Salmonella, rfbE, oprL, femA and invA have been selected for primer design using PrimerExplorer V4. The DNA samples of E. coli, P. aeruginosa, S. aureus and Salmonella, including both standard and clinical strains, were isolated using the DNA extraction kit. LAMP reaction was processed under 65°C for 45 mins. Results determination was performed by observation by naked eye and SYBR Green I, electrophoresis and SPR biosensor. For application, a total of 212 clinical blood samples were used. All samples were subjected to the LAMP platform in one 96-well plates at one time. For a single 96-well plate, a total of 24 samples were detected for 4 pathogens at one reaction. Besides, regular PCR detection was also performed as control.

Result

According to the development of LAMP assays, positive results had been obtained from DNA amplification of standard strains, with color change from orange to green by SYBR Green I and typical ladder bands pattern from electrophoresis, as well as amplification curve with similar tendency with that of Q- PCR. LAMP platform, a total of 22 E. coli, 59 P. aeruginosa, 65 S. aureus and 5 Salmonella were identified. In compar-

ison with PCR whereas 22 E. coli, 56 P. aeruginosa, 63 S. aureus and 5 Salmonella were determined, the LAMP platform was in highly accordance with PCR. However, the LAMP platform only requires 45 mins from DNA to results determination, and PCR (with different denaturation temperature) requires a total of 16 h, including 4 separate PCR reaction for 2.5 h and 1.5 h for electrophoresis.

Conclusions

This study has developed a bacteremia pathogens detection platform based on loop-mediated isothermal amplification, which is capable of detecting E. coli, P. aeruginosa, S. aureus and Salmonella, from DNA to results determination within one hour. This study may significant aid in further early diagnostic of bacteremia, using a rapid and easy-operating platform.

doi: 10.1016/j.cca.2022.04.889

W135

Molecular characterization of glucose-6-phosphate dehydrogenase deficiency specific variants among selected populations in malaria endemic areas of Ethiopia

G. Abebe [a], D. Seifu [a], M. Wolde [a], A. Assefa [b], Y. Mamuye [c]

[a] *Addis Ababa University, Ethiopia*
[b] *Ethiopian Public Health Institution, Ethiopia*
[c] *St. Paul's Hospital Millennium Medical College, Ethiopia*

Background-aim

Background: Glucose 6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect, affects an estimated 400 million people worldwide. Severe clinical manifestation associated with G6PDd (e.g., chronic hemolytic anemia) depends on the type of G6PD molecular variants and exposure to hemolytic triggers (e.g., anti-malarial like Primaquine). However, scarce studies on G6PDd render the use of Primaquine for effective therapeutic treatment of malaria. Therwefore; the aim of this study was To determine the availability and characterize selected molecular variants of G6PDd specific genes among selected populations in malaria endemic area of Ethiopia.

Methods

Method: A cross sectional study was conducted among selected populations in malaria endemic areas of Ethiopia from July 30, 2014 to January 30, 2015. A total of 523 dried blood spot samples were randomly selected from stored samples of national malaria indicator survey of 2011. Polymerase chain reaction and restricted fragment length polymorphism technique was applied to characterize G6PDd variants as G6PD*A, G6PD*A- and/or G6PD*Mediterranean.

Result

Result: Of 523 studied dried blood spot samples, 514 (98.28%) had G6PD genotype available, among which G6PDd were detected on 46 (9.0%) samples. G6PD*A (100%) was the only genotype characterized, while neither G6PD*A- nor G6PD*Mediterranean genotypes were detected. Of all 46 (9.0%) G6PD*A mutation, 25 (4.9%) were male hemizygous, 4 (0.8%) were homozygous females and 17 (3.3%) were heterozygous females. The result also showed G6PDd prevalence variation among regions with 12.06% in Southern Nations Nationalities Peoples, 10.62% in Tigray, 8.51% in Somali, 6.41% in Amhara and 5.26% in Afar.

Conclusions

Conclusion: G6PD*A variant was the only G6PDd genotype detected in this study. G6PD*A variant has almost (90%) the same enzymatic activities with the wild type. Therefore; this result supports the safe use of primaquine, especially the single low dose for transmission interruption of P.falciparum gametocyte and radical cure of P.vivaxas a part of malaria elimination toolkit.

doi: 10.1016/j.cca.2022.04.890

W136

Clinical and genetic profiling of gorlin syndrome in Korean patients

B. Kim [b], J. Mun [a], M.J. Kim [b], S.S. Park [b], M. Seong [b]

[a] *Department of Dermatology, Seoul National University Hospital, Seoul*
[b] *Department of Laboratory Medicine, Seoul National University Hospital, Seoul*

Background-aim

Gorlin syndrome is cancer predisposition syndrome characterized by multiple basal cell carcinomas and diverse developmental abnormalities. Germline mutations have been reported in 2 susceptibility genes: PTCH1 and SUFU. However, clinical and genetic data regarding patients with Gorlin in Korea is limited. We aimed to analyze clinical phenotypes and to characterize the genetic spectrum and characteristics in Korean Gorlin syndrome patients through analyzing causative genes.

Methods

In this study, we included patients with clinically confirmed Gorlin syndrome who were visited in the Seoul National University Hospital in Korea. Clinical data were analyzed using hospital data retrospectively. Whole exome sequencing using peripheral blood was performed in all patients to identify the mutation of BCBS-related genes including PTCH1 and SUFU.

Result

A total of 18 patients with Gorlin syndrome were included. The median age was 34 (range 12-66) years. Thirteen were female (72.2%). Fourteen unconnected patients and 2 family members (mother and daughter) were included. Basal cell carcinoma was found in the majority of patients (92.3%). Whole exome sequencing study revealed that 84.6% harbored mutations in PTCH1 genes. When we excluded 2 patients in the same family, genetic data from 16 unrelated Korean revealed 87.5% harbored causative mutations in PTCH1 genes: splice site variants (1 patient), nonsense variants (4 patients), frameshift variants (2 patients), and gross deletion (2 patients). Two patient showed missense variant which was classified into variant of uncertain significance. None of SUFU variants were found in our cohort.

Conclusions

This study showed loss of function mutations of PTCH1 gene are the main genetic cause of Gorlin syndrome patients in Korea. As BCCs were commonly detected in Korean Gorlin syndrome, a detailed periodic der-

matologic examination is necessary to treat the carcinomas in a timely manner.

doi: 10.1016/j.cca.2022.04.891

W137

Comparison of two RT PCR methods with two different genes for detection of severe acute respiratory syndrome coronavirus 2 (SARS-COV2)

M. Alabdullatif [b], A. Hazizi [a], A. Alsadoon [e], F. Albloui [a], Y. Hawsawi [d], F. Alseraye [a], W. Tamimi [c]

[a] *Department of Pathology & Laboratory medicine, Security Forces Hospital, Riyadh, Saudi Arabia*
[b] *Imam Mohammad Ibn Saud Islamic University, Riyadh, Saudi Arabia*
[c] *King Abdulaziz Medical city, KIng Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*
[d] *King Faisal Specialist Hospital & Research Center, Jeddah, Saudi Arabia*
[e] *Molecular Laboratory, SmartLab, Riyadh, Saudi Arabia*

Background-aim

World Health Organization (WHO) announced that diagnostic testing for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV2) should be performed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Most of these methods use different gene props and therefore the sensitivity and specificity of each method may different. In this study, we have compared two RT-PCR methods using two different genes for detection SARS-COV2.

Methods

A total of random 40 nasopharyngeal swab samples were collected, transported and received in iced box shipment. All samples were performed on two separate semi-automated PCR systems (Qiagen and Abbott m2000). For Qiagen method, 200uL from each sample were added in 96-well QiAcube plate which loaded in QiAcube HT (SN:019658; Qiagen, Germany) to extract RNA. Following extraction, master mix prepared using SARS-COV2-RT-PCR kit 1.0 (REF: 821005; Altona, Germany) for 44 samples including 40 patient samples, two negative controls using nuclease-free water (with and without internal control), and two positive controls (with and without internal control). Extraction elute of each sample (20uL) added to master mix (10uL) to have a total volume of 30uL which uploaded into Rotor-Gene Q (SN: R0219307; Qiagen, Germany). The primer pair used to amplify S gene and E gene in SARS-COV2. Amplifications were done as follow: reverse transcriptase (20 minutes at 55oC); initial denaturation (2 minutes at 95oC); 45 cycles of denaturation (15 seconds at 95oC), annealing for (45 seconds at 55oC), and extension (15 seconds at 72oC). Results reported as valid for internal control less than 35 cycle threshold (CT).

The Abbott m2000 System uses SARS-CoV-2 assay was a dual target assay for the RdRp and N genes. All 40 samples were extracted using m2000sp (Abbott, United States) as recommend by manufacture using 100uL. An RNA sequence that was unrelated to the SARS-CoV-2 target sequence was introduced into each specimen at the beginning of sample preparation. This unrelated RNA sequence was simultaneously amplified by RT-PCR and serves as an internal control (IC) to demonstrate that the process has proceeded correctly for each sample. Following extraction, master mix (20uL) added to extraction elute of each sample (30uL) to have a total volume of 50uL which uploaded into m2000rp (Abbott, United States). Amplification were done as follow: reverse transcriptase (25 minutes at 55oC); initial denaturation (5 minutes at 94oC); 40 cycles of denaturation (20 seconds at 94oC), annealing for (55 seconds at 55oC), and extension (15 seconds at 72oC).

Result

All samples had valid extraction process with a CT value of internal control between 26.97 to 28.89. A total of 30 samples displayed positive results and 10 samples exhibited negative results with 100% agreement for both methods. This has resulted with a 100% accuracy between both methods.

Conclusions

Both semi-automated methods from Qiagen and Abbott are comparable and accurate despite different technology and different primer genes.

doi: 10.1016/j.cca.2022.04.892

W138

Interpretative cut-off for alpha-1-antitrypsin concentration in detection of alpha-1-antitrypsin deficiency among adults – a pilot study in the Republic of Serbia

A. Beletić [a], A. Dudvarski-Ilić [c], L. Nagorni-Obradović [c], B. Milenković [c], M. Ljujić [b], D. Radojković [b], V. Đorđević [b], S. Stanković [a]

[a] *Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia*
[b] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia*
[c] *School of Medicine, University of Belgrade & Clinic for Lung Diseases, Clinical Center of Serbia, Belgrade, Serbia*

Background-aim

Alpha-1-antitrypsin deficiency (AATD) is a genetic risk factor for lung diseases in adults. In AATD, deficiency alleles of the AAT-encoding gene are present in homozygous or compound heterozygous combination and a heterozygous carrier is a subject with one deficient and one functional allele. Reduced AAT level in blood is the first-line laboratory hallmark of AATD. Since a number of acquired factors can influence AAT level in blood, we assessed the interpretative cut-off for AAT concentration – the level below which the presence of AATD should be investigated.

Methods

We retrospectively analyzed the group of 121 subjects (70 men and 51 women, age 45 (26-76) years), tested for AATD between 2007 and 2015. Laboratory methods included immunonephelometry, PCR-reverse allele specific hybridization, isoelectric focusing and DNA sequencing. Kruskal-Wallis test and ROC analysis were used in statistical evaluation.

Result

In total, there were 14 cases of AATD and 35 heterozygous carriers. Level of AAT in blood [median (min-max)] was significantly different ($P < 0.001$) between AATD cases [0.23 (0.11–0.53) g/L], heterozygous carriers [0.86 (0.55–2.30) g/L] and patients with no AATD [1.40 (0.88–2.35) g/L]. Seventeen carriers had AAT concentration in the reference range (0.9–2.0 g/L), while in four subjects with no AATD the AAT level was below the reference range. The level of 1.25 g/L was identified as the cut-off to distinguish group comprising both AATD and

heterozygous carriers from subjects in whom AATD was not detected [AUC (95% Confidence interval (CI)) = 0.87 (0.80–0.92), P < 0.001; sensitivity (95% CI) = 89.6 (77.3–96.5)%, specificity (95% CI) = 65.7 (53.7–76.5)]. Also, AAT concentration below 0.53 g/L was shown to identify the AATD cases [AUC (95% Confidence interval (CI)) = 1.00 (0.98–1.00), P < 0.001] with both sensitivity and specificity reaching 100%.

Conclusions

In the investigated group the AAT level of 1.25 g/L was the cut-off below which the testing for the presence of the AATD-associated alleles is necessary. The AAT concentration lower than 0.53 g/L allowed for an initial differentiation of the AATD cases from the heterozygous carriers and subjects no AATD detected, which should be confirmed with further analyses of AATD-associated alleles.

doi: 10.1016/j.cca.2022.04.893

W139

Molecular characterization of glucose 6-phosphate dehydrogenase deficiency among selected populations in malaria endemic area of Ethiopia

A. Getachew [b], S. Daniel [a], W. Mistire [a], A. Ashenafi [c], M. Yeshiwondim [d]

[a] *College of Health Science, Addis Ababa University, Ethiopia*
[b] *College of Medicine and Health Sciences, Debre Tabor University, Ethiopia*
[c] *Ethiopian Public Health Institute, Addis Ababa, Ethiopia*
[d] *Saint Paul's Millennium Medical College, Addis Ababa, Ethiopia*

Background-aim

Glucose 6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect that affects an estimated 400 million people worldwide and which is most frequently seen in Africa, Asia & Mediterranean region. Approximately 186 specific molecular variants were recently identified worldwide and G6PD*B, G6PD*A (A376G) and G6PD*A- (G202A) were the commonest G6PDd variant seen in subSaharan Africa. The severity of clinical manifestations associated with G6PDd, for example; chronic hemolytic anemia, depends on the type of G6PD molecular variants and exposure to hemolytic triggers like Primaquine antimalarial drugs. However, lack of testing for G6PDd specifically in our country Ethiopia limits the usefulness of Primaquine for effective therapeutic treatment and eradication of malaria in regions of high G6pDd prevalence.

Methods

A cross-sectional study was conducted among selected populations in malaria-endemic areas of Ethiopia from July 30, 2014, to January 30, 2015. A total of 523 dried blood spot samples were randomly selected from stored samples from the 2011 national malaria indicator survey. Polymerase chain reaction and restriction fragment length polymorphism analysis was applied to characterize G6PDd variants as G6PD*A, G6PD*A-, and/or G6PD* Mediterranean.

Result

Of 523 studied dried blood spot samples, 514 (98.28%) had a G6PD genotype available, G6PDd variants were detected in 46 (9.0%) samples. G6PD*A was the only genotype present (100%); neither G6PD*A- nor G6PD*Mediterranean genotypes were detected. Twenty-five of the 46 positive cases (54.35%) were hemizygous males, 4 (8.69%) were homozygous females, and 17 (36.95%) were heterozygous females. The results of this study also showed a difference in the prevalence of G6PDd among regions: 12.06% in Southern Nations Nationalities Peoples, 10.62% in Tigray, 8.51% in Somali, 6.41% in Amhara and 5.26% in Afar.

Conclusions

This study result (Both G6PD*G202A and G6PD*C563T mutation that can cause mild to severe hemolysis, is not detected) supports the safe use of primaquine especially the single low dose for transmission interruption of Pf malaria in selected malarial endemic areas of Ethiopia. Consequently, the present finding supports the country's policymakers to formulate the policy relating to malaria elimination for selected malaria-endemic regions.

doi: 10.1016/j.cca.2022.04.894

W140

Development of multiplex loop mediated isothermal amplification (LAMP) assay and rapid DNA extraction method for detection of mycobacterium tuberculosis

K.V. Sarath, V.S. Arun, T.E. Biby, M.K. Dhanesh, K. Jofy Paul, D.M. Vasudevan

R&D- Molecular Diagnostics, Agappe Diagnostics LTD

Background-aim

Tuberculosis (TB) remains the most dreaded disease to human health around the world. Early and accurate diagnosis of TB is very crucial to reduce morbidity and mortality rates and risk of transmission. Present diagnostic methods for Mycobacterium tuberculosis (MTB) are expensive, complex, laborious, and technically challenging.

Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification method used for the diagnosis of infectious diseases. LAMP is more simple, quick, and cost-effective compared to other molecular diagnostics methods such as polymerase chain reaction (PCR).

This study was aimed to develop a Multiplex LAMP assay that can amplify MTB and Internal control simultaneously in a single assay. We developed the assay with a simple DNA extraction method that can extract the TB DNA within 10 minutes.

Methods

Two sets of LAMP primers were designed using Primer explorer software by Eiken chemical. One primer set is specific to the IS6110 gene of MTB and the second one is specific to the Human GAPDH gene, which will act as the internal control. Specific probes with different fluorophores were also designed. LAMP assay is optimized with an in-house LAMP master mix. Liquified sputum samples heated at 95 °C for 10 minutes with Lysis buffer were used as the sample for the assay. All validation studies were done with MTB controls and clinical sputum samples. All clinical samples were confirmed as positive or negative by real-time PCR (20 Positive and 10 Negative). All tests were done in Bio-Rad CFX 96 real-time PCR instrument.

Result

The limit of detection of the assay is detected as 4 copies/ reaction. The total time taken for LAMP assay was 35 minutes and TB DNA extraction 10 minutes. 20 PCR positive sputum samples were detected as positive with internal control amplification and 10 PCR negative samples were detected as negative with amplification plot only in internal control channel.

Conclusions

We have developed a rapid TB DNA extraction method and Multiplex TB LAMP assay that can detect a minimum of 4 copies of TB DNA per reaction which is equal to PCR. This assay takes only 45 minutes for the sample to result having high sensitivity will be very useful for rapid detection of MTB.

doi: 10.1016/j.cca.2022.04.895

W141

Evaluation of interleukin-22 and its' expression in tuberculosis: A pilot study

S. Gupta [a], K. Gauba [a], P. Mitra [b], M. Banerjee [a], D. Yadav [a], P. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India*
[b] *Department of Biochemistry, PGIMER, Chandigarh, India*

Background-aim

Disease progression of Tuberculosis (TB) is determined mainly by the balance between the microorganism and the host defense systems. T cell-mediated immune response begins after dissemination of Mycobacterium tuberculosis in the body and many distinct types of T helper cells are present at the site of infection. Interleukin-22 (IL-22) helps in cell proliferation, regeneration, and provides protection against microbial diseases. IL-22 producing T cells can migrate into the granulomas during TB infection. However, disparity exists in literature regarding its role. The present study aims to compare serum IL-22 levels and its' expression in TB patients and healthy controls to shed more light on existing literature.

Methods

85 sputum positive TB patients and 85 asymptomatic healthy subjects were enrolled in the study taking into account the exclusion and inclusion criteria. After obtaining due informed consent, 5mL venous blood was withdrawn in plain and EDTA vacutainers from all participants enrolled. Serum IL-22 levels were estimated using Human IL-22 ELISA kit (Krishgen BioSystems, India). Total RNA was isolated from whole blood, converted to cDNA and gene expression done using Sybr green real time PCR technology. Statistical analysis was performed using SPSS.

Result

The median (IQR) of serum IL-22 was significantly lower in TB patients compared to controls (18.55 (5.08) vs 49.38 (162.88) pg/mL); $p < 0.0001$). IL-22 expression was significantly upregulated with a fold change value of 29.44 in TB patients. On ROC analysis, IL-22 discriminated TB patients from healthy controls at 21.67 pg/mL with a sensitivity and specificity of 82% and 82% respectively and an AUC of 0.904. Whereas, relative expression of IL-22 discriminated TB patients from healthy controls at <1.9 X 10-5 with sensitivity and specificity of 87% and 81% respectively.

Conclusions

IL-22 levels are found to be significantly decreased in patients contradictory to its expression which is upregulated. This is the first study on gene expression of IL-22 which indicates that active site for IL-22 production might be at the site of disease. It is crucial for the modulation of tissues in response to TB infection.

doi: 10.1016/j.cca.2022.04.896

W142

Gene chimeras involving CYP21A2 and TNXB genes in Spanish patients with congenital adrenal hyperplasia (CAH)

L. Martínez Figueras [c], A. Escribano Muñoz [b], A. Carcavilla [a], E. Llorente Martín [d], M. Arriba Domènech [d], B. Ezquieta Zubicaray [c]

[a] *Endocrinología Pediátrica. Hospital Universitario La Paz (HLP), Madrid*
[b] *Endocrinología Pediátrica. Hospital Virgen Arrixaca (HVA), Murcia*
[c] *Instituto Investigación Sanitaria Gregorio Marañón and Laboratorio Diagnóstico Molecular. Bioquímica. Hospital General Universitario Gregorio Marañón, Madrid*
[d] *Laboratorio Diagnóstico Molecular. Bioquímica. Hospital General Universitario Gregorio Marañón, Madrid*

Background-aim

Gene rearrangements between CYP21A2, TNXB and their high homologous pseudogenes (CYP21A1P, TNXA) result in the generation of chimeric genes, responsible for the recently described CAHX syndrome. CAHX patients show CAH and Ehlers-Danlos syndrome (EDS) clinical symptoms. Three CAHX chimeras with different clinical severity are described: CH1 (120bp deletion in exon 35), CH2 (point mutation in exon 40) and CH3 (a cluster variant in exons 41,43). The small size and few series reported so far warrant further studies in other populations.

AIM: To learn about the number of CAH Spanish patients at risk of having CAHX chimeras, their distribution, and the clinical manifestations in two tertiary hospitals.

Methods

Molecular analysis:

A. CYP21A2 chimeras junction-site determination: intra or downstream to the gene

B. Multiplex Ligation Probe Amplification (MLPA) to detect CH1 chimeras

C. Sequencing of TNXB exons 40,41,43 to detect CH2, CH3 chimeras

Medical history review and clinical evaluation were performed in 13/17 (76%) patients.

Result

- CAH patients carrying alleles "at risk" of CAHX chimeras:

Among the 4210 CAH patients (classical, nonclassical and hyperandrogenism) analyzed in our laboratory since 1995, 394 were carrying, at least, one allele with CYP21A2 deletion. In 189, junction-site was

located before CYP21A2 exon 6. In 205 (52%) deletion extended downstream and may involve TNXB; MLPA performed in 128 patients revealed 37 (29%) CH1 chimeras. Sequencing of TNXB exons performed in 90 samples detected 12 (13%) CH2 chimera.

- CAHX chimeras distribution and clinical evaluation:

HVA: Intragenic CYP21A2 chimeras (not involving TNXB) were more frequent (71%, $p < 0,05$) in this area. Only one CH2 patient (14%) with no clinical symptoms was detected.

HLP: CH1 and CH2 were found in 2/10 (20%) patients each. A CH2 patient showed EDS related clinical symptoms without hypermobility (Beighton score: 2/9). One patient with EDS manifestations was negative.

Conclusions

CAHX chimeras seem less frequent in our population (29% vs 39% for CH1, and 13% vs 32% for CH2) but a not homogeneous distribution is observed, and larger studies are required.

MLPA, the first approach allowing the systematic search of CAHX chimeras, only detects CH1. TNXB sequencing must be performed to discard CAHX.

doi: 10.1016/j.cca.2022.04.897

Clinica Chimica Acta 530 (2022) S348–S352

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Neurodegenerative Disease

W148

Effects of methanolic extract of vernonia amygdalina (MEVA) and ocimum gratissimum (MEOG) on blood pressure, blood volume, and angiotensin converting enzyme activities in hypertensive male wistar rats

O.B. Onyema-Iloh [b], S. Meludu [a], E.O. Iloh [d], S.O. Usman [c]

[a] *Basic Medical Sciences, Nnamdi Azikiwe University, Awka, Anambra State*
[b] *Chemical Pathology Department, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State*
[c] *Chemical Pathology Department, Nnamdi Azikiwe University, Awka, Anambra State*
[d] *Industrial Chemistry Department, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State*

Background-aim

High blood pressure is a significant risk factor for cardiovascular diseases and can lead to life-threatening complications when not managed properly. Antihypertensive drugs are used in the management of hypertension but with some side effects. The effect of methanolic extract of Vernonia amygdalina (MEVA) and Ocimum gratissimum (MEOG) on blood pressure, blood volume, and angiotensin converting enzyme activities in hypertensive male wistar rats.

Methods

Fifty-six wistar rats (100-110)g were assigned to seven groups of eight rats each. Group 1-7 constitutes the normal, hypertensive group, MEVA (200 mg/kg bwt) group, MEVA (400 mg/kg bwt), MEOG (200 mg/kg bwt) group, MEOG (400 mg/kg bwt) group and reference drug (lisinopril, 30 mg/kg) group respectively. The extract and reference drug were given through oral gavage. All groups except group 1 were induced with 8% NaCl from 0-4weeks before treatment with extract and reference drug from 5-8 weeks. Angiotensin converting enzyme (ACE) activities was assayed using spectrophotometric method.

Results

At 0 week (before induction), there were no significant differences ($p < 0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups. At 4 weeks (after induction), there were significant increase ($p < 0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1. At 8 weeks (after treatment), there were significant decrease ($p < 0.05$) in the blood pressure, blood volume and angiotensin converting enzyme activities in all the groups except group 1.

Conclusions

The decrease in elevated blood pressure, blood volume and serum angiotensin converting enzyme activities showed that MEVA and MEOG may possess angiotensin converting enzyme inhibitory effect and diuretic effect. Hence may be useful in hypertensive conditions.

doi: 10.1016/j.cca.2022.04.899

W149

Neuroprotection of umbelliferone beta glucososide ameliorates cerebral ischemic stroke/reperfusion injury via antioxidant and anti-inflammatory mechanism

K. Vikas [c], B. Prakash [a], A. Firoz [b]

[a] *Jamia Hamdard*
[b] *King Abdulaziz University*
[c] *SHUATS*

Background-aim

Cerebral Ischemic is one of the neurological disorders and is categorized via insufficient blood circulation to the brain for its metabolic requirement. A lacking of blood supply leads to poor oxygen supply to the brain tissue cells and inducing the death of brain tissue or a cerebral ischemia stroke. Umbelliferone beta glucososide (UFG) (7-hydroxy coumarin) is a potent free radical scavenger and used for the treatment of various diseases. However, the beneficial effect of UFG against the apoptotic cell death in the brain has not explored. We investigated the protective effect of UFG against the cerebral ischemia reperfusion injury and explore the possible mechanism of action.

Methods

The cerebral ischemia reperfusion injury was induced via occluding bilateral common carotid arteries for 30 min, followed via reperfusion (4h). End of the experimental study, the rats were sacrificed and brain was removed for the estimation of various biochemical, antioxidant, inflammatory and apoptosis marker.

Results

The result showed that UFG significantly enhanced the passive avoidance memory, increased sensorimotor sign and attenuated reactive hyperemia. UFG ameliorates the cerebral ischemia/reperfusion injury via alteration the antioxidant parameters such as MDA (65%), SOD (73%), CAT (70%), GSH (72%); pro-inflammatory cytokines viz., TNF-⟨ (80%), IL-6 (73.4%), IL-1® (69.4%); inflammatory mediators including PGE2 (65.5%), NF-kB (49%) and apoptosis maker such as caspase-3 (59%), caspase-9 (63%) and caspase-12 (60.5%), respectively.

Conclusions

This study exhibited that UFG may have potential effect in the treatment of cognitive impairment and help improve the brain dysfunction induced via ischemia reperfusion injury.

doi: 10.1016/j.cca.2022.04.900

W150

Proxy measures of obesity and selected lipids as risk factors of vascular cognitive impairment in adult hypertensives

O.B. Aborisade [a], M.A. Charles-Davies [a], M.O. Owolabi [b], O.E. Agbedana [a]

[a] *College of Medicine, department of Chemical Pathology, University of Ibadan, Ibadan*
[b] *Department of Medicine, College of Medicine, University of Ibadan, Ibadan*

Background-aim

Chronic hypertension exerts profound deleterious effects on the structure of the cerebral vasculature, resulting in Vascular Cognitive Impairment (VCI). The role of abdominal obesity and plasma lipids, shown as risk factors in development of VCI in adults with hypertension is unclear and is investigated in this study.

Methods

Normoglycaemic individuals (n = 216) aged 40-75 years were enrolled by convenient sampling method. They included newly diagnosed hypertensives with (NDHCI, n = 69). and without cognitive impairment (NDH, n = 81) age matched with apparently healthy individuals (Controls). Anthropometric measures [height, weight, Waist Circumference (WC), Hip Circumference (HC)], Waist Hip Ratio (WHR), Body Mass Index (BMI)] and blood pressure were obtained by standard methods. Plasma lipids [Total cholesterol (TC), High Density Lipoprotein cholesterol (HDL-c) and triglyceride were determined spectrophotometrically, while Low Density Lipoprotein cholesterol (LDL-c) was calculated using Friedewald formula. Neuropsychological assessment was based on Cognitive Score (CS) using Community Screening Instrument for Dementia. Data analysed by appropriate statistical tools were significant at $p < 0.05$.

Results

Obesity indicators (WHR, WC and BMI) and dyslipidaemia indicators (triglycerides, TC, LDL-c levels were significantly higher in NDHCI and NDH relative to control ($p < 0.01$). Significantly low Cognitive scores were found in NDHCI compared with NDH and control ($p < 0.001$). Also, HDL-c level was significantly lower in NDHCI and NDH relative to control ($p < 0.001$). WHR and LDL-c had inverse relationships (R2 = 0.306, 0.119; ® = -14.627,-0.031) with cognitive score in NDH and NDHCI respectively.

Conclusions

Obesity and atherogenic dyslipidaemia may facilitate the progression of hypertension to cognitive impairment in hypertensive individuals.

doi: 10.1016/j.cca.2022.04.901

W151

Antioxidants and biomarkers of inflammation as risk factors of vascular cognitive impairment in adult hypertensives

O.B. Aborisade [a], M.A. Charles-Davies [a], M.O. Owolabi [b], O.E. Agbedana [a]

[a] *College of Medicine, Department of Chemical Pathology, University of Ibadan, Ibadan*
[b] *Department of Medicine, College of Medicine, University of Ibadan, Ibadan*

Background-aim

Impairment in cognition and attention involving memory loss characterize Vascular Cognitive Impairment (VCI), a complication of hypertension. The involvement of antioxidants and inflammatory biomarkers in the progression of hypertension to VCI is controversial and is investigated in Nigerians in this study.

Methods

A total of 216 normoglycaemic individuals (aged 40-75 years) were enrolled into this study by convenient sampling method. They included 81 Newly Diagnosed Hypertensives (NDH) without cognitive impairment, 69 Newly Diagnosed Hypertensives with Cognitive Impairment (NDHCI) attending Medical Outpatient Clinic and 66 apparently healthy individuals (Controls), who are members of staff of University College Hospital. Socio-demographic indices and lifestyle were obtained through a semi-structured questionnaire while Systolic and Diastolic Blood Pressure (SBP and DBP, respectively) were obtained using standard methods. Neuropsychological assessment based on Cognitive Score (CS) was performed using Community Screening Instrument for Dementia. Antioxidants [Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD)] and inflammatory biomarkers [Interleukin-6 (IL-6), High sensitivity C-Reactive Protein (Hs-CRP)] in serum were estimated by ELISA. Data were subjected to descriptive statistics and analysed using ANOVA, Pearson's correlation and multiple linear regressions at $p < 0.05$.

Results

The inflammatory biomarkers hs-CRP and IL-6 levels were significantly higher while the activities of catalase, GSH; ($p < 0.001$), and SOD; ($p < 0.007$) and cognitive scores; ($p < 0.001$) were lower in NDHCI and NDH relative to control ($p < 0.007$).

Conclusions

Increase in inflammatory biomarkers with decrease in antioxidants facilitates progression of hypertension to cognitive impairment in Nigerian hypertensive adults.

doi: 10.1016/j.cca.2022.04.902

W152

Oxidative stress, trace and toxic metal levels in Alzheimer's disease in an African population

I. Omotosho, M. Ngwube, J. Abdulmalik

University of Ibadan

Background-aim

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by loss of memory resulting from neurodegenerative disorders, this has been attributed to oxidative stress and accumulation of Amyloid (A®) protein in the brain. Environmental and genetic alterations have been implicated as the pathogenesis of the disease. This work investigated levels of selected trace (Iron, Zinc and Copper) and toxic (Cadmium and Lead) metals in AD patients.

Methods

In this case-control study, a total of 38 participants aged >60years consisting of 18 clinically diagnosed with AD and 20 apparently healthy age-matched adults were recruited from the University College Hospital Ibadan Geriatric Centre. Semi- structured questionnaires were used to obtain demographic information, clinical history, lifestyle and dietary patterns from participants. Plasma levels of iron, copper, zinc and blood levels of lead and cadmium were analyzed using Atomic Absorption Spectrophotometry (AAS). Plasma levels of malondialdehyde (MDA), total antioxidant capacity (TAC), hydrogen peroxide (H2O2), and total plasma peroxide (TPP) were determined spectrophotometrically, while oxidative stress index (OSI) and copper to zinc ratio (Cu:Zn) were calculated.

Results

Mean plasma level of zinc was significantly lower in the cases (86.04 ± 11.07μg/dl) compared to controls (108.80 ± 12.47μg/dl), while blood lead (13.85 ± 2.96μg/dl, 8.32 ± 2.10μg/dl) and cadmium (1.34 ± 0.71μg/L, 0.71 ± 0.14μg/L) levels were significantly higher in cases than in controls respectively. Although Fe and Cu levels were similar in cases and controls, Cu:Zn ratio was significantly elevated in cases compared to controls (p = 0.000). Though other OS markers were not significantly different in both groups, TPP was significantly higher in cases (64.96 ± 7.20 μmol/H2O2 vs. 55.41 ± 2.38μmol/H2O2) while MDA correlated inversely with TAC in cases (r = –0.477, p = 0.045).

Conclusions

The low plasma Zn coupled with high blood Pb and Cd levels may precipitate the elevated TPP and Cu:Zn ratio in cases. The reduced metallothionine defense of the system as indicated by the elevated Cu:Zn ratio in cases may also exacerbate this problem. Increased oxidative stress influenced by the high toxic metal level in cases may be participatory in the progression of AD.

doi: 10.1016/j.cca.2022.04.903

W153

Vitamin D receptor mutations influence on course of Parkinson's disease in patients treated with L-DOPA

B. Zapala[c], A. Spychalowicz[d], M. Piwowar[a], U. Cialowicz[b], M. Kosciuszko[e], O. Chmura[e], A. Wasinska[e]

[a] *Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College, Cracow, Poland*
[b] *Department of Clinical Biochemistry Jagiellonian University Medical College, Cracow, Poland*
[c] *Department of Clinical Biochemistry, Jagiellonian University Medical College, Cracow, Poland*
[d] *Department of Rehabilitation, The Center of Movement Organ Rehabilitation "KRZESZOWICE" SP ZOZ, Poland*
[e] *Jagiellonian University Medical College, Cracow, Poland*

Background-aim

Parkinson's disease (PD) is second most often occuring neurodegenerative disease after Alzheimer's disease. Age is being considered the most important factor for PD risk. Vitamin D(VD) is steroid hormone crucial for calcium homeostasis and bone metabolism. Contrary to other vitamins, VD is being produced in human organism in presence of sunlight. VD metabolism is multi-factorial process which involves specific enzymes of liver and kidneys with 1,25-D3 being active product. Latest research indicated that VD modulates over 1000 genes involved in cellular growth, protein synthesis and immunological processes. Several animal studies showed potential protective attributes of VD in dopamine cells. The aim of the study was to search for the conncections between VDR gene mutations and course of PD development.

Methods

Sequential analysis of VDR gene was performed on genomic DNA isolated from peripheral blood leukocytes of 100 patients with diagnosed Parkinson's Disease treated with Levodopa. Sequencing was performed in 3130xl Genetic Analyzer(Applied Biosystems) and statistical analysis was conducted using AB DNA Sequencing Analysis Software v. 5.2. (Applied Biosystems).

Results

From analyzed VDR gene fragments splicing region of exon 1 turned out to be the most interesting one. Mutation of "start"(ATG) codon was detected in most cases. In examined patients C/C genotype was present 32 times, C/T 53 times and T/T 23 times. Patients in research group had statistically significant prevalence of SNP. We found that dominant C/C alleles showed statistically earlier average age of diagnosis. In addition, the presence of each subsequent T allele significantly delayed the onset of the disease (p = 0.014). The T/T genotype could have also extended the time from diagnosis to the implementation of l-dopa treatment, but data did not reach statistical significance (p = 0.07) We have also connected C/T genotype of rs2228570 variant with higher chance of levodopa-induced dyskinesias. We will also present results of Vitamin D metabolome assessment and vitamin D levels which are currently under statistical processing.

Conclusions

We conclude that due to connections between VDR Gene mutations and clinical consequences gene sequencing may in the future be a viable way to predict future Parkinson Disease course.

Acknowledgment

The study was financially supported by the Ministry of Science and Higher Education (grant No: K/DSC/003558).

doi: 10.1016/j.cca.2022.04.904

W154

Genetic variability and the clinical course of Parkinson's disease and efficacy of levodopa treatment

B. Zapala [b], A. Spychalowicz [d], M. Piwowar [a], S. Czekalska [c], M. Hadasik [e], M. Zawada [c], J. Koper [e], A. Wasinska [e]

[a] *Department of Bioinformatics and Telemedicine, Jagiellonian University Medical College Cracow, Poland*
[b] *Department of Clinical Biochemistry, Jagiellonian University Medical College Cracow, Poland*
[c] *Department of Hematology Diagnostics, The University Hospital, Cracow, Poland*
[d] *Department of Rehabilitation, The Center of Movement Organ Rehabilitation "KRZESZOWICE" SP ZOZ, Poland*
[e] *Jagiellonian University Medical College, Cracow, Poland*

Background-aim

Parkinson's disease is the second most common neurodegenerative disorder in the world with Levodopa being the gold therapeutic standard. The response to levodopa treatment may be varied, possible from the fact that the genetic variability may determine the response to the treatment.

Methods

The aim of this work was to investigate the impact of genetic variants in the genes coding monoamine oxidase B (MAOB), dopamine receptor D2 (DRD2) and DOPA decarboxylase (DDC) on the observed differences in the clinical course of Parkinson's disease and the effects of levodopa treatment in the diagnosed patients. Patients diagnosed with Parkinson's disease n were included into the study group (126 patients: women and men aged 39 to 95). The whole peripheral blood was drawn from the patients and protected, then the genetic material in the form of DNA was extracted and the genotyping of single nucleotide polymorphisms (SNP) was performed using the TaqMan probes.

Results

It was stated that rs2283265 and rs1076560 genetic variants of the DRD2 gene determine more frequent presence of dementia and higher patients' scores in the II and III part of the UPDRS ($p < 0,05$). Furthermore, they do not affect the presence of the levodopa treatment complications and the need of deep brain stimulation in the patients diagnosed with Parkinson's disease ($p > 0,05$). It was also demonstrated that the genetic variants of rs1799836 of the MAOB gene and rs921451 of the DDC gene do not affect the clinical course of Parkinson's disease and the effects of levodopa treatment in diagnosed patients ($p > 0,05$).

Conclusions

We believe that genetic sequencing may in future serve as an tool to assess the future course of PD.

doi: 10.1016/j.cca.2022.04.905

W156

Dietary quercetin modulates ouabain-sensitive NA+/K+- atpase in mammalian brain model

A.T. Ogundajo

CHEMICAL PATHOLOGY DEPARTMENT,OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITAL COMPLEX, ILE-IFE, NIGERIA

Background-aim

The Na+, K+-ATPase is also known as the sodium pump, is a ubiquitous transmembrane enzyme that transports Na+ and K+ across the plasma membrane by hydrolyzing ATP. Quercetin is one of several naturally-occurring dietary flavonoids compounds, ubiquitously found in fruits, vegetables, herbs, tea, wine and as supplement. This study investigated in vivo modulatory effect of quercetin on normal rat brain.

Methods

Male adult Wistar rats (200-250 g) were randomly divided into the OR group (oral), IP group (Intraperitoneal), SC group (subcutaneous) and IV group (intravenous). These groups were administered with quercetin at a dose of 50 mg/kg once daily for five (5) days based on route of administration. Quercetin was pre-dissolved in ethanol. Animals were anesthetized with ether and euthanized by decapitation, the whole brain was quickly removed and placed on ice and the homogenate was prepared in 0.05 M Tris-HCl, pH 7.4 and the supernatant was used for the assay of Na+/K+-ATPase. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. Na+/K+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphorous (Pi) was measured by the method of Fiske and Subbarow.

Results

This present study showed that quercetin has inhibitory activity on cerebral sodium pump in a concentration-dependent manner irrespective of the route of administration while the effect was highly significant in IP group.

Conclusions

Quercetin modulates Na+/K+- ATPase activity in mammalian brain model by exerting dose -dependent inhibitory effect irrespective of the route of administration.

doi: 10.1016/j.cca.2022.04.907

W157

Prostaglandin-D-synthase levels in cerebrospinal fluid associated with altered tau proteins in patients with suspected Alzheimer's disease

D. Morell-Garcia [b], M.A. Ballesteros-Vizoso [a], J.M. Bauça [b], A. Garcia-Martin [c], S. Tarongi [c], G. Amer [c], A. Barceló [b]

[a] *Health Research Institute of Balearic Islands, Palma de Mallorca, Spain*
[b] *Laboratory Medicine Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[c] *Neurology Department, Son Espases University Hospital, Palma de Mallorca, Spain*

Background-aim

Cerebrospinal fluid (CSF) t-tau, p-tau (phosphorylated at threonine 181) and amyloid beta 42 (A®42), are considered classic core biomarkers to support Alzheimer's disease (AD) diagnosis. Moreover, hyperphosphorylation of tau is implicated in A®-induced cell death, possibly via a toxic gain-of-function mechanism. Some studies have reported different hypotheses to explain the mechanisms of neurodegeneration involved in the development of AD, including iron toxicity and prostaglandin pathways.

The aim of this study was to detect other CSF biomarkers that are associated with altered CSF classic biomarkers profile in patients with suspected AD.

Methods

A prospective observational study in a tertiary care hospital, between March 2019 and May 2020, was done. We recruited adult patients admitted consecutively into Cognitive Impairment Unit and subjected to a lumbar puncture. CSF samples were tested for NT-proBNP, ferritin, calcium, glucose, total protein, lactate and 25(OH)-D vitamin (Architect ci16200,Abbott Diagnostics) and after two months of freezing at -80°C for t-tau, p-tau, A®42 (Innotest,Fujirebio) and prostaglandin-D-synthase (PDS) [BN-II, Siemens]. Spearman's correlation and a Kruskal-Wallis' test for multivariate analysis were performed, considering a statistical significance of 5%.

Results

A total of 38 patients were included (42% male, median age of 67 [p5-p95: 53-78] years old), no age nor gender differences were found between subgroups. Ten patients presented pathological results in all three CSF classic Alzheimer's biomarkers, eighteen patients presented some tau protein values altered and ten patients presented normal results.

We found significant associations between ferritin levels and t-tau protein ($r = 0.335$, $p = 0.039$); also between PDS and t-tau and p-tau ($r = 0.545$ and $r = 0.464$ respectively, $p = 0.003$).

Differences were seen in CSF ferritin between patients with CSF tau protein values altered and those without (7.65 vs 6.30 ng/mL; $p = 0.041$) and also in PDS levels (21.2 vs 15.1 mg/L; $p = 0.036$). No differences were found in the rest of studied CSF biomarkers.

Conclusions

For patients with suspected Alzheimer's disease, CSF ferritin and PDS levels may have a role as neurodegeneration biomarkers and could be used for patient screening to help early identify altered CSF tau protein values.

doi: 10.1016/j.cca.2022.04.908

W158

Sleeping apnea' connection to neurodegenerative diseases

V. Manolov [b], V. Pencheva-Georgieva [f], M. Dayova [a], O. Georgiev [f], V. Vasilev [a], R. Emilova [c], I. Petrova [e], S. Hadjidekova [d], K. Tzatchev [b], L. Traykov [e]

[a] *Clinical laboratory Ramus, Sofia, Bulgaria*
[b] *Dept. of Clinical Laboratory, Medical University, Sofia, Bulgaria*
[c] *Dept. of Immunology, NCIPD, Sofia, Bulgaria*
[d] *Dept. of Medical Genetics, Medical University, Sofia, Bulgaria*
[e] *Dept. of Neurology, Medical University, Sofia, Bulgaria*
[f] *Dept. of Propaedeutics of Internal Diseases, Medical University, Sofia, Bulgaria*

Background-aim

Hepcidin leads to the deposition of iron in macrophages in atherosclerotic plaques by an increase in lipid peroxidation and progression of foam cells, which leads to the risk of atherosclerosis. Obstructive sleep apnea is associated with the development of insulin resistance, arterial hypertension, metabolic syndrome, systemic atherosclerosis and increased cardiovascular risk.

Methods

75 patients with obstructive sleep apnea (OSA) and neurodegenerative disease (NDD) were included. Their results were compared to sex and age matched healthy control. CBC, iron homeostasis parameters, hsCRP, and hepcidin were measured in the included groups. For evaluation of atherosclerotic changes IMT and FMT were applied.

Results

We found increased serum hepcidin levels in OSA and NDD patients (104.9 ± 9.1 g/L and 135.6 ± 12.7 g/L) compared to healthy controls (19.5 ± 0.8 g/L); $P < 0.001$. A positive correlation was found in OSA ($r = 0.819$, $r = 0.866$) and NDD ($r = 0.859$, $r = 0.881$) patients with atherosclerotic changes between IMT and FMD ($P < 0.01$).

Conclusions

Our findings suggest serum hepcidin quantification as a marker for iron deposition in atherosclerotic plaques and diagnosis of atherosclerotic changes. Brain-vascular disease risk factors are connected to obstructive sleep apnea syndrome. Dysregulation of iron homeostasis is one of the main risks atherogenesis factors. Early hepcidin quantification might predict an atherosclerosis occurrence in OSA patients, which might be especially important for better clinical diagnosis and practice.

doi: 10.1016/j.cca.2022.04.909

Clinica Chimica Acta 530 (2022) S353–S356

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

NeurologicalDisorder

W143

Diagnostic utility of intraoperative squash cytology in central nervous system tumors with histopathological correlation

B. Kc

Grande International Hospital

Background-aim

Background:

Intra-operative squash cytology is an important diagnostic tool for rapid and accurate diagnosis of central nervous system tumors. It has shown to play a vital role to determine determining further surgical management of patients undergoing surgery and can be ascertained as an alternative method to frozen section in resource limited settings.

Aim and objectives:

General

To evaluate the value of squash smear cytology in rapid intra-operative diagnosis of central nervous system tumors and its correlation with final histological diagnosis.

Specific:

i. To evaluate the CNS tumors in relation to site, age and sex.
ii. To compare the accuracy of squash cytological diagnosis of CNS tumors with that of histopathological diagnosis.
iii. To establish the role of squash cytology in rapid assessment of CNS tumors in a resource limited setting.

Methods

This study was carried out over a period of one year from 1st December, 2015 to 30th November, 2016. The study was performed on CNS tumor patients undergoing surgery at TUTH, Maharajgunj, whose specimen were received intra-operatively for squash cytology and postoperatively for histopathological examination.

Research design: Cross-sectional (Descriptive)

Study Population: All the patients with CNS tumors undergoing surgery whose samples were received for squash cytology and biopsy in the department of pathology, over one year period.

Place of study: Cytopathology and Histopathology unit, Department of Pathology, TUTH.

Sample size: 57 consecutive cases

Inclusion and Exclusion Criteria:

Inclusion criteria:

- All the patients operated for CNS mass lesions and intra-operative as well as post-operative sample received at Department of pathology.

Exclusion criteria:

- Cases in which histopathological sample were not available.

Specimen Collection:

Intra-operative specimens were received in normal saline. Squash smears were prepared by placing the tissue on the dry slide and crushed or squashed using another glass slide. The smears were then fixed in 95% propanolol for 10 minutes and then processed for giemsa and pap stain. The post-operative specimens received in 10% formalin were processed for histopathological examination; sections were stained with H&E stain (Annex 3-5).

Data Collection:

Specimen of intra-operative and post operative CNS tumors received at Department of Pathology was taken for study, after informed consent. A brief history along with radiological findings was taken from requisition form and filled in Proforma structured for this study (Annex 1).

Data Management:

All the data were entered in Microsoft Excel XP (20010). The results were then analyzed by IBM SPSS Statistics programme, version 20.

Results

During a period of one year of the study, a total number of 58 squash cytology specimens were received for intra-operative evaluation. One case was excluded from study because the biopsy specimen was not available for histopathological evaluation. Out of 57 patients evaluated, the mean age of the patient was 39 years, ranging from four to 73 years. A slight male predilection of CNS tumor was observed, the Male: Female ratio being 1.1:1.

The frequency of CNS tumors increased with age and a peak was seen at 41-50 year (22.8%). Thereafter, the frequency declined with only 15.8% in sixth decade of life. Most cases 94.8% were seen in upto 60 years of age. There were only three cases above 60 year of age.

With respect to CNS tumor grades, the widest age distribution was observed in grade I followed by grade IV tumors; Grade I ranging from first to ninth decade with a peak incidence at fifth decade and grade IV tumors ranging from first to eighth decade with a peak incidence at sixth decade. The age distribution of grade II tumors was from first to fourth decade. The narrowest age distribution was in grade III tumors, ranging from third to fifth decade only.

Number of cases in brain exceeded the spinal cord. Only 13 cases (23%) were seen in the spinal cord. Frontal lobe was the most common location constituting 36.8%, followed by temporal lobe.

The most common tumor diagnosed in squash cytology was glial tumour (24 cases) followed by meningioma (12 cases) and tumour of nerves (10 cases). Glioblastoma was most common glial tumor overall, in both histopathology and squash cytology. In the last four cases, only descriptive opinion was available.

One case each of oligodendroglioma and metastastic tumor diagnosed in squash cytology was diagnosed as glioblastoma in histopathology. Similarly, two cases of glioblastoma in squash cytology were diagnosed as anapalstic oligodendroglioma and pilocytic astrocytoma in histopathology. Thus, total number of cases of GBM both in histology and squash cytology is 13. There were four cases of pilocytic astrocytoma in squash cytology which were compatible with histopathology diagnosis. In addition, one case of neurofibroma and one case of GBM in squash cytology were diagnosed as pilocytic astrocytoma in histolopathological examination. Eight cases of schwannoma in squash cytology correlated completely with histopathology. However, additional three cases of schwannoma were diagnosed in histopathology which in cytology was diagnosed as ependymoma, neurofibroma and only few spindle cells respectively.

Neuroepithelial tumors constituted the most common type of tumor (24 cases, 43.2%) followed by meningeal tumor (13 cases, 22.1%) and tumor of cranial/spinal nerves (10 cases, 20.9%) diagnosed both in squash and histopathological diagnosis. Astrocytic tumor was the most common, comprising 32.8% (19 cases) in histopathology diagnosis and 29.8% (17 cases) in squash cytology.

Regarding frequency, Grade I CNS tumors were the most common, constituting 34 cases (59.6%). Among the grade I tumor, meningioma was the most common type (44%), followed by schwannoma (11%) and pilocytic astrocytoma (10.5%). Grade IV tumour (Glioblastoma) was the second most common tumor followed by grade II and III tumors respectively.

Out of 57 cases, 46 cases were correctly diagnosed in both squash cytology and histopathological examination, the concordance being 80.7%. All 12 cases diagnosed as meningioma in squash cytology showed complete concordance in histopathological diagnosis (100% concordance). The lowest concordance was noted in pilocytic astrocytoma and oligodendroglioma, the concordance being 66.6% each. One of the cases of anaplastic oligodendroglioma is included within the category of oligodendroglioma. Eleven cases were discordant.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of squash cytology to diagnose malignant tumor, in our study, are as follows:

1. Sensitivity: 86.3%
2. Specificity: 88.5%
3. PPV: 82.6%
4. NPV: 91.8%

Conclusions

Squash cytology is a reliable, fairly accurate and rapid tool in intraoperative diagnosis of CNS tumors. The present study supports the feasibility of squash cytology for intra-operative diagnosis and as an alternative to frozen section.

The concordance rate between squash cytology and histopathology was as high as 80 to 100%. There was 100% accuracy of squash cytology in case of meningioma and ependymoma followed by glioblastoma and schwannoma. The accuracy was slightly less for pilocytic astrocytoma and oligodendroglioma. Concordance of 100% was also seen in six other tumors but number of cases was just one for each of them, limiting the generalization of data.

Peak age was in fifth decade and grade I tumors were most common followed by grade IV and both of these showed wide age distribution ranging from first to ninth decade for former and up to eighth decade for later.

Glial tumours were most common nervous system tumor and glioblastoma constituted slightly more than 50% of them. Tumors of brain exceeded the spinal cord and frontal lobe was the most common location.

doi: 10.1016/j.cca.2022.04.911

W144

T helper 17 and interferon gamma positive T helper 17 cells in major depressive disorder

R. Ghosh [a], P.K. Kumar [a], P. Mitra [a], P. Purohit [a], N. Nebhinani [b], P. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur*
[b] *Department of Psychiatry, All India Institute of Medical Sciences, Jodhpur*

Background-aim

Recent advances in neuro-immunology has led to compelling evidence that inflammatory pathways may also be involved in the pathophysiology of Major depressive disorder (MDD). Animal model studies and some of the clinical studies have reported elevated levels of T helper 17 (Th17) along with decrease in T regulatory (Treg) cell count. However, the clinical studies investigating Th17 cells in MDD have not reached consensus in their results. Furthermore, the role of pathogenic subset of Th17 such as the “T helper 1” like Th17 in depression has not been explored, despite significant findings in animal models of neuro-inflammation. The current study was designed to assay the circulating Th17, Th1, Interferon © positive Th17(IFN©+ Th17) and Treg cell counts in MDD patients.

Methods

The study population included 53 patients of first episode, drug naïve MDD patients diagnosed and classified as per the ICD-10 classification of MDD and Hamilton Depression Rating Scale. 53 non psychiatric volunteers satisfying the exclusion criteria were taken as healthy controls in the study. Th17, Th1, IFN©+ Th17 and Treg cell counts were assessed by flowcytometry.

Results

The mean (± SD) percentage of Th17 cells in cases (1.87 ± 1.03) was significantly higher than the mean in controls (1.13 ± 0.94) (p value < 0.001). There was non-significant difference in the Treg counts between the cases (2.12 ± 2.0) and controls (2.27 ± 0.95) though the mean (± SD) was higher in the controls compared to the cases. The Th17: Treg ratio was significantly higher in cases (5.41 ± 10.84) compared to the controls (0.69 ± 0.89) (p value < 0.05). Further analysis of data has also revealed that “Th1” like IFN©+ Th17 subsets secreted more IL-17 in cases (515.68 ±423.44) compared to controls (509.55 ± 337.36). Intracellular IL-17 levels in this subset of Th17 cells also showed a weak but significant positive correlation with HDRS scores in the cases ($R = 0.28$, $p = 0.04$). However, the differences between cases and controls in IFN©+ Th17 count and intracellular IL-17 levels in this cell subset were not significant.

Conclusions

Elevated circulating Th17 count and Th17: Treg ratio in MDD cases add to the evidences suggesting Th17 mediated pro-inflammatory state in MDD. Findings also suggest a role of more pathogenic IFN© + Th17 cell subset in driving the disease progression in MDD.

doi: 10.1016/j.cca.2022.04.912

W145

Relationships between stress hyperglycemia, mortality prevalence and some laboratory parameters in patients with acute ischemic stroke

Z. Yaneva [b], M. Boyadzhieva [b], Y. Bocheva [a]

[a] *Central Clinical Laboratory, St Marina University Hospital-Varna, Bulgaria, Medical University - Varna, Bulgaria*
[b] *Department of Endocrinology, St Marina University Hospital-Varna, Bulgaria, Medical University-Varna, Bulgaria*

Background-aim

Stress hyperglycemia (SH, hyperglycemia on acute admission) is usually defined as blood glucose (BG)ε 7.8 mmol/L in patients without known diabetes mellitus or established HbA1c > 6.5%. It is commonly found in patients with acute ischemic stroke (AIS) and is associated with higher morbidity and mortality.

Aim:To examine retrospectively the prevalence of fatal outcome in patients with AIS according to the BG level at admission and to look for possible contributing factors.

Methods

We studied consecutive patients hospitalized for AIS in a neurological intensive care unit from May 2016 to April 2017. The cohort of 555 patients was divided into three groups – with SH, type 2 diabetes mellitus (T2DM) and normoglycemia (NG). We studied the mortality prevalence and some laboratory parameters.

Results

In the total study population, SH was found in 20.9%. Fatal endpoint prevalence was 32.8%, 33.8% and 19.05% in SH, T2DM and NG groups respectively (SH vs NG, $p = 0.003$; T2DM vs NG, $p = 0.0007$; SH vs T2DM, ns). Patients with SH showed the highest white blood cell (WBC) count ($p = 0.0003$). There was a positive correlation between WBCs level and nonfatal AIS severity ($r = 0.38$, $p = 0.0008$). As expected, survivors were younger ($p < 0.05$), but showed higher total cholesterol (SH, $p = 0.003$; T2DM, ns; NG, $p = 0.009$) and LDL-cholesterol (SH, $p = 0.005$; T2DM, ns; NG, ns) compared with non-survivors in all groups. Moreover, we observed a positive association between AIS severity and age in survivors with SH ($r = 0.22$; $p = 0.049$) and NG ($r = 0.18$; $p = 0.005$), but not in those with T2DM.

Conclusions

The similar mortality prevalence in SH and T2DM groups, significantly higher than in NG group, suggests an association of hyperglycemia with an increased risk of fatal outcome. The association established between AIS severity and age in survivors with SH and NG, but not in those with T2DM, could be interpreted as a possibility of equally severe course of AIS in diabetic patients regardless of their age. Further investigation is needed on the observed trends in WBCs and lipid parameters.

doi: 10.1016/j.cca.2022.04.913

W146

Temporal platelet agglutination shown in in vitro samples from elderly patient with advanced Parkinson's disease – Case report

A. Kanzaki

Department of Internal Medicine, Hyogo College of Medicine, Sasayama Medical Center, Sasayama, Hyogo, Japan

Background-aim

An 83-year-old woman with advanced Parkinson's disease (PD) and treated with intravenous levodopa was admitted to our hospital for her entrance to a geriatric health services facility. Results of routine blood tests incidentally revealed isolated thrombocytopenia (143×109/L) in an ethylenediaminetetraacetic acid (EDTA) tube, approximately half the amount as compared with the same test conducted three days prior and microscopy findings showed platelet agglutination without fibrin deposition. Vital signs were not remarkable and other blood test results did not support the existence of any infection or an autoimmune disease, though it was notable that the count in a citric acid tube was rather low (12×109/L).

Methods

Reproducibility of the results with EDTA, citric acid, heparin, and FC-Mix (Terumo) tubes using blood samples was examined. Furthermore, considering the microscopic findings, cluster designation 41 and 42b and time-course changes of their counts were also examined.

Results

Lower platelet counts were reproducibly observed in citric acid and heparin tubes (1.0×109/L and 0.8×109/L, respectively) as compared to the results obtained with EDTA and FC-Mix tubes (minimum 213×109/L and 220×109/L, respectively), while cluster designation 41 and 42b were elevated (99.7% and 92.2%, respectively). Time-course changes of the counts in the in vitro samples indicated a drastic increase with disagglutination in citric acid and heparin tubes (maximum 106×109/L and 165×109/L, respectively), whereas results with both EDTA and FC-Mix tubes were within a normal range (maximum 239×109/L and 224×109/L, respectively). To assess the effect of levodopa, additional blood sample tests were performed after dripping levodopa, which showed that the counts were not related to the drug.

Conclusions

Thrombocytopenia was especially notable in citric acid and heparin tubes with elevated platelet surface markers, and agglutination in vitro was only temporal, while levodopa did not have an effect. It might be explained at least in part as a consequence of weaker anticoagulant potency, as shown in citric acid and heparin as compared to EDTA, and may contribute to reveal the etiology of PD. Nevertheless, the significance in regard to etiology remains unknown, thus additional studies are needed.

doi: 10.1016/j.cca.2022.04.914

W147

Urinary metabolomics using gas chromatography mass spectrometry: Potential biomarkers for autism spectrum disorder

Z.U.N. Khan [a], D.P. Chand [a], D.H. Majid [a], D.S. Ahmed [a], D.A. Habib [a], D.L. Jafri [a], K.A. Khan [b]

[a] *Aga khan university hospital, Karachi, Pakistan*
[b] *Bolan Medical College, Quetta , Pakistan*

Background-aim

Diagnosis of autism spectrum disorder (ASD) is generally made phenotypically and the hunt for ASD-biomarkers continues. The purpose of this study was to compare urine organic acids profiles of ASD versus typically developing (TD) children to identify potential biomarkers for diagnosis and exploration of ASD etiology.

Methods

This descriptive cross-sectional study was performed in the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine in collaboration with the Department of Pediatrics and Child Health, Aga Khan University, Pakistan. Random urine samples were collected from children with ASD diagnosed by a pediatric neurologist based on DSM-5 criteria and TD healthy controls from August 2019 to June 2021. The urine organic acids were analyzed by Gas Chromatography-Mass Spectrometry. To identify potential urinary biomarkers for ASD canonical linear discriminant analysis was carried out for the organic acids, quantified in comparison to an internal standard.

Results

A total of eighty-five subjects were enrolled in the current study. The mean age of the ASD (n = 65) and TD groups (n = 20) was 4.5 ± 2.3 and 6.4 ± 2.2 years respectively with 72.3% males in the ASD group and 50% males in the TD group. Parental consanguinity was 47.7% and 30% in ASD and TD groups, respectively. The common clinical signs noted in children with ASD were developmental delay (70.8%), delayed language skills (66.2%), and inability to articulate sentences (56.9%). Discriminant analysis showed that 3-hydroxyisovalericc, homovanillic acid, adipic acid, suberic acid, and indole acetic were significantly different between ASD and TD groups. The biochemical classification results reveal that 88.2% of cases were classified correctly into 'ASD' or 'TD' groups based on the urine organic acid profiles.

Conclusions

The urine organic acids that were good discriminators between ASD and TD groups were 3-hydroxy isovaleric acid, homovanillic acid, adipic acid, suberic acid, and indole acetic. The discovered potential biomarkers could be valuable for future research in children with ASD.

doi: 10.1016/j.cca.2022.04.915

Clinica Chimica Acta 530 (2022) S357–S368

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Nutrition, Vitamins, Trace Elements

W159

Determination of free vitamin D in serum by ultrafiltration with liquid chromatography-tandem mass spectrometry

D. Wang

Peking union medical college hospital

Background-aim

Free 25-hydroxyvitamin D3 [25(OH)D3] may be a more meaningful marker of vitamin D function than total serum 25(OH)D; however, current direct (ELISA) and indirect (calculation) methods for serum free 25 (OH)D levels suffer from concentration-dependent bias and a poor concordance index. Thus, we aimed to establish a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to measure free 25 (OH)D3 and evaluate consistency in levels obtained by LC-MS/MS, ELISA, and calculation. The potential role of free 25(OH)D3 in predicting bone status was also assessed.

Methods

Samples were prepared by ultrafiltration (UF), and then liquid-liquid extraction followed by derivatization before LC-MS/MS analysis. Passing-Bablok regression and Bland-Altman plots were used to estimate the relation and bias between free 25(OH)D levels obtained by LC-MS/MS and by ELISA or calculation based on albumin and vitamin D binding protein. The potential role of free 25(OH)D3 in predicting bone status was compared with that of 25(OH)D3, 1,25(OH)2D3, and 24,25 (OH)2D3 in 50 bone loss and 45 normal cases.

Results

Free 25(OH)D was obtained by adjusting PH values with buffer and rinsing the UF tube twice. Repeatability of the LC-MS/MS method ranged from 6.0% to 9.3% and the within-laboratory coefficient of variation ranged from 11.9% to 17.7%. Passing-Bablok equation showed R2 range of 0.62–0.72. Bland-Altman plots showed that the mean bias among assay pairs ranged from -1.4% to 0.8%. The slope and intercept of the equation between LC-MS/MS and calculation method ($r = 0.79$) were 1 and 0, respectively.

Serum free 25(OH)D3 significantly positively correlated with 25 (OH)D3 ($r = 0.326$, $p = 0.022$) and calcium ($r = 0.488$, $p < 0.001$). Serum 25(OH)D3 and free 25(OH)D3 in the normal bone group were higher than those in the bone loss group. Reference interval of free 25 (OH)D was 1.68–14.65 pg/mL.

Conclusions

A simple LC-MS/MS method for measuring free 25(OH)D was developed, and can be used in future research.

doi: 10.1016/j.cca.2022.04.917

W160

Evaluation of the urine and serum iodine status in Tibet, China: A multicenter study

Y. Zou

Peking Union Medical College Hospital

Background-aim

As an important trace element, iodine plays important roles in the synthesis of thyroid related hormones. However, few studies reported the iodine status of Tibet in China. Thus, this study was aimed to evaluating the urine iodine concentration (UIC) and serum iodine concentration (SIC) in Tibet, China based on a multicenter study.

Methods

In total, 1499 apparently healthy individuals in Tibet, including Ali (altitude: more than 4700 meters; 238 males, 264 females), Nyingchi (altitude: 3500 to 4500 meters; 142 males, 294 females), as well as Shigaze and Lhasa (altitude: 2500 to 3500 meters; 250 males, 311 females) were enrolled. UIC and SIC were measured by inductively coupled plasma mass spectrometry (ICP-MS), and UIC was also adjusted by urine creatinine (Cr) concentrations. According to CLSI C28-A3, reference intervals (RIs) of UIC and SIC in Tibet were established.

Results

The median concentration of UIC, adjusted UIC and SIC in Tibet was 137.9g/L and 118.4g/L, and 54.7g/L, respectively. The prevalence of iodine insufficient, adequate, excessive were 30.4%, 63.0%, 6.6%, respectively. RIs of UIC and SIC in Tibet was [23.3g/L,426.8g/L] and [31.1g/L, 90.6g/L]. Furthermore, age, sex, drinking and smoking were the major source of variation of UIC but not for SIC.

Conclusions

Based on this multi-center cross-sectional study, both urine and serum iodine status in Tibet, China were evaluated and analyzed, which were significantly different from that of Chinese living in plateau. The iodine status of Tibetan region was considered sufficient.

doi: 10.1016/j.cca.2022.04.918

W161

Profile of vitamin b12 and folate in population of central Serbia

M. Petrovic [a], M. Stanojevic Pirkovic [b], I. Nikolic [b], M. Andjelkovic [b]

[a] *Clinical Center Kragujevac*
[b] *University of Kragujevac, Faculty of Medical Sciences, Department of Biochemistry*

Background-aim

Vitamin B12 is important for metabolism of all human cells – especially for cells of nervous system and red blood cells, so its deficiency leads to serious health conditions – both neurological and hematological. The most common cause of vitamin B12 deficiency is low inatake, followed by malabsorption and intestinal disorders. Folate – vitamin B9 is essential for nucleic acids synthesis and metabolism of amino acids, so its deficiency causes megaloblastic anemia, glossitis, diarrhea, depression, and neural tube defects. The aim of the study was to evaluate status of vitamin B12 and folate among population of the region in central Serbia.

Methods

Blood samples were taken from 100 healthy adult individuals (34 male and 66 female) aged 18 to 87 years. Exclusion criteria were vitamin supplementation, medical history, alcohol consumption and use of oral contraceptives. Serum vitamin B12 and folate concentrations were measured on the Access2 (Beckman Coulter Inc.). Variables were tested for gender and age group differences by using the Mann-Whitney's U-test. P-values below 0.05 were considered significant.

Results

Mean serum concentration of vitamin B12 was 256.69 pg/mL (ranged from 10.00 pg/mL to 824 pg/mL), and for folate 6.74ng/mL (ranged from 1.70 ng/mL to 14.3 ng/mL). Among the population 33.0% were found to be deficient in vitamin B12 (41,2% of male and 28.8% of female) considering 180 pg/mL as a cut-off value. Folate was deficient in 7% of participants (1% of male and 9,1% of female) using 3.1 ng/mL as a cut-off value. Mean concentration for both vitamins did not show statistically significant difference between male and female group as well as between group aged 18-65 years and group of those older than 65 years.

Conclusions

The results of our investigation demonstrated serum vitamin B12 deficiency in one third of the adult population of central Serbia, while serum folate was deficient in 7% of examined adults. Further studies on a larger population should be performed in order to establish exact vitamin B12 and folate state, and, possibly, new reference interval.

doi: 10.1016/j.cca.2022.04.919

W162

Maternal perspectives on probiotics, intake of probiotic food and occurrence of atopic dermatitis among Filipino children

C.A. Durante [b], E. Bullecer [b], F. Durante [a]

[a] *College of Medicine, University of the East*
[b] *College of Public Health, University of the Philippines – Manila*

Background-aim

Atopic dermatitis (AD), also known as eczema, is the most common chronic relapsing skin disease in children, affecting approximately 10% to 30% of children worldwide. For this reason, the research community investigated possible innovative prevention and treatment strategies for AD. One of these strategies was the manipulation of the intestinal flora through probiotics.

Methods

This study is a cross-sectional, analytic study on 680 mothers and 680 children recruited in selected urban communities in Laguna, Philippines. This research used a semi-quantitative food frequency questionnaire to gather data on probiotic consumption; and ISAAC diagnostic tool to confirm atopic dermatitis.

Results

The study showed that most mothers (92%) had highly positive attitude to probiotics. Path analysis was run to check for association and the resulting path showed significant association between attitude and intention, attitude and intake, behavioral control and intake, behavioral control and intention, and intention and intake of probiotic food. Female children (42%) have higher intake than males (31%). High intake was also noted among children of mothers with educational attainment of College and higher (41%); among low- (39%) and middle-income families (44.06%); and among those without family history of AD (39%). Three out of ten of the respondents reported daily intake of at least one bottle of the probiotic foods enumerated. Among 680 respondents, 18% (n = 123) were diagnosed as having atopic dermatitis, while 82% (n = 557) were without AD. This study showed that controlling for the effect of family history of AD, the odds of having atopic dermatitis was 2.1 times higher among those with low intake status compared to children with high intake status. Furthermore, the odds of having atopic dermatitis was 3.9 times higher among those with no intake compared to children with high intake status. With both results having a p-value of < 0.0001, the association is significant. One out of three children consumed a probiotic drink daily.

Conclusions

Based on the results of this study, intake of probiotic food was a protective factor against atopic dermatitis.

doi: 10.1016/j.cca.2022.04.920

W163

Effects of cassava processing methods on insulin index, glyceamic profile, glyceamic load and index of cassava (manihot esculeuta) meals in type 2 diabetes mellitus patients seen in Benue State University Teaching Hospital, Makurdi, Nigeria

B. Myke-Mbata, S. Adebisi

Department of Chemical Pathology, Benue state university Makurdi, Benue State, Nigeria

Background-aim

The major challenge of dietary modification is high cost and non-availability of prescribed food items favoured by dietary modification expert. Hence, the need to assess the impact of the affordable staple starchy foods. Cassava is the most consumed staple starchy food; therefore, this study evaluated its impact on glycaemic and insulin response.

The aim of this study is to determine the effect of food processing methods on insulin index, glycaemic profile, glycaemic load and glycaemic Index of cassava food products in type 2 diabetes mellitus patients at Medical out patient department, Benue State University Teaching Hospital, Makurdi, Benue State.

Methods

This was a cross-sectional study carried out on randomly selected 66 individuals with diabetes mellitus attending the diabetic clinic of Benue State University Teaching Hospital and 65 age matched apparently health controls. Each subject ingested three cassava processed products (cassava fufu, abacha and garri) (equivalent of 50g glucose) and 50 g of reference meal which is anhydrous glucose solution. Samples were taken for blood glucose and insulin at intervals of 0,30,60,90,120,180 mins. Proximate analysis was done on the cassava food products.

Results

The Glycaemic Index for fufu, garri and chips for diabetics and controls were found to be (Mean ± SD) 92.59 ± 8.07 vs 93.26 ± 17.31; p = 0.78,79.89 ± 24.33 vs 95.92 ± 10.34;p 0.01),76.24 ± 3.31 vs 91.94 ± 27.69 p = 0.01 respectively. The insulin index for fufu, garri and chips for diabetics and controls were 97.22 ± 10.29 vs 55.83 ± 35.47; p = 0.0001, 88.54 ± 10.02 vs 69.36 ± 31.03;p = 0.023;94.90 ± 13.15 vs 97.02 ± 20.23;p = 0.05 respectively. The glycaemic load for fufu, garri and chips for diabetics and controls were46.29 ± 4.04 vs 46.62 ± 8.66; p = 0.782;39.94 ± 12.17 vs 47.96 ± 5.18;p = 0.0001,38.12 ± 1.66 vs 45.97 ± 13.85;p = 0.0001 respectively. The glycaemic profile index is 71.61 ± 6.76 vs 37.34 ± 1.16 p = 0.0001; 73.99 ± 2.29vs41.41 ± 1.60 p = 0.0001, 94.53 ± 9.38 vs 46.19 ± 2.48 p = 0.0001 respectively. The proximate analysis for the meals taken by the participants shows protein, moisture, fibre, fat, ash and carbohydrate content as follows the cassava (%) (crude form)1.07 ± 0.02;72.00 ± 0.21;0.80 ± 0.07;0.58 ± 0.03;0.35 ± 0.02;25.07 ± 0.03,Abacha1.44 ± 0.04;59.13 ± 0.15;0.73 ± 0.01;1.71 ± 0.02; 36.83 ± 0.03,garri1.82 ± 0.10;67.36 ± 0.09;0.15 ± 0.02;0.91 ± 0.09;0.25 ± 0.05;39.64 ± 0.03 and fufu 1.56 ± 0.021;67.51 ± 0.19;0.21 ± 0.02; 0.52 ± 0.02;0.20 ± 0.01;30.22 ± 0.021 respectively.

Conclusions

Glycaemic index, insulin index, glycaemic profile and glycaemic load of fufu, garri and cassava chips was found to be high in both diabetic and control subjects. The glycaemic profile of fufu, garri and cassava chips was significantly ($p < 0.05$) elevated in diabetics compared to control subjects while glycaemic Index and glycaemic load was elevated significantly ($p < 0.05$) in control subjects compared to diabetics. There is an inverse relationship between insulin index and glycaemic Index amongst the diabetic subjects. Proximate analysis of the different cassava products shows that there is increased carbohydrate and protein in cassava meals studied compared to the crude form as well as reduced fibre content with the exception of cassava chips which exhibited high fibre content. Quantitative insulin sensitivity check index [QUICKI] was significantly reduced among the subjects studied while Homeostatic model assessment of insulin resistance was found to be significantly increased in diabetic subjects studied, QUICKI had significantly positive correlation with insulin index while HOMA-IR was significantly, negatively correlated with insulin index.

doi: 10.1016/j.cca.2022.04.921

W164

Maternal perspectives on probiotics and probiotic food consumption of children in the Philippines

C.A. Durante, E. Bullecer

College of Public Health, University of the Philippines – Manila

Background-aim

The objective of the study is to investigate attitude, perceived behavioral control and perspectives of mothers regarding commercially-available probiotic-containing food and its effect on the actual consumption of children of these foods in selected urban communities in the Philippines.

Methods

A survey questionnaire was used to gather information on mother's attitude, perceived behavioral control and intention of giving probiotics to her child. A semi-quantitative food frequency questionnaire was designed to measure amount of daily intake of probiotic food. Using a cross-sectional, analytical study design, composite scores were computed for attitude, behavioral control and intention as it relates to actual consumption.

RESULTS

Most mothers (92%, n = 625) had highly positive attitude to probiotics. Path analysis was run to check for association and the resulting path showed significant association between attitude and intention, attitude and intake, behavioral control and intake, behavioral control and intention, and intention and intake of probiotic food. Female children (42.43%) have higher intake than males (30.61%). High intake was also noted among children of mothers with educational attainment of Vocational (45.45%) and College and higher (41.09%); and among low- (38.92%) and middle-income families (44.06%). As for the frequency of consumption of probiotic food, 3 out of 10 respondents reported daily intake of at least one bottle of the probiotic foods enumerated.

Conclusions

Mother's attitude, perceived control and intention are positively associated with higher total intake among children. The intake of probiotics among children in the sample population was high. However,

mothers giving probiotics to their children lack correct information regarding probiotics.

doi: 10.1016/j.cca.2022.04.922

W165

Gonadal status, related micronutrients and antioxidant capacity in males occupationally exposed to lead

C. Obi-Ezeani [a], S. Meludu [b], E. Dioka [b], I. Onuora [b]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

Background-aim

Occupational exposure to lead (Pb), a well-known endocrine disruptor may alter biochemical indices including reproductive hormones and micronutrients like selenium (Se) and zinc (Zn) which are necessary for testosterone synthesis and antioxidant defense system. The study assessed the gonadal status, some micronutrients and total antioxidant capacity (TAC) in males occupationally exposed to lead.

Methods

A total of 82 male subjects between 18 and 54 years, comprised of 41 motor mechanics (MM) and 41 occupationally unexposed control subjects at Thinker's Corner, Enugu were recruited for the study. Five milliliters of blood was collected from each subject, 3ml was dispensed into K2EDTA tube for Pb, Se and Zn analyses while 2ml was dispensed into plain vacutainer tubes, for testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and TAC analyses and centrifuged at 3000g for 5 minutes and the sera obtained used for Pb, Se and Zn were determined by Atomic Absorption Spectrophotometer (AAS), testosterone, FSH and LH were analyzed by Enzyme Linked Immunosorbent Assay (ELISA), and TAC was analyzed spectrophotometrically. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0, and $p < 0.05$ was considered significant.

Results

The mean blood Pb and serum FSH and LH levels were significantly higher while the mean blood Se, Zn and serum testosterone and TAC levels were significantly lower in the motor mechanics compared with the control ($p < 0.05$). 65.9% of the MM were eugonadal whereas 34.1% had compensated hypogonadism. The compensated hypogonadic MM had lower testosterone and higher FSH and LH levels compared with the eugonadal MM ($p < 0.05$). Pb showed significant negative correlations with Se ($r = -0.762$, $p = 0.0001$), Zn ($r = -0.604$, $p = 0.0001$), TAC ($r = -0.457$, $p = 0.003$) and testosterone ($r = -0.743$, $p = 0.0001$), and significant positive correlations with FSH ($r = 0.356$, $p = 0.022$) and LH ($r = 0.340$, $p = 0.029$).

Conclusions

This study suggests that occupational exposure to lead may be associated with impaired gonadal function, micronutrients levels and antioxidant defense which could result to disturbances in reproductive function or fertility in men.

doi: 10.1016/j.cca.2022.04.923

W166

Glycaemic index of dough staple foods commonly eaten in Nigeria

S. Meludu [b], B. Myke-Mbata [b], E. Dioka [b], C. Obi-Ezeani [a]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

Background-aim

Starchy dough food is the most commonly consumed food in Nigeria. However, the recent disease epidemiological changes from communicable diseases to non-communicable diseases, has implicated changes in occupational choice from farming to sedentary works as well as consumption of predominantly dough starchy food as a notable culpable cause. Hence, this study examines the glycaemic impact of the commonly eaten staple starchy food in Nigerian subject.

Methods

This was a cross-sectional study carried out on 16 healthy staff from Benue State University Teaching Hospital. Each participant ingested food products (pounded yam, amala, fufu and garri) (equivalent of 50g glucose) and 50 g of reference meal which is anhydrous glucose solution. Samples were taken for blood glucose at intervals of 0,30,60,90,120 mins. The area under curve was determined using trapezoid method for different time intervals. A plot of concentration against time was used for the calculation, Area under the curve(AUC) = (Conc2 + Conc1)/ 2 x (time2-time1). The sum of area under curve for each test food was divided by the sum of area under curve for standard glucose and multiplied by 100 to determine the glycaemic index of the food products respectively.

Results

The glycaemic index for apparently healthy group after intake of pounded yam, amala, garri and fufu were 80.81%, 71.63%, 80.59% and 94.81% respectively. Cassava meals had highest glycaemic index while amala has the lowest glycaemic index.

Conclusions

Glyceamic index in diabetic subjects for pounded yam, amala, fufu and garri is high in healthy subjects. Consumption of this Starchy dough food should be less frequent and in smaller portions especially in people with sedentary life style.

doi: 10.1016/j.cca.2022.04.924

W167

Serum level of some trace elements and vitamin c in women with polycystic ovarian syndrome in Nnewi south east Nigeria

C. Njoku [b], S. Meludu [b], E. Dioka [b], C. Obi-Ezeani [a]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

Background-aim

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine diseases in women, influencing 5% to 10% of women of

reproductive age. Stressful lifestyle lead to increased prevalence of PCOS in young adolescent and early reproductive population and its association with many ongoing complications such as infertility, obesity, insulin resistance, dyslipidemia, endothelial dysfunction and overt diabetes mellitus. The aim of the study was to evaluate the serum level of zinc, copper, iron and vitamin C in PCOS women.

Methods

A case-control study that included a total of 60 (30 patients with PCOS and 30 healthy controls) women. Serum zinc, copper, iron levels were analyzed using atomic absorption spectrophotometric method. Vitamin C was analyzed using high performance liquid chromatography (HPLC).

Results

Vitamin C was significantly higher in women with PCOS compared to the controls ($P < 0.05$). There were no significant differences in body mass index, levels of serum zinc, copper and iron in women with PCOS compared to the controls ($P < 0.05$).

Conclusions

PCOS patients are not at risk of deranged trace elements metabolism. The significant increase in Vitamin C could be as a result of Vitamin C supplements to the patients, and as an antioxidant may help in fight against free radicals, enhance fertility and prevent complications.

doi: 10.1016/j.cca.2022.04.925

W168

Evaluation of micronutrients zinc, copper and magnesium in some patients suffering from depressive illness in Ibadan, southwestern Nigeria

A. Onuegbu [a], E. Agbedana [b], K. Adebayo [c], J. Olisekodiaka [a]

[a] *Department Of Chemical Pathology College Of Health Sciences Nnamdi Azikiwe University Awka, Anambra State*
[b] *Department Of Chemical Pathology College Of Medicine University Of Ibadan Ibadan, Oyo State*
[c] *Department Of Psychiatry College Of Health Sciences, Osogbo Ladoke Akintola University Of Technology Ogbomoso, Nigeria*

Background-aim

Depressive illness is a common psychiatric disorder that occurs worldwide but is often neglected among Africans. It is a leading cause of disability. There is an indication its prevalence rate is increasing in Africans. Various predisposing factors have been implicated in the pathogenesis of depression but detailed studies on the role of micronutrients zinc, copper and magnesium in depression still remains scanty and inconclusive especially in Nigerian patients. This study was therefore aimed at measuring the plasma comcentrations of these micronutrients in some Nigerian patients to determine their potential involvement in this disease.

Methods

Sixty subjects (mean age 40.3 ± 12.3) diagnosed by the Consultant Psychiatrist as suffering from depression and who fulfilled the inclusion criteria were enrolled from the Psychiatry Department of the University College Hospital, Ibadan using the Diagnostic and Statistical Manual Technique (DSM-IV criteria). They were classified as either suffering from mild, moderate and severe depression with the use of the Hamilton Depression Rating Scale (HDRS). Forty apparently healthy subjects (mean age 40.1 ± 10.1) served as controls. Anthropometric indices of all patients and controls were measured using standard methods and the Body Mass Index (BMI) calculated. Plasma zinc, copper and magnesium were determined using the Atomic Absorption Spectrophotometer (AAS).

Results

The results showed that the mean age and BMI (21.9 ± 3.4 vs 21.6 ± 2.7 Kg/m2) did not vary significantly between depressed subjects and controls. Plasma zinc (μg/L), copper (mg/dl) and magnesium (mg/L) in the patients were also not significantly different from control values (709 ± 135 vs 669 ± 120, 1.38 ± 0.11 vs 1.37 ± 0.06, 21.8 ± 4.0 vs 20.9 ± 3.6 respectively). None of the micronutrients significantly correlated with the level of severity of depression in the patients.

Conclusions

This study suggests that zinc, copper and magnesium may not be important contributory factors in Nigerians suffering from depression.

doi: 10.1016/j.cca.2022.04.926

W169

Serum vitamin profile and its relationship with other biomarkers in Korean breast cancer patients

J.A. Kim [b], J.E. Lee [a], S. Lee [b]

[a] *Breast Division, Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine*
[b] *Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine*

Background-aim

Numerous studies have shown that vitamins reduce risk of cancers by playing important roles as coenzyme or enzyme in metabolic pathways. However, the relationship between serum vitamin levels and breast cancer is still unknown. Furthermore, data relating vitamin levels to breast cancer risk among Asian population are sparse. In this study, we investigated serum vitamin status in Korean breast cancer patients and their association with clinical and laboratory parameters.

Methods

A total of 104 patients with breast cancers, 62 patients with benign breast diseases, and 75 healthy Korean females were prospectively enrolled in this study. Serum concentrations of vitamins A, D, and E, along with homocysteine and methylmalonic acid to evaluate vitamin B12 deficiency were measured by HPLC or HPLC-MS/MS. We investigated demographic or clinical characteristics (age, BMI, menopausal status) and other biochemistry test results (total protein, albumin, AST, ALT, ALP, HDL, LDL, iron, TIBC, cholesterol), and analyzed their correlation with serum vitamin levels. Additional assessments were done in subgroup analyses to investigate the possible associations between vitamin levels and tumor stages, the presence of lymph node metastasis, and breast cancer subtypes.

Results

The serum concentrations of vitamins A and E were significantly lower in patients with breast cancer than in controls (1.50 vs. 1.86 umol/L, $P<0.001$ for vitamin A; and 27.19 vs. 31.07 umol/L, $P<0.001$ for vitamin E). Severe vitamin D deficiency (<10 ng/mL) was more prevalent in patients with breast cancer than in controls (27.89 % vs. 13.87 %). Vitamin D level was significantly lower in breast cancer patients with ER-negative (12.65 vs. 17.71 ng/mL, $P<0.05$), or with triple-negative subtypes (9.75 vs. 17.57 ng/mL, $P<0.05$) than in those with other subtypes. Vitamin B12 deficiency (methylmalonic acid >350 nmol/L) was only observed in two patients with breast cancer, but none in controls. Vitamin E level showed positive correlation with LDL ($r=0.42$, $P<0.0001$) and total cholesterol ($r=0.59$, $P<0.0001$).

Conclusions

We found that breast cancer patients have lower serum vitamin levels than controls, suggesting the potential role of antioxidant vitamins in breast cancer. Further studies with large patient cohorts are required to elucidate the significance of vitamins in breast cancer.

doi: 10.1016/j.cca.2022.04.927

W170

Association between serum vitamin d status and metabolic risk factors in a rural Korean population

H. Park [a], H. Kim [b], Y.S. Je [a], H. Lim [a], K. Lee [a]

[a] *Department of Laboratory medicine, Seoul Clinical Laboratories, Yongin, Korea*
[b] *Departments of Obstetrics and gynecology, Konkuk University School of Medicine, Chungju, Korea*

Background-aim

The association of serum vitamin D deficiency and metabolic risk factors in adults has been recently investigated. This study focused the correlation between serum vitamin D and metabolic risk factors with a typical cohort of rural Korean individuals.

Methods

This study involved 875 participants (men 541, women 334), ranging in age from 19-84 years old, from the Center for Physical Examination, Chungju Hospital of Konkuk University in Korea. Fasting blood samples were collected and serum concentrations of 25(OH)D3, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured. Multiple linear regression analysis was performed to estimate the associations between serum 25(OH)D and lipids. The association between the occurrences of dyslipidemias and vitamin D levels was assessed via multiple logistic regression analysis. Confounding factors including age and body mass index (BMI) were used for adjustment.

Results

The overall percentage of vitamin D deficiency, vitamin D insufficiency, and vitamin D sufficiency were 27%, 46%, and 27%, respectively. Mean serum 25(OH)D3 level was 25.9 ± 17.7 ng/mL. Dyslipidemia was strongly associated with sex ($P<0.001$), BMI ($P<0.001$), TC ($P<0.001$), LDL-C ($P<0.001$), HDL-C ($P<0.001$), TG ($P<0.001$), and serum 25(OH)D3 levels ($P=0.001$). The serum 25(OH)D3 levels were inversely associated with TC ($P<0.001$), TG ($P<0.001$), and LDL-C ($P=0.026$) in all groups.

Conclusions

The prevalence of vitamin D deficiency is determined herein is lower than that determined previously in Korea, probably owing to differences in the study group. Serum 25(OH)D3 concentrations were associated with the serum lipid levels, except for HDL-C. The incidence of dyslipidemia decreases with an increase in serum 25(OH)D3 levels.

doi: 10.1016/j.cca.2022.04.928

W171

Assessment of serum level of vitamin d, selenium and calcium in women with polycystic ovarian syndrome in Nnewi south east Nigeria

C. Njoku, S. Meludu, E. Dioka, C. Okafor

Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Background-aim

Polycystic ovarian syndrome (PCOS) is a common endocrinopathy affecting 5% to 10% of women of reproductive age. It is characterized by anovulation, menstrual irregularities, hyperandrogenism and infertility.Increased prevalence of PCOS in early reproductive population may be attributed to stressful lifestyle. The aim of the study was to evaluate the serum level of vitamin D, selenium and calcium in PCOS women.

Methods

A case-control study that included a total of 60 (30 patients with PCOS and 30 healthy controls) women. Serum vitamin D was analyzed using high performance liquid chromatography (HPLC), Selenium was analyzed using atomic absorption spectrophotometric method while serum calcium was measured by spectrophotometer.

Results

Vitamin D, selenium and calcium were significantly reduced in women with PCOS compared to the controls ($P<0.05$). There was no significant difference in body mass index in women with PCOS compared to the controls ($P<0.05$).

Conclusions

PCOS women are at risk of vitamin D, selenium and calcium deficiency. There are increase evidence that indicate the role of vitamin D deficiency as a risk factor for cardiovascular disease and several malignant tumors. There is possibility that selenium which is an essential trace element may affect female fertility. While calcium plays vital role in activation and maturation of oocyte, hence, abnormalities in calcium metabolism may play an important role in pathogenesis of PCOS. Therefore, there is need for supplement therapy and food fortification in PCOS women.

doi: 10.1016/j.cca.2022.04.929

W172

Relationship between bivalent essential trace elements in human serum

A. Sadykov, I. Zolkina, A. Skryabin, I. Mamedov, V. Yurasov

Scientific laboratory "Chromolab"

Background-aim

Essential trace elements are cofactors of large amount of key reactions in the human body. Therefore, their excess or deficiency can significantly affect health and in case of serious violations lead to death. Trace elements, especially those with the same charge, can compete with each other, which may indirectly affect their concentration in body biofluids. The aim of this study was to evaluate correlation relationships in order to further study by molecular biology methods.

Methods

Serum samples (n = 724) were obtained from 18-70 years old patients. Concentration of trace elements were measured by using inductively coupled plasma mass spectrometry (ICP-MS). Cu, Co, Mn, Mo, Zn, Cr, Ni, V, W and Se were measured. The correlation analysis was performed by non-parametric Spearman method.

Results

Correlation coefficients were generally less than 0.3, mostly positive. Highest correlations were found between Fe and Ni (0.47, $p < 0.01$), Cr and V (0.45, $p < 0.01$), Ni and Mn (0.3, $p < 0.01$), Se and Zn (0.33, $p < 0.01$), V and W (0.46, $p < 0.01$), Co and Mn (0.32, $p < 0.01$). The number of detected negative correlations was significantly less and the values were lower: Se and Mo (-0.1, $p < 0.01$), Ni and Cu (-0.04, $p < 0.1$).

Conclusions

The predominance of positive correlations over negative ones may indicate a positive shift and overestimation of the coefficients due to technological reasons or the contribution of nutritional reasons. In addition, negative connections may be greater in reality. However, we believe that coefficients > 0.3 cannot be explained by technological errors, and these relationships can be used in the diagnosis of combined micronutrient deficiencies.

doi: 10.1016/j.cca.2022.04.930

W173

Levels of 25-hydroxyvitamin d in serum of Mongolian children

T. Enkhjargal[a], R. Lander[b]

[a] *National Centre for Public Health, Ulaanbaatar*
[b] *University of Colorado*

Background-aim

Rickets in young Mongolian children is a significant problem. The high levels of rickets have been attributed to low intakes of vitamin D. Serum concentration of 25-hydroxyvitamin D (25(OH)D) is the best indicator of vitamin D status. In this survey we have investigated the status of vitamin D among young children.

Methods

98 children (54 male and 44 female) 6-36 months of age from Ulaanbaatar city and two western and eastern provinces of Mongolia were included in the survey. Serum 25(OH)D was performed by using a radioimmunoassay procedure.

Results

The mean concentration of serum was 29.00 nmol/L. The indicator was lower in boys than in girls (25.30 nmol/ L vs. 33.65 nmol/L), and was lowest in the youngest age group (21.86 nmol/L in 6-11.9 month-old children vs. 30.73 nmol/L in the 12-36 months age group). The mean level of 25(OH)D in children from the eastern province was lower (23.24 nmol/L) than from the capital city (29.15 nmol/L) and the western region (31.77 nmol/L), but the differences were not statistically significant.

Vitamin D deficiency (<25 nmol/L) was detected in 61.2% of the surveyed children with higher frequencies in boys (66.7% vs. 55.8% in girls), in UB (65.7% vs. 51.9% in rural areas) and in younger children (73.7% in 6-11.9-month-olds vs. 58.9% in the 12-36 months of age group).

Conclusions

The high level of vitamin D deficiency indicates that there is a need to promote the expansion of the coverage of vitamin D supplements among young children.

doi: 10.1016/j.cca.2022.04.931

W174

Levels of serum selenium and IL-6 in occupationally lead exposed workers in Rajasthan

S. Gangam, S. Sharma, P. Mitra, T. Goyal, P. Singh, P. Sharma

All India Institute of Medical Sciences, Jodhpur

Background-aim

Lead (Pb), a toxic heavy metal extensively used in industries is capable of inducing several adverse health effects in the workers exposed to this metal. Chronic occupational lead exposure leads to increase in levels of pro-inflammatory cytokines leading to inflammatory damage to various organs. Lead also interacts with some of the essential metals like selenium which has a major role in ameliorating oxidative stress induced by lead toxicity.We aimed at estimating levels of IL 6 and selenium in occupationally Pb exposed individuals.

Methods

74 chronically Pb exposed workers were recruited for the study after taking informed consent. Venous blood samples were collected taking due aseptic precautions. Blood Pb levels (BLL) and Serum Se were analyzed using Dual Atomic Absorption Spectrophotometer (ICE 3500 Thermofischer). Serum IL- 6 levels were measured by using ELISA (Krishgen Biosystems). Commercial reference materials were obtained from Bio-

Rad (Lyphochek® Whole Blood & Serum Metals Control) for the internal quality assurance and control program.

Results

Data was expressed as Median (IQR) and Spearman correlation, Mann Whitney U test was used for comparison as the data was not normally distributed. Median BLL was 4.24 ug/dL (Range: 0.68-31.76). Median serum selenium levels were 102.15 ng/mL (Range: 46.9 – 189.6). The levels of selenium were found to be negatively correlated with lead levels (r value -0.35), while IL -6 levels were positively correlated with lead (r value 0.3). Further, when the population was divided into two groups with BLL < 4 ug/dL & BLL > 4 ug/dL, the levels of selenium and IL-6 were also found to be statistically significant with p value 0.02 and 0.01 respectively.

Conclusions

In our study we found that selenium was negatively correlated, and IL 6 was positively correlated with lead. Negative correlation may suggest the protective role of selenium in lead toxicity and positive correlation with IL 6 suggests the occupational Pb exposure promotes inflammatory processes by increasing the levels of pro-inflammatory cytokines.

doi: 10.1016/j.cca.2022.04.932

W175

Possible sample contamination in the quantification of manganese before and after a complete blood count

M.A. Ballesteros Vizoso, J.M. Bauça, R. Ortiz Montero, P. Argente Del Castillo Rodríguez, D. Morell-Garcia, J.A. Delgado Rodríguez, F. Forteza, I. Martín Navas

Department of laboratry medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

Manganese (Mn) is an essential trace element with greater requirements during fetal and neonatal development, thanks to its crucial role in skeletal and cerebral development. It crosses the placental barrier, and higher values have been described for pregnant women and in umbilical cord blood. Both excess and deficiency may be neurotoxic, and have been related to the weight birth and to parenteral nutrition (PN).

Its determination in premature infants under PN could be of great interest, as these may also contain an extra contribution of Mn due to contamination of the constituents used in its preparation.

Premature children are under multiple analytical controls, including several complete blood counts (CBCs) and trace element determinations, for which whole blood samples are needed.

Our aim was to assess the possible contamination of samples after introducing a metal probe for the realization of the CBC, in order to avoid collecting extra tubes for the determination of Mn.

Methods

A total of 31 healthy adults were included in our observational study in which one tube of whole blood with EDTA was collected to each participant. Mn was determined before and after introducing a metal probe from the flow cytometer Cell-Dyn Sapphire(Abbott Diagnostics, USA).

Mn determinations were performed on the ICP-MS NexION 300X platform (PerkinElmer, Finland) using a calibration by standard addition.

Integration and analysis times were 3000ms and 1min 43s, respectively. Imprecision and accuracy were $<6\%$.

Normality was assessed with the non-parametric Kolmogórov-Smirnov test. Comparison of means was made using the Student's t test for paired data. Statistical significance was considered when $p<0.05$.

Results

A total of 62 samples of EDTA total blood were analyzed.

The mean value(standard deviation) obtained in the 31 samples without introducing the metallic probe was 12.85(4.03)μg/L, and 11.42(5.30)μg/L after the introduction of the metallic probe. P value was 0.071.

Conclusions

No statistical difference was found after the introduction of the metal probe in the whole blood sample. As no alteration was detected in the concentration of Mn were seen, a single whole blood sample may be used for CBC and Mn for newborn in baseline conditions and after PN control.

doi: 10.1016/j.cca.2022.04.933

W176

Sumultaneous determination of b vitamins in human blood by LC/MS method for diagnosis of various pathological conditions and drug therapy

I. Zolkina, I. Mamedov, A. Sadykov, V. Yurasov, S. Aushkap

LLC "Laboratory of chromatographic systems"

Background-aim

Vitamins B play an important role in the functioning of the cardiovascular system. So, thiamine promotes the biosynthesis of actin and myosin involved in myocardial contraction, accelerates the physiological myocardial hypertrophy in myocardial infarction. The value of vitamin B6 in the diet of patients with coronary heart disease is associated with its participation in the conversion of amino acids, glycogen breakdown, and fatty acid metabolism. In cardiovascular diseases, vitamin B6 is involved in reducing elevated levels of homocysteine. By affecting the sympathetic nervous system, vitamin B6 has a positive effect on blood pressure. That is why the determination of these vitamins in biological fluids is very important for diagnosis.

Methods

The quantitative determination of B vitamins and their coenzyme forms in whole blood and plasma of patients (more than 600 patients of different age groups) was performed by liquid chromatography with mass-spectrometry detection (LC/MS-MS) using Shimadzu and ABSciex equipment. Complex analysis allowed to simultaneously determine all the analyzed vitamins in various forms in one sample. The multi-stage preparation of biological samples consisted of precipitation, extraction, and concentration of the samples. Based on the data on the quantitative content of vitamins, their reference intervals (RI) were calculated, using the methods of Hoffmann, Bhattacharya and the method of mixed Gaussian models.

Results

The analytical method was validated, which allowed quantitative simultaneous determination of various B vitamins forms. The validated method allowed obtaining reliable data on the quantitative content of B vitamins in the blood. According to the data obtained, RI in the blood were calculated for the vitamins and their coenzyme forms. For plasma: vitamin B2 56-97 nmol/l, vitamin B2 4-43 nmol/l, vitamin B3 13-161 nmol/l, vitamin B5 54,5 – 604,4 nmol/l, vitamin B6 11,3-302 nmol/l, vitamin B7 0,025-5,647 nmol/l. For whole blood: vitamin B1 82 – 239 nmol/l, vitamin B2 116 – 393 nmol/l, vitamin B6 3,5 – 80 nmol/l.

Conclusions

The calculated RI allow us to use them to interpret the results of the quantitative content of vitamins and their coenzyme forms in the blood of patients. The data obtained by LC/MS-MS, which is necessary for making the correct diagnosis, especially during conditions caused by cardio-vascular diseases.

doi: 10.1016/j.cca.2022.04.934

W177

Altered neurotransmitter levels in lead exposed children

M. Lingeswaran, P. Mitra, S. Sharma, .. Abhilasha, S. Gangam, P. Sharma

Department of Biochemistry, AIIMS, JODHPUR

Background-aim

Lead is an universal environmental pollutant released in the environment from various sources such as automobile exhaust, paint, battery industries etc. Lead has shown to have deleterious effects on neuronal development and plasticity. Impaired Neurobehavioural and cognitive development have been observed among children with high lead levels. The mechanism underlying these changes are poorly understood. Neurotransmitters such a serotonin and dopamine have shown to play a critical role in cognitive and neurobehavioural development among children. The aim of this study was to evaluate changes in peripheral serotonin levels in association with blood lead among school going children.

Methods

The study included 72 school going children under the age of 15 years. Their lead levels were assessed by atomic absorption spectrophotometry. Based on their blood lead levels (BLL) they were categorised into two groups, high blood lead level (BLL >5 microgram/dl, N $=33$) and low lead level (BLL $<$ 5 microgram/dl, N $=39$). Peripheral serotonin levels were measured by ELISA.

Results

There was significant difference between peripheral serotonin levels between the two groups ($p<0.01$). Serotonin levels were significantly lower in High lead level group when compared to low lead level group.

Conclusions

These findings suggest that the deleterious effects of lead on cognition and neural plasticity could be via altered neurotransmitter levels.

doi: 10.1016/j.cca.2022.04.935

W178

Optimization of chromatographic and sample pretreatment conditions for thiamine determination in human plasma using an internally developed reversed high performance liquid chromatography coupled with fluorescence detector method

Z. Chellouai, S. Chellouai, M.H. Charmat, I. Belhadj, R. Moussaoui

Faculté de Médecine, Université Oran 1, B.P 1510 El Menaouer 31000 - Oran, Algeria

Background-aim

Thiamine (vitamin B1) is a water-soluble vitamin found in yeast, cereal sprouts. It is a nutrient essential for the proper functioning of neurological and cardiovascular system. The aim of this study was to develop and pre-validate a plasma thiamine assay by a reversed high performance liquid chromatography coupled with fluorescence detector (HPLC-fluo) method .

Methods

An HPLC-fluo method has been developed and pre-validated including the optimization of sample pretreatment (temperature , time incubation , pH) and chromatographic conditions (flow rate , injection volume and mobile phase composition) . Perchloric acid was used to precipitate proteins in plasma. Thiamine was derivatized using potassium ferricyanide sodium hydroxyide solution to thiochrome. The HPLC technique used an octadecyl silica (ODS) column, with a mobile phase (phosphate buffer: methanol) in isocratic mode, with a fluorimetric detection (ex/em 367/435 nm). During the pre-validation phase, selectivity , specificity , the limit of quantification,the limite of detection and the measurment range were assessed .

Results

Sample pretreatment uneeded an incubation time of 20 min and a temperature of 25˚C . The flow rate was 1 ml/min and injection volume was 50 µl , with a mobile phase phosphate buffer (pH 7): methanol (70: 30 v / v) . The method developed exhibits satisfactory selectivity and specificity , the limit of quantification was 3 nmol / l and the limit of detection was 2 nmol / l. The measurement range was between 12.5 and 1500 nmol / l.

Conclusions

In this work, we optimized HPLC and sample pretreatment conditions . The performances of the technique are compatible with a hospital use in terms of analysis time does not exceed six minutes (CV$<$ 5%). The proposed method could ultimately serve as a suitable method for a routine analysis.

doi: 10.1016/j.cca.2022.04.936

W179

Influence of parenteral nutrition on blood manganese concentrations in preterm newborn

M.A. Ballesteros Vizoso [a], B. Badal Cogul [a], P. Argente Del Castillo Rodríguez [a], J.A. Delgado Rodríguez [a], S.I. Vetter [b], M. Tejedor Mestre [b], I. Martín Navas [a], J.M. Bauça [a]

[a] *Department of laboratry medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain*
[b] *Paediatric service, Hospital Universitari Son Espases, Palma de Mallorca, Spain*

Background-aim

Manganese(Mn) is an essential trace element with special requirements during fetal and newborn development, thanks to its vital role in brain and bone development as well as for proper hematologic and immune functions.

Mn crosses the placental barrier. High Mn concentrations have been described in pregnant women and in umbilical cord blood. Individuals under parenteral nutrition may show toxic Mn concentrations due to possible Mn contamination of nutrition constituents. Therefore, the quantification of this trace element may be of great interest for the newborn to assess and prevent potential neurotoxic effects related with its accumulation.

The aim of this study was to assess the influence of parenteral nutrition(PN) on blood Mn levels of preterm infants.

Methods

Prospective observational study performed in 50 preterm newborn admitted in the Intensive Care Unit, with a birth weight <1500g and under PN. Informed consent was obtained from parents. The study was approved by the Ethics Committee of our institution.

Blood was collected into a K2EDTA tube on days 1, 15 and 30 after birth. Manganese measurements were performed in whole blood on the NexION 300X(PerkinElmer) ICP-MS platform using 103Rh as internal standard.

Manganese concentrations were compared in 3 ways using the Mann Whitney's U-test:those with Mn measurements on day 1 vs day 15 ($N=24$), those with measurements on day 1 vs day 30($N=19$) and those with measurements on day 15 vs day 30($N=39$). Statistical significance was set at 0.05.

Results

Mean duration of parenteral nutrition was 8 days. Mean concentration(standard deviation) of Mn was 48.7(24.0)µg/L on day 1, 38.2 (18.9)µg/L on day 15, and 26.0(12.5)µg/L on day 30. Although no differences were seen between day 1 and 15, statistical significance was found between day 1 and 30($p=0.030$) and between day 15 and 30 ($p=0.016$).

Conclusions

Upon birth, preterm infants show variable Mn concentrations in blood, which are higher than those described in adults and also after 30 days of life. The start of PN does not seem to modify their concentrations after 15 days of life.

The decrease in blood Mn levels found on day 30th could be associated with possible removal of PN or also a physiological reduction related to the normal growth of the babies.

doi: 10.1016/j.cca.2022.04.937

W180

Vitamin D levels in snoring children

D. Morell-Garcia [b], C. Ribot [a], M. De La Peña [d], J.A. Peña-Zarza [c], A. Alonso-Fernández [d], P. Giménez [a], J. Piérola [a], A. Barceló [b]

[a] *Health Research Institute of Balearic Islands, Palma de Mallorca, Spain*
[b] *Laboratory Medicine Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[c] *Pediatric Sleep Unit, Son Espases University Hospital, Palma de Mallorca, Spain*
[d] *Respiratory Department, Son Espases University Hospital, Palma de Mallorca, Spain*

Background-aim

Hypovitamosis D is a common health problem. The purpose of this study was to investigate the interrelationship between vitamin D serum levels and: 1) metabolic profiles, 2) sleep parameters, and 3) paternal and maternal vitamin D status in a sample of snoring children referred to a sleep unit.

Methods

A prospective observational study where we selected children and their parents for whom serum Vitamin 25(OH) D had been measured. All children underwent overnight polysomnography evaluation. Measurement of serum 25(OH) D vitamin was performed by an standardized chemiluminescence immunoassay. Serum glucose, lipids, liver enzymes, PTH (parathyroid hormone), insulin and glycated hemoglobin (HbA1C) levels were also measured. Glucose and insulin levels were used to estimate insulin resistance using the homeostasis model assessment (HOMA-IR).

Results

A total of 137 families were included, vitamin D insufficiency (<30 ng/mL) and deficiency (<20 ng/mL) were found in 40.9 % and 17.5% of the children. We found significant associations between serum 25 (OH) D levels in parents and in their children (paternal 25(OH) D $r=0.441$, $p<0.001$; maternal 25(OH) D $r=0.482$, $p<0.001$;). After adjustments for age, BMI z-score and seasonality the odds ratio for the risk of vitamin D insufficiency according to the vitamin D status of parents were: OR(95% CI): paternal insufficiency 15.1 (2.7-35.7), p 0.002; maternal insufficiency 7.2 (2.4-22.0) p 0.001.

An inverse correlation was observed between 25(OH) D levels and HOMA-IR ($r=-0.144$, $p=0.043$) and GGT levels ($r=-0.139$, $p=0.048$) in the total sample of children. However, in stepwise linear multivariate regression, these associations did not remain significant after adjusting for covariates. When the children with vitamin D deficiency were analyzed separately, serum 25(OH) D concentration was found to be associated with apnea-hypopnea index (AHI) ($r=-0.647$, $p=0.009$) and arousal respiratory index ($r=-0.669$, $p=0.034$).

Conclusions

Hypovitaminosis D is common among snoring children. Family patterns of vitamin D could be helpful for the early identification of children at risk of metabolic and sleep disturbances, also when considering strategies to improve vitamin D status.

doi: 10.1016/j.cca.2022.04.938

W181

Evaluation of the interchangeability of blood collection tubes for the measurement of trace elements

J.A. Delgado RodrÍguez, F. Forteza Ferrer, S. GutiÉrrez SÁnchez, J. Robles Bauza, J.M. BauÇa

Department of Laboratory Medicine, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Background-aim

In the light of the tiny proportions of trace elements in the human body, potential contamination sources need to be carefully minimized and prevented, both in the preanalytical and in the analytical phase.

Our objective was to estimate the degree of interference associated with the separating gel and metal probes used in autoanalyzers in the determination of trace elements in serum.

Methods

Thirty individuals referring to our laboratory were selected and, after signing the informed consent, two blood tubes were collected from each of them: normal serum tube (with separating gel, used for general biochemistry tests) and a trace element-specific tube (acid-washed) (Vacutainer, BD).

Copper, selenium and zinc were quantified in the normal serum tube after the performance of biochemistry tests and also in the trace element-specific tube.

Trace element testing was performed on the inductively coupled plasma-mass spectrometer NexION 300X (PerkinElmer) using germanium as internal standard, and calibrated by standard addition.

Mean difference was calculated between paired data (bias,%). Trace element-specific tube was taken as reference. The maximum allowable biases (MAB) were 5.4%, 4.61% and 3.31% for Cu, Se and Zn, respectively, according to our quality specifications.

Analytically significant interferences were considered if calculated bias was greater than MAB.

Results

Result intervals were 816.2-1856.8µg/L for copper, 62.0-110.1µg/L for selenium and 545.0-1050.6µg/L for zinc, covering the clinically relevant range.

The bias associated with the use of the normal serum tube (with separating gel and after introduction of metal probe) was 2.58% for copper, 3.33% for selenium and 14.16% for zinc.

The calculated bias for zinc exceeded MAB, according to the specifications in our laboratory.

Conclusions

Since the interference is not analytically significant for copper and selenium, both serum tubes are interchangeable for their testing. However, the error found in the measurement of zinc advises against the use of the normal serum tube with separating gel.

Although this observation about zinc has already been suggested elsewhere, we interestingly found that the normal serum tube may be used as alternative in the case of copper and selenium when the trace element-specific tube is missing, grossly hemolyzed or sample is insufficient.

doi: 10.1016/j.cca.2022.04.939

W182

Association between nutritional and inflammatory biomarkers in COVID-19 patients with chronic kidney disease

V. Khurana, B. Goswami, S. Kaushik

Department of biochemistry, Maulana azad medical college, New Delhi, India

Background-aim

The world witnessed the emergence of a new disease, COVID-19 caused by SARS–CoV-2 which is now a global pandemic. This proinflammatory disease, establishes a scenario of an acute on chronic condition on the already nutritionally and immunologically compromised CKD patients. Much has been speculated on the possible link between nutrient status and COVID-19 mortality. The aim of our study was to assess the association between nutritional and inflammatory biomarkers in these patients in the Indian population.

Methods

A prospective, cross sectional study was performed on 100 adult known cases of CKD, who were recently diagnosed as COVID-19 positive by rRT-PCR in a designated tertiary-care hospital in India. On the day of admission prior to initiation of any treatment, the serum levels of nutritional and inflammatory biomarkers of the subjects were measured and analysed.

Results

High serum levels of inflammatory biomarkers – IL6 and hsCRP, and low serum levels of nutritional biomarkers – Albumin and Cholesterol, with Prealbumin levels near the lower limit of the normal reference range were found in the study population. A significant negative correlation between inflammatory biomarker IL6 and nutritional biomarkers Vitamin C ($r = -0.21$, $p = 0.03$*) and Prealbumin ($r = -0.30$, $p = 0.00$*), and between inflammatory biomarker hsCRP and nutritional biomarkers Albumin ($r = -0.21$, $p = 0.03$*), Zinc ($r = -0.24$, $p = 0.01$*) and Folate ($r = -0.20$, $p = 0.04$*)was noted.

Conclusions

Since a negative association was found between some of the measured nutritional and inflammatory biomarkers, it is suggested that achieving a good nutritional status might indirectly, slow down the pro-

gression of both CKD and COVID-19 and thus aid in better management and prognosis of both. There is thus a need to reassess the nutrition regimens of patients with kidney diseases in the midst of COVID-19.

doi: 10.1016/j.cca.2022.04.940

W183

Dosage of vitamin D2 and D3 by HPLC in the population of eastern Morocco

E. Sebbar, I. Elmezgueldi, B. Mouhoub, O. Nassiri, A. Amrani, S. Farih, A. Idrissi, S. Belmahi, M. Choukri

Faculty of Medecine and Pharmacy of Oujda

Background-aim

The objective of our study was to determine the vitamin D status in the population of Eastern Morocco.

Methods

We recruited 419 patients (240 women and 179 men) from different localities of Eastern Morocco. Serum determination was performed by high performance liquid chromatography (HPLC) separative method which allows the determination of vitamin D2 and D3.

Results

In our work the mean level of total vitamin D was 18.66 ng/ml. 21.66 ng/ml for men and 16.78 ng/mL for women. For vitamin D3, the mean level was 18.17 ng/ml. 21.48 ng/ml for men and 16.21 ng/mL for women. For vitamin D2, the mean level was 0.5 ng/ml, we note that 82.58% of the participants had undetectable serum levels of vitamin D2. Analysis of vitamin D status by geographic distribution found that all provinces in the Oriental region had high prevalences of hypovitaminosis D.

Conclusions

In our study, the prevalence of hypovitaminosis D in the population of the Oriental region was very high, in the different provinces, and affected both sexes and different age groups. This result indicates the fact that even in a region like ours, which benefits from a strong sunshine all year round and during the summer season, the prevalence of hypovitaminosis D is very high, and even “endemic” in the general population.

doi: 10.1016/j.cca.2022.04.941

Clinica Chimica Acta 530 (2022) S369–S385

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Obesity, Metabolic Syndrome, Diabetes

W184

Levels of homocysteine and high-density lipoprotein cholesterol are associated with increased risk of peripheral artery occlusive disease in patients with coexisting type 2 diabetes mellitus and hypertension

Y. Wang

Department of Neurology, National Taiwan University Hospital, Yun-Lin Branch, R.O.C

Background-aim

The coexistence of type 2 diabetes mellitus (T2DM) and hypertension (HTN) significantly increases the probability of developing peripheral artery occlusive disease (PAOD). Early identifications of those coexisting T2DM and HTN patients at high risk of PAOD with subsequent interventions are important.

Ankle brachial index (ABI) is a simple, noninvasive method for the estimation of PAOD. The aim of the present study was to determine whether there is an association between ABI and cardiovascular risk factors in patients with coexisting T2DM and HTN.

Methods

This was a cross-sectional study with a total of 90 patients with coexisting T2DM and HTN who had no apparent history of cerebro-cardiovascular disease. After careful clinical examinations, and biochemical evaluations, the enrolled subjects underwent ABI examinations by using VP-1000 Automatic Arteriosclerosis Measurement System. We used linear regression models to assess the relationship between cardiovascular risk factors and ABI in studied subjects.

Result

The mean age of patients with coexisting T2DM and HTN was 62.4 ± 8.2 years and the mean duration of diabetes was 12.3 ± 6.7 years. The mean ABI value was 1.09 ± 0.07. We found that values of ABI had statistically significantly correlations with the male sex ($r = 0.343$, $p < 0.001$), smoking status ($r = 0.246$, $p = 0.02$), drinking status ($r = 0.297$, $p = 0.005$), pulse pressure (PP) ($r = -0.241$, $p = 0.02$), red blood cell count (RBC) ($r = 0.253$, $p = 0.02$), hemoglobin (Hb) ($r = 0.274$, $p = 0.009$), hematocrit (Hct) ($r = 0.242$, $p = 0.02$), high-density lipoprotein cholesterol (HDL-c) ($r = 0.189$, $p = 0.08$), folic acid ($r = -0.246$, $p = 0.02$). In a multiple linear regression analysis, HDL-c (95% CI: 0.001-0.003; $p = 0.002$) and log Homocysteine (Hcy) (95% CI: -0.237 to -0.006; $p = 0.04$) were independently associated with levels of ABI in patients with coexisting T2DM and HTN after adjustment of confounding risk factors.

Conclusions

Our results suggest that lower HDL-c and higher Hcy increase the risk of PAOD in asymptomatic patients with coexisting T2DM and HTN.

doi: 10.1016/j.cca.2022.04.943

W185

Phytocompounds from pedalium murex inhibits polyol pathway enzymes: Implication in prevention of microvascular diabetic complications through in-vitro, kinetics and molecular docking studies

D.K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

The secondary diabetic complications are mediated by the activation of polyol pathway, increased intracellular glucose flex in diabetes causes lens tissue damage through osmotic as well as oxidative stress due to aldose reductase (AR) enzyme in polyol pathway. Pedalium murex is used to treat gonorrhea, dysuria, pain, fever, asthma, headache, diarrhea etc.

Methods

In this study, In-vitro and In-silico analysis has been used to screen out the efficacy of phytoconstituents of Pedalium murex for targeting the molecules in polyol pathway. Physico-chemical investigation, phytochemical analysis and aldose reductase inhibitory activity in rat lens aldose reductase (AR) enzyme was investigated. HPTLC fingerprinting analysis was performed to know the phytochemical composition in chloroform: methanol (8:2) solvent system. Healthy adult Wistar albino rats (150-200 g) were taken for aldose reductase inhibitory potential using rat lens AR enzyme. In order to prove the above results, a combination of computational prediction and enzyme kinetics has been also performed through docking simulation for aldose reductase enzyme.

Result

HPTLC fingerprint analysis revealed the presence of eleven prominent phytoconstituents. The study revealed that phytoconstituents interacts with AR of polyol pathway and inhibits the activity of enzymes. Significant aldose reductase inhibitory potential was found against AR enzyme [IC50 value (57.20 ± 3.68) μg/mL], which was further found

to be non-compititive inhibition as per the values of Vmax, Km and Ki. Docking simulation demonstrated negative binding energies which might potentiate tighter binding to the active site of the enzyme and more effective AR inhibitors. Physicochemical and phytochemical test confirmed the presence of various secondary metabolites.

Conclusions

The study suggests the possible role of phytochemical in reducing secondary diabetic complications, which could be useful for the treatment of diabetic cataract in the future. Docking study of pure phytoconstituents was found to be significant compared to commercially available AR inhibitors.

doi: 10.1016/j.cca.2022.04.944

W186

Folic acid and vitamin b12 supplementation in subjects with type 2 diabetes mellitus: A multi-arm randomized controlled clinical trial

D. Bandyopadhyay [a], S. Satapathy [a], B.K. Patro [b], S. Khan [a], S. Naik [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India*
[b] *Department of Community Medicine and Family Medicine, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India*

Background-aim

This study was conducted to investigate and compare the effects of add-on folic acid and vitamin B12 supplementation on glycaemic control, insulin resistance and serum lipid profile in subjects with type 2 diabetes mellitus.

Methods

This study was a randomized, multi-arm, open-label clinical trial. 80 patients with type 2 diabetes and on stable oral antidiabetics were enrolled and 20 patients each were randomly allocated to one of the four groups – Group A: add-on Folic acid (5 mg/day); Group B: add-on Methylcobalamin (500 mcg/day); Group C: add-on Folic acid (5 mg/day) + Methylcobalamin (500 mcg/day) and Group D: Standard oral anti-diabetic drugs. The patients were followed up after 8 weeks.

Result

HbA1c improved significantly in Groups B and C [median changes from baseline – 1.2% (– 13 mmol/mol) and – 1.5% (– 16 mmol/mol) respectively, p values 0.04 and 0.02 respectively] compared to Group D. Groups B and C also showed significant improvements in plasma insulin, insulin resistance and serum adiponectin compared to Group D. Serum homocysteine declined significantly in all three groups with add-on supplementation compared to standard treatment. No improvement in lipid profile was noted in any of the groups.

Conclusions

Add-on supplementation with vitamin B12 improved glycaemic control and insulin resistance in patients with type 2 diabetes mellitus.

doi: 10.1016/j.cca.2022.04.945

W187

Carbohydrate and fat metabolism complications in patients kidney transplant recipients: Impact of calcineurin inhibitors

H. Benghezel [b], M. Sadelaoud [b], W. Toumi [b], A. Boussebat [c], F. Rahmine [c], A. Bougroura [a]

[a] *Nephrology service Batna University Hospital Center*
[b] *Sadelaoud Laboratory Batna, Algeria*
[c] *University batna 2, Algeria*

Background-aim

In Algeria, maintenance immunosuppression in the context of renal transplantation is based mainly on two pharmacological molecules, cyclosporin A and tacrolimus. However, these molecules have a poor tolerance profile, especially in terms of metabolic complications. The objective of this present work was to study the impact of cyclosporine A and tacrolimus on lipid and carbohydrate metabolism in a population grafted at the Batna University Hospital Center.

Methods

We realised a descriptive prospective study which spanned a period from 31/03/2014 to 01/26/2017. A collection of patient data, including pre-transplant assessments (specifically: blood sugar, HbA 1 c, total cholesterol, low lipoprotein (LDL) and high density (HDL) and triglycerides) was performed from the associated medical records to the determination of these biochemical parameters on a Cobas 6000 Roche diagnostics machine module 501 ® at the level of the biochemistry laboratory at the Batna university hospital center. 59 patients (subpopulation A) were selected to study the impact of the two immunosuppressants on carbohydrate metabolism, an identical number (sub-population B) was selected for a similar study on lipid metabolism. Statistical analysis was performed by SPSS IBM Statistics ® version 20, using three hypothesis tests: the Chi-square test, the kruskal wallis test and the wilcoxon test.

Result

A low prevalence of HbA 1c levels ≥6% was found (5.1%), with an optimal glycemic profile. No statistically significant difference was found between cyclosporine A and tacrolimus in terms of impact on blood sugar and HbA 1 c. Hypertriglyceridemia was the most common dyslipidemia (42.4%). The prevalence of hypercholesterolemia (CT), hypertriglyceridemia and low HDLc levels were respectively: 5.1%; 30.5% and 15.2% in the case of cyclosporine A and: 1.7%; 11.9% and 6.8% in the case of tacrolimus.

Conclusions

The effect disruptive to the carbohydrate metabolism of the two immunosuppressants was not enough.

obvious. On the other hand, the disruptive effect on the lipid metabolism of the two immunosuppressants has been found, with clear superiority to cyclosporineA.

doi: 10.1016/j.cca.2022.04.946

W188

Hypogonadism in type 2 diabetic male patients with and without erectile dysfunction

D. Tamrakar [c], D.S. Bhat [a], B.K. Yadav [b], E.T. Tuladhar [b], V.K. Sharma [b]

[a] *Bachelor of Science in Medical Laboratory Technology student, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu*
[b] *Department of Biochemistry, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu*
[c] *Department of Biochemistry, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu*

Background-aim

Type 2 Diabetes Mellitus (T2DM) is the most prevalent chronic metabolic disorder resulting hyperglycemia that leads to serious effects on every body system. Its complications are not only limited to neuropathy and vasculopathy but also instigating an endocrinological changes, which all together have detrimental effects on the patient's quality of life. Lower Serum Testosterone (LST) and increased Sex Hormone Binding Globulin (SHBG) with Erectile Dysfunction (ED) are the distressing complications in T2DM male patients. In the developing country like Nepal, where patients find it embarrassing talking about sexual health, patients usually under report their sexual problems. Owing to these concerns, this study was designed to evaluate hypogonadism among T2DM Nepalese male population visiting the major tertiary health care center of Nepal.

Methods

Male patients visiting medicine department of Tribhuvan University Teaching Hospital with the history of T2DM were enrolled in this cross-sectional study. A pre-structured questionnaire was used for demographic data whereas a validated questionnaire, an abridged 5-item version of the International Index of Erectile Function (IIEF-5), was used to assess ED denoted by score below 22.

Result

A total of 160 patients were enrolled in the study with a mean age of 45 years. The prevalence of ED with varying severity among diabetic male was found to be 76.87% and the frequency of LST was 48.75% at 95% confidence interval. The reduction in total ($p = 0.005$) and free ($p = 0.03$) testosterone were significantly associated with ED among study population. Total testosterone below 300 ng/dL in this study showed statistically significant with the presence of ED ($p = 0.02$) and reduced IIEF5 score ($p = 0.04$). There was a significant positive correlation of IIEF5 Score with total ($r = 0.224$, $p = 0.004$) and free ($r = 0.23$, $p = 0.002$) testosterone while negative correlation was found with other comorbidities like duration of T2DM ($r = -0.416$, $p = 0.000$) and HbA1c ($r = -0.391$, $p = 0.000$).

Conclusions

The higher prevalence of ED among T2DM patients were associated with LST level which were also attributed with other comorbidities like increased duration of illness and higher HbA1c level.

doi: 10.1016/j.cca.2022.04.947

W189

Remnant cholesterol, fasting plasma glucose and other lipid fractions in some obese and non-obese individuals in Nnewi, south east Nigeria

C. Nnamdi, U. Amah, A. Onuegbu, C. Okpara, J. Okwara

DEPARTMENT OF CHEMICAL PATHOLOGY, COLLEGE OF HEALTH SCIENCES, NNAMDI AZIKIWE UNIVERSITY, AWKA, ANAMBRA STATE NIGERIA

Background-aim

Obesity is one of the features of metabolic disorders, and has been shown to be involved in cardiovascular diseases. Remnant cholesterol has been suggested to be a link from obesity to coronary heart diseases, hence a predisposing factor in the development of cardiovascular disease by obese individuals. We compared the level of non-fasting remnant cholesterol, fasting lipid profile and fasting blood sugar in overweight, obese and normal weight participants in Nnewi, a community in south east Nigeria.

Methods

A total of 90 apparently healthy obese, overweight and normal weight participants who met the inclusion criteria were randomly enrolled into the study. They were grouped using their body mass index of 18.5 to 24.9 kg/m2 (normal weight), 25 to 29.9 kg/m2 (overweight) and ≥30 kg/m2 (obese). The parameters were analyzed using standard methods. statistical analysis was performed using statistical package for social science 20.0.

Result

There were no significant differences ($p > 0.05$) in the mean calculated remnant cholesterol (mmol/L) between the obese (0.63 ± 0.19), overweight (0.53 ± 0.27) and normal weight individuals (0.46 ± 0.27). Mean fasting very low density lipoprotein (VLDL) and triglyceride (mmol/L) were only significantly higher ($p < 0.05$) in overweight compared to normal weight individuals (0.71 ± 0.31 vs 0.44 ± 0.23 and 1.56 ± 0.69 vs 0.98 ± 0.50) respectively while plasma glucose (FPG) levels (mmol/L) were however significantly lower in obese subjects when compared with overweight persons (5.36 ± 1.24 vs 5.46 ± 0.17) but significantly higher on comparison with normal weight subjects (5.36 ± 1.24 vs 3.74 ± 0.80). Remnant cholesterol did not correlate with body mass index (BMI) in those that are obese, overweight or normal weight.

Conclusions

The result obtained from this study indicates that remnant cholesterol does not appear to have a relationship with obesity. Further studies could be carried out with a larger population size.

Keywords: remnant cholesterol; Obesity; BMI; cardiovascular disease; lipid profile.

doi: 10.1016/j.cca.2022.04.948

W190

Mody prevalence in a mixed-ancestry population in Bellville, Western Cape, South Africa

S. Raghubeer [b], A.M. Tshivhase [b], S. Davids [b], G. Hon [b], A.E. Zemlin [a], R.T. Erasmus [a], T.E. Matsha [b]

[a] *Department of Pathology, Chemical Pathology Division, National Health Laboratory Service (NHLS) and Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa*
[b] *SAMRC/Cardiometabolic Health Research Unit, Department of Biomedical Sciences, Faculty of Health & Wellness Sciences, Cape Peninsula University of Technology, Bellville Campus, Cape Town, 7530, South Africa*

Background-aim

Maturity onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus (DM) and is thought to account for roughly 1-5% of all DM cases. Often, patients with MODY are misdiagnosed with type 1 or type 2 diabetes, resulting in incorrect treatment. At the time of reporting, no data is available on the prevalence of MODY in populations from Africa. Therefore, our study aimed to investigate and report on the incidence of MODY, specifically mutations in HNF1A (MODY 3) and GCK (MODY 2) in a population from the Western Cape, South Africa.

Methods

Study participants were recruited (1643 in total, 407 males, 1236 females) and underwent anthropometric tests. Thereafter, blood was collected, and real-time PCR was used to screen for polymorphisms in HNF1A (rs1169288, rs115080759, rs140491072, rs137853245, and rs142318174) and GCK (rs4607517) genes.

Result

The participants' mean age was reported as 47.1 ± 15.6 in males and 49.9 ± 15.1 females. Ninety-seven individuals (5.9%) were identified with a specific HNF1A gene polymorphism (rs1169288) and twelve (0.9%) with a GCK polymorphism (rs4607517). In total, 6.6% of the study population expressed MODY mutations. Our results bear similarity to studies conducted globally.

Conclusions

To our knowledge, we are the first to report on MODY incidence in Africa. This research provides the basis for MODY prevalence studies in South Africa, as well as data on non-Caucasian populations.

doi: 10.1016/j.cca.2022.04.949

W191

What can we gain from holidays? The important festival's effect on metabolic syndrome

Z. Yutong [b], W. Danchen [b], G. Xuehui [b], C. Xinqi [b], X. Liangyu [b], L. Songbai [a], Y. Songlin [b], L. Honglei [b], Y. Yicong [b], M. Chaochao [b], X. Tengda [a], Q. Ling [b]

[a] *Department of Health Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, No. 1 Shuaifu Yuan, Dongcheng District, Beijing 100730, China*
[b] *Department of Laboratory Medicine, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing 100730, China*

Background-aim

Largely influenced by an unhealthy lifestyle, the common human indexes for metabolic syndrome change during the traditional Spring Festival in China. In this study, we aimed to evaluate the effect the Spring Festival made on common metabolic indexes.

Methods

This is a retrospective cross-sectional study based on clinical data. In total, 117,650 adults were enrolled from Peking Union Medical College Hospital from 2014 to 2018. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glucose were analysed using a Roche C8000 automatic analyser. The seven working days before the Spring Festival were defined as Group A, while that after the vacation was defined as Group B.

Result

The average age of the participants was 39 ± 12 years; the average body mass index (BMI) was 25.0 ± 3.3 and 22.4 ± 3.3 kg/m2 for males and females, respectively. Common basic and biochemical measurements were significantly lower in females than in males but higher for HDL-C ($p < 0.001$). The distribution of glucose and lipid profiles in February was higher than that in January and March. All indexes were higher (lower for HDL-C) in Group B than that in Group A, and the deviation of average/median weight, BMI, waist, SBP, DBP, TC, TG, HDL-C, LDL-C and glucose was 0.51 kg for female and 0.25 kg/m2, 0.41 cm, 0.40 mmHg, 0.05 mmHg, 0.15 mmol/L, 0.14 mmol/L, -0.03 mmol/L, 0.13 mmol/L and 0.03 mmol/L for the whole people, respectively. Of all indexes, lipid profiles especially in dyslipidaemia group increased more obviously. Accordingly, the prevalence of metabolic syndrome after the Spring Festival rose from 10.0% to 12.7% in male, and from 6.0% to 7.5% in female, but the increase was more obvious for the prevalence of obesity [1.28 (95% CI: 1.01–1.62)] and dyslipidaemia [1.35 (95% CI: 1.15–1.58)]. Furthermore, the distribution of almost all indexes got highest (lowest for HDL-C) in the first and second working day after the Spring Festival vacation, and the values of glucose and lipid profiles were significantly higher in Monday than that in other days.

Conclusions

The lifestyle during China's Spring Festival could affect some human metabolic measurements especially the lipid profiles, which could lead to inappropriate evaluation, especially overdiagnosis. Therefore, it is recommended that, for check-ups, a rest period of 2 days is maintained after important festivals to ensure precise evaluation. Furthermore, it is possible that what is gained during holidays will not revert to pre-holiday levels, which could accelerate metabolic syndrome incidence.

doi: 10.1016/j.cca.2022.04.950

W192

Effect of vitamin d supplementation on glycose homeostasis, islet function and common metabolic indexes in diabetes and prediabetes: A systematic review and meta-analysis

Z. Yutong [b], G. Bo [a], Y. Songlin [b], W. Danchen [b], Q. Ling [b]

[a] *Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, No. 1 Shuaifu Yuan, Dongcheng*

District, Beijing 100730, China
[b] *Department of Laboratory Medicine, Peking Union Medical College Hospital, Peking Union Medical College & Chinese Academy of Medical Science, Beijing 100730, China*

Background-aim

To evaluate the effect of vitamin D supplementation on glycose homeostasis, islet function and common metabolic indexes in diabetes and prediabetes with vitamin D insufficiency or deficiency.

Methods

Literatures were searched via Medline, Embase, Web of science, Cochrane Library, Open Grey and www.controlled-trials.com from the earliest available time to the end of May 2020, together with a historical search. Only RCTs initially designed for vitamin D insufficient or deficient diabetes and prediabetes were included [25(OH)D < 30 ng/mL]. All data were extracted and analyzed based on the Cochrane guideline, and presented according to PRISMA guideline.

Result

In total, 27 articles (n = 1,932) with variable quality were enrolled in this study. Meta-analysis showed that vitamin D supplementation significantly improved fasting blood glucose (FBG), postprandial blood glucose (PPBG) and QUICKI index in diabetes and prediabetes with baseline 25(OH)D levels < 30 ng/mL. The effect size was -3.36 (-5.83, -0.89), -14.72 (-28.15, -1.29) and 0.02 (0.01, 0.03), respectively. With low heterogeneity, higher percentage regressing from prediabetes to normal glucose status [1.60 (1.19, 2.17), p = 0.002, n = 564], and lower percentage progressing from prediabetes to diabetes [0.68 (0.36, 1.27), p = 0.23, n = 569] were found in vitamin D supplementation group. The positive effects of vitamin D supplementation on body mass index (BMI), waist, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-) and C-reactive protein (CRP) were also recognized in this study.

Conclusions

Modest improvements of vitamin D supplementation on short-time glycose homeostasis, insulin sensitivity and disease development in diabetes and prediabetes with vitamin D insufficiency or deficiency were recognized in this study, but more studies need to be conducted in this future to support the clinical application.

doi: 10.1016/j.cca.2022.04.951

W193

Feasibility of community-based health education intervention to control non-communication diseases in Bangladesh: An implementation research

T.C. Nath [c], S. Islam [a], K. Nag [b], S. Das [c]

[a] *International Centre for Diarrhoeal Disease Research, Bangladesh*
[b] *Shaheed Suhrawardy Medical College, Bangladesh*
[c] *Sylhet Agricultural University, Bangladesh*

Background-aim

Bangladesh is experiencing an epidemiological transition from communicable diseases to non-communicable diseases (NCDs) and is assigned as one of the leading causes of death among adults. To best deliver the NCDs control intervention and addressing the compliance of a wider target population, community-based health education (CBHE) intervention was adopted as an affordable, potential, and cost-effective strategy. The study aimed to assess the feasibility and barriers of CBHE intervention to reduce NCD risk factors among the selected rural population of Bangladesh.

Methods

A mixed-method study, utilizing PRISM (Practical Robust Implementation Sustainability Model) framework, was conducted between February 2018 to January 2019 in Dhaka and Sylhet districts of Bangladesh. A total of 320 adult community residents selected from four different areas were divided into intervention and control groups. Four focus group discussions (FGDs) adults and four in-depth interviews (IDIs) with health workers were also conducted. Both thematic and descriptive statistical approach was used for analyzing data.

Result

Quantitative findings revealed that CBHE Intervention had a significant role (P = 0.00) to improve the mean knowledge score in the intervention group (3.35) compared to the control group (0.29). NCDs preventive behaviors and NCDs preventive attitude were also significantly increased in the intervention group (P < 0.05) compared to the control group. Some of the significant barriers associated with CBHE integration identified in the qualitative study were budget deficiencies, inadequate training of health workers, and information gaps. In contrast, local political context and positive community attitudes were observed as supportive factors for the CBHE intervention.

Conclusions

In this study, significant increased in both mean knowledge score and behavior changes were demonstrated due to CBHE intervention. Community-based HE is feasible and promising and can serve as a companion tool for reducing NCDs risk factors in the local context of Bangladesh. However, allocation of the adequate budget, as well as coordination and collaboration with research and control, should need to be addressed for the sustainability of the intervention.

doi: 10.1016/j.cca.2022.04.952

W194

Association of insulin resistance with diabetic kidney disease in Nepalese type 2 diabetes at diagnosis

S. Shrestha [a], P.K. Shrestha [b], B.K. Yadav [a], M. Raut [a], U.P. Khanal [c]

[a] *Department of Biochemistry, Institute of Medicine, Maharajgunj, Kathmandu, Nepal*
[b] *Department of Internal Medicine, Institute of Medicine, Maharajgunj, Kathmandu, Nepal*
[c] *Department of Radiology, Institute of Medicine, Maharajgunj, Kathmandu, Nepal*

Background-aim

Insulin resistance (IR) is a central metabolic anomaly in the pathogenesis of Type 2 Diabetes Mellitus(T2DM) and is believed to be related to visceral fat deposits and altered adipose organ signaling. The dysregulated metabolic milieu of IR has now been increasingly associated with

kidney damage in T2DM patients with potential effect on podocyte insulin signaling leading to microalbuminuria and Diabetic Kidney Disease (DKD).This vicious circle encompassing IR along with its metabolic risk factors and loss of kidney function needs to be identified promptly at diagnosis to prevent or delay the occurrence of end stage renal disease (ESRD) among T2DM patients.

This study aims to determine IR using C-peptide modified Homeostatic Model Assessment 2(HOMA2) in newly diagnosed Nepalese T2DM patients at diagnosis and evaluate the association of the calculated IR index with markers of DKD. It also aims to explore other simple and indirect measures to identify undergoing IR responsible for initiating the kidney damage.

Methods

84 newly diagnosed treatment naive T2DM patients fulfilling the inclusion criteria were selected from outpatient department (OPD) visiting Endocrinology unit in Tribhuvan University Teaching Hospital (TUTH). Anthropometric and biochemical measurements were carried out in these patients. HOMA2 was used for assessment of IR and ultrasound (USG) of abdomen was performed to examine fatty liver and fatty pancreas. For comparison, diabetic patients were divided into two groups – one with HOMA2-IR≤1.8(Insulin Sensitive Group (ISG)) and other with HOMA2-IR > 1.8 (Insulin Resistant Group (IRG)). SPSS ver. 21.0 was used to analyze the data. Mean comparison was done by independent t-test. Group comparison for dichotomous variables was done by Chi-square test. Spearman correlation was used to establish the correlation between the variables.

Result

The mean age of T2DM patients at diagnosis was 47.70 ± 11.58 years, which included 42 males and 42 females. Greater magnitude of IR was observed in new T2DM patients with 66.7% (n = 56 with 28 males and 28 females) being insulin resistant and only 33.3% (n = 28 with 14 males and 14 females) being insulin sensitive. Significant mean differences were found between IRG and ISG in terms of Waist Circumference ($p = 0.008$) and Waist to Hip ratio ($p < 0.001$) underlying the increased propensity for South Asians to develop IR owing to greater accumulation of visceral fat.

Regarding biochemical measurements, significant mean differences were noted for unhealthy levels in IRG in post prandial blood glucose ($p = 0.031$), glycosylated hemoglobin (HbA1c) ($p = 0.04$) fasting C-peptide($p < 0.001$), high density lipoprotein (HDL)($p = 0.025$), total cholesterol to HDL ratio(TC/HDL) ($p = 0.009$), triglyceride to HDL ratio (TG/HDL)($p = 0.024$), low density to HDL ratio (LDL/HDL)($p = 0.03$) and albumin to creatinine ratio (ACR) ($p = 0.03$) compared to ISG. These findings can obviously be instrumental in defining a panel of biochemical tests in detecting IR among Nepalese T2DM patients at diagnosis.

IR was also positively correlated with ACR ($r = 0.25$ & $p = 0.021$) with statistically significant association ($p = 0.006$). IRG had higher rate of microalbuminuria than ISG. IR was inversely correlated with estimated glomerular filtration rate (eGFR) ($r = -0.22$ & $p = 0.043$) with statistically significant association ($p = 0.034$) which could possibly justify that individuals with IR at diagnosis may be at elevated risk for developing structural changes in the kidney and eventual decline in GFR leading to DKD. However, there was no significant association of diabetic retinopathy (DR) with albuminuria and declining eGFR. In fact, DR was very rare finding in our study at diagnosis of T2DM irrespective of IR or IS suggesting DR to be less related to IR and more related to duration of hyperglycemia.

USG examination of liver and pancreas revealed significant association of fatty liver and fatty pancreas with IR. IRG had higher index of fatty liver (77% versus 23%, $p = 0.001$) and fatty pancreas (84% versus 16%, $p = 0.009$) than ISG.

Conclusions

HOMA2IR can be used as an effective tool to identify undergoing IR responsible for kidney damage in T2DM patients at diagnosis. Easy surrogates such as WC, WHR, TC/HDL, TG/HDL, LDL/HDL and USG for fatty liver and fatty pancreas can also be assessed for IR. As insulin resistant T2DM patients are more prone to develop DKD, measurement of ACR and eGFR to monitor kidney function should be made mandatory in these patients for targeted therapy from diagnosis to prevent or delay ESRD.

doi: 10.1016/j.cca.2022.04.953

W195

Effect of ethanolic extract of gongronema latifolium leaves on malondialdehyde level and antioxidant enzymes activities in tissue samples of streptozotocin induced diabetic male wistar rats

R.A. Analike [a], J.E. Ahaneku [a], G.I. Ahaneku [c], D.L. Ajaghaku [e], S.C. Meludu [b], P.I. Ezeugwunne [b], C.E. Onah [d], E.C. Ogbodo [d]

[a] *Department of Chemical Pathology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[b] *Department of Human Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[c] *Department of Internal Medicine, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[d] *Department of Medical Laboratory Science, Faculty of Health Science and Technology, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[e] *Department of Pharmacology, Enugu State University, Enugu, Nigeria*

Background-aim

The study evaluates the Effect of Ethanolic Extract of Gongronema latifolium Leaves (GLE) on Malondialdehyde Level and Anti-oxidant Enzymes in Tissue samples of Streptozotocin (STZ) induced Diabetic Male Wistar Rats.

Methods

Forty (40) Male Wistar Rats weighing between 100-138g were used for the study. The rats were divided into five groups of eight animals in each. Animals in group one were not induced while those in groups two to five were induced diabetes with single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight. Group one (Normal control) and two (Diabetic control) were fed with rat chow and Water for eight weeks. Groups three, four and five received oral gavage of 200mg/kgbw/day, 400mg/kgbw/day ethanol extract of GLE and100mg/kgbw/day Metformin respectively, and Rat Chow and Water for eight weeks. After Eight weeks, the rats were sacrificed and the Heart, liver and kidney were dissected out. The tissues were rinsed immediately with 0.9% ice-cold normal saline to remove blood and 0.5g of each was homogenized in 5ml of phosphate buffered saline (PBS) (pH 7.4) using a Mortar and Pestle homogenizer and the homogenate was centrifuged using TGL-20M Ultra Refrigerated Centrifuge at 12,000g for 20minutes at 4oC to get the post mitochondrial supernatant. The supernatants were used for the estimation of Malondialdehyde,

Total Antioxidant Capacity, Glutathione Peroxidase, Catalase and Superoxide Dismutase using standard Laboratory Methods.

RESULT

Result showed that the Liver, kidney and Heart Malondialdehyde (nmol/ml) were significantly increased while the Total antioxidant Capacity (mol/l), Glutathione Peroxidase (U/mL), Catalase (kU/L) and Superoxide Dismutase (U/ml) were significantly reduced in Group two compared with Groups one, three, four and five ($P < 0.05$).

Conclusions

Ethanolic Extract of Gongronema latifolium Leaves significantly reduced the liver, kidney and heart Malondialdehyde concentration and increased the antioxidant enzymes activities in the treated groups.

doi: W195

Effect of ethanolic extract of gongronema latifolium leaves on malondialdehyde level and antioxidant enzymes activities in tissue samples of streptozotocin induced diabetic male wistar rats

W196

Weight management and perceived body weight and shape in an urban South African population

T. Matsha [a], G.M. Hon [a], S. Davids [a], R. Erasmus [b]

[a] *Cape Peninsula University of Technology*
[b] *Stellenbosch University*

Background-aim

Misperception of own body image is wide-spread, and might basically prevent any corrective measures, either from the affected individual, or from any intervention programs.

Methods

A cross-sectional study involving 1889 adults aged 20 years or older participating in the Vascular Health and Metabolic study (VMH) was undertaken. Participants were required to estimate their weight prior to anthropometric (weight, height, waist and hip circumferences) measurements and the Stunkard figure rating scale (FRS) was used for participants to select a silhouette that closely resembled them. Metabolic syndrome (MetS) components, blood pressure, fasting blood glucose, high density lipoprotein and triglycerides measurement were performed on all participants. Metabolic syndrome (MetS) classification was adapted from Joint Interim Statement (JIS).

Result

The mean ± SD age was 47.7 ± 15.8 years for the men ($n = 452$) and 50.3 ± 14.9 years for the women ($n = 1437$) $p = 0.0014$. Approximately 20% overweight participants estimated their weight correctly whilst over 40% obese individuals underestimated their weight and this was exaggerated in obese men where only 9.5% estimated their weight correctly compared to 26.9% women. Only ≤30.6% overweight or obese participants were engaged in a weight loss programme although 90% of obese and over 65% overweight individuals desired to be thinner. In a logistic regression adjusted for age, sex and MetS components, only the waist circumference was associated with the likelihood for weight loss management, odds ratio (95% confidence interval), 5.7 (3.8-8.4), $p = < 0.0001$).

Conclusions

Our data shows that both men and women underestimate their weight and this has a bearing on participation in a weight loss programme. Furthermore, correct realisation of overweight or obesity as shown by increased waist circumference is associated with a high likelihood to engage in weight reduction programme. We therefore recommend that in health facilities for obesity management, a figure represent the individual weight should be included.

doi: 10.1016/j.cca.2022.04.955

W197

Antidiabetic activities of peptides isolated from soft – shelled turtle yolk hydrolysates

T.P.N. Nong [b], J.L. Hsu [a]

[a] *Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan*
[b] *Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 912, Taiwan*

Background-aim

Bioactive compounds present in food could potentially have beneficial effects on human health. In traditional Chinese medicine, Chinese soft-shelled turtle (SST) (Pelodiscus sinensis) possesses many health-function properties. The aim of the present study is to screen the inhibitory capacity of peptides released from soft-shelled turtle yolk (SSTY) proteins against dipeptidyl peptidase IV (DPP-IV); an enzyme known to deactivate incretins, hormones involved in insulin secretion. Therefore, the inhibition of DPP-IV activity is seen as a promising treatment method in type 2 diabetes.

Methods

Soft-shelled turtle yolk hydrolysates were generated by using gastrointestinal enzyme digestion. Peptide fractions of molecular weight lower than 3 kDa were further subjected to the reversed phased high-performance liquid chromatography (RP-HPLC) and strong cation exchange (SCX) chromatography. Then, the inhibitory activities of resulting fractions were examined using DPP IV assay. The bioactive peptides from SSTY were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled with database-assisted peptide sequencing.

Result

Their DPP IV half maximal inhibition concentration (IC50) of SSTY hydrolysate ranged 1.017 ± 0.0356 mg/ml. The peptide sequences were determined as LPLF, simultaneously found in the active RP-HPLC and SCX fractions. The synthesis peptides showed dose-dependent inhibition effects on DPP IV with IC50 value 496.3 ± 111.21 μM. Additionally, preincubation with DPP IV revealed that LPLF is a substrate of DPP IV and its interaction towards DPP IV was proposed using molecular docking.

CONCLUSIONS

To current study suggests that LPLF has potential as suitable candidates for investigations as natural and multifunctional substances against type 2 diabetes.

doi: 10.1016/j.cca.2022.04.956

W198

Effects of ethanolic extract of gongronema latifolium leaves on renal function of streptozotocin induced diabetic male wistar rats

R.A. Analike [b,], J.E. Ahaneku [c], G.I. Ahaneku [e], S.C. Meludu [d], D.L. Ajaghaku [f], O.B. Onyema-Iloh [a], C.M. Njoku [c]

[a] *Department of Chemical Pathology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria*
[b] *Department of Chemical Pathology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[c] *Department of Chemical Pathology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[d] *Department of Human Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[e] *Department of Internal Medicine, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria*
[f] *Department of Pharmacology, Enugu State University, Enugu, Nigeria*

Background-aim

The study evaluates the effects of ethanolic extract of Gongronema latifolium leaves (GLE) on renal function of Streptozotocin (STZ) induced Diabetic male wistar rats.

Methods

Forty (40) Male Wistar Rats weighing between 100-138g were used for the study. The rats were divided into five groups of eight animals in each. Animals in group one were not induced while those in groups two to five were induced diabetes with single intraperitoneal injection of streptozotocin (STZ) at a dose of 65 mg/kg body weight. Group one (Normal control) and two (Diabetic control) were fed with rat chow and Water for eight weeks. Groups three, four and five received oral gavage of 200mg/kgbw/day, 400mg/kgbw/day ethanol extract of GLE and100mg/kgbw/day Metformin respectively, and Rat Chow and Water for eight weeks. Blood samples were collected from the animals after overnight fasting through retro orbital puncture before treatment, four weeks and eight weeks following treatment. 3.5ml of blood was collected by retro-orbital puncture method, 0.5ml was transferred into fluoride oxalate sample containers, 3ml was transferred into plain sample containers. The plasma and serum were used for the estimation of fasting Plasma glucose and serum sodium, potassium, chloride, bicarbonate, urea, creatinine, uric acid respectively using standard Laboratory methods.

Result

Result showed that at four weeks following treatment, there was significant reduction in serum sodium (mmol/l), potassium, chloride (mmol/l) and bicarbonate (mmol/l) levels with significant increase in the fasting plasma glucose (mmol/l), urea (mmol/l) and creatinine (mmol/l) of the animals in the diabetic control group, groups three, four and five compared with normal control ($P < 0.05$). At eight weeks following treatment, there was no significant difference in the serum sodium, potassium, chloride, bicarbonate, creatinine and fasting plasma glucose levels of group four compared with the normal control. There was no significant difference in the serum uric acid (μmol/l) levels of the treated and untreated groups ($P > 0.05$).

Conclusions

Ethanolic Extract of Gongronema latifolium have no toxic effect on the kidney and possess the potential to reverse streptozotocin induced renal parameter derangement and can compete with Metformin in the management if diabetes.

doi: 10.1016/j.cca.2022.04.957

W199

Association of cardiometabolic risk biomarkers with waist-height index in children and adolescents from San Luis Potosí, México

J.M. Vargas-Morales [a], F.S. Boer-Pérez [a], P.E. Cossío-Torres [b], M.G. De La Cruz-Maldonado [c], P. Hernandez-Morales [a], A. Rodríguez-Rodríguez [a], B.B. Jurado-Manzano [a]

[a] *Facultad de Ciencias Químicas, Universidad Autónoma de San Luís Potosí, México*
[b] *Facultad de Medicina, Universidad Autónoma de San Luis Potosí, México*
[c] *Laboratorio Clínico de la Cruz, Matehuala, San Luis Potosí, México*

Background-aim

Currently obesity represents a public health problem in México and the world; in addition, the frequency of obesity in children has increased. The diagnosis of obesity is determined with the body mass index (BMI), but there are other anthropometric indexes such as the waist-height index (WHI) and there are reports that present advantages over BMI in the diagnosis of obesity; the cut-off value for diagnosis of obesity in children is greater than or equal to 0.5. The objective of the study is to evaluate the association between cardiometabolic risk biomarkers and WHI in children and adolescents from San Luis Potosí, México.

Methods

The study was cross-sectional, the Spearman correlation test for WHI with glucose and lipids was performed; of the associated variables, simple linear regression was performed using the SPSS statistics® 20 software.

Result

952 children and adolescents from 3 to 20 years old participated; a median of 0.46 was obtained for the value of WHI. A positive and significant association was found between triglycerides ($r = 0.317$, $p < 0.001$), total cholesterol ($r = 0.121$, $p < 0.05$), LDL cholesterol ($r = 0.134$, $p < 0.001$) with WHI. In addition, a negative and significant association between HDL cholesterol ($r = -0.281$, $p < 0.001$) and the WHI.

Conclusions

For each 1 mg/dL increase in triglycerides, LDL cholesterol and total cholesterol, ICT increases by 0.0004, 0.0004 and 0.0003 respectively and for every 1 mg/dL of HDL cholesterol that is increased, the WHI

is reduced by 0.002 in the sample study; and possibly higher cardiometabolic risk during the study period and when they are adults.

doi: 10.1016/j.cca.2022.04.958

W200

Relationship between vitamin d deficiency and nutrient metabolism in obese children

Y. Jiang, Y. Zhang

Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Chengdu, China

Background-aim

This study aimed to investigate the relationship between vitamin D level and nutrient metabolism level in obese children.

Methods

A total of 533 obese children, aged 5–16 years, were enrolled from January 2015 to January 2019. Weight, height, body mass index (BMI) were obtained. All children underwent serum biochemical tests including vitamin D (25(OH)D) level, glucose, lipids, insulin, uric acid, cysteine and were divided into three group: the vitamin D-deficient group(< 20ng/ml), the vitamin D-insufficiency group(20-30ng/ml) and normal group(> 30ng/ml).

Result

The weight, BMI, fasting plasma glucose(FPG), fasting insulin, C-peptide, homeostasis model assessment insulin resistance index(HOMA-IR) and apolipoprotein(Apo) A1 level in vitamin D-deficiency group (n = 220) were significantly higher than those in vitamin D-insufficiency group(n = 267) and vitamin D normal group(n = 46) ($P < 0.05$). There was no significant difference between vitamin D-insufficiency group and vitamin D normal group ($P > 0.05$). The level of vitamin D was negatively correlated with the concentration of Apo-A1 after adjusting BMI standard deviation scores(BMI-SDS), but not with FPG, fasting insulin and C-peptide.

Conclusions

Vitamin D deficiency can lead to abnormal levels of glucose and insulin in children, and Apo-A1 may play a role in this process.

doi: 10.1016/j.cca.2022.04.959

W201

Inverse correlation between circulating protein 53 (P53) levels and HS-CRP levels in central obesity elderly men

R. Triana [b], T. Yulianti [b], A. Meiliana [b], E. Halimah [a]

[a] *Padjajaran University, Indonesia*
[b] *Prodia Education and Research Institute*

Background-aim

In obesity, there is a concept of low-grade chronic inflammation called meta-inflammation. However, along with aging, the concept of low-grade chronic inflammation is known as inflammaging (inflammation and aging). The mechanism of aging goes along with age, one of which is characterized by cellular senescent, which occurs mostly in adipose tissue. Adipose tissue is the site of accumulation of large cell senescent, in regulation of obesity and aging. Proteins 53 (p53) is marker for cell senescent, which are also known to induce inflammation. However the correlation of circulating p53 and hsCRP in central obese men with inflammaging is unknown yet, so the objective of this study was to determine the correlation of circulating p53 as cell senescent marker and hsCRP in central obese men with inflammaging.

Methods

The study design was an observational study with cross sectional approach. The subjects were 64 men with central obesity (waist circumference > 90 cm), aged ≥ 45 years old, and fulfilled exclusion criteria. Subjects were divided into 2 age groups, those are middle age group: 45-59 years old (50.7%) and elderly group:≥ 60 years old (49,3%). Serum circulating Protein 53 (p53) were quantified by ELISA principles. Serum hsCRP were quantified by Immulite 2000. All assays were performed according to the manufacture instruction. Statistical analysis was performed with SPSS for windows ver 24. Significance value were define as alpha level < 0.05 based on two-tailed tests.

Result

In this study it was found that the mean waist circumference in elderly was higher in elderly group (103.11 ± 7.72 cm) compared to middle-aged group (102.45 ± 8.18 cm). The mean serum level of hsCRP was significantly higher ($p = 0.035$) in middle-aged group (2.82 ± 1.80 mg/mL), compared to elderly group (2.00 ± 1.40 mg/mL). But the mean serum level of circulating p53 was higher in elderly group (1.22 ± 2.80 IU/mL), compared to middle-aged group (1.21 ± 3.24 IU/mL), however there was no significant difference. To see the interaction between circulating p53 and inflammatory variable, Spearman correlation test was performed. The correlation result showed circulating p53 correlated with hsCRP in elderly with $r = -0.414$ and $p = 0.011$. Meanwhile there was no correlation shown in the middle age group ($r = -0.127$, $p = 0.449$).

Conclusions

This study provides new evidence that the correlation between hsCRP and p53 in circulation is inversely correlated, whereas in cells based on previous studies positively correlated.

doi: 10.1016/j.cca.2022.04.960

W202

Oral glucose tolerance testing for diagnosis of gestational diabetes mellitus in a poor resource setting: Associated factors, challenges and opportunities

L. Imoh, J. Istifanus, J. Asieba, E. Ella, N. Yersib, Z. Muhammad, J. Gungrit

Department of Chemical Pathology, University of Jos, Jos, Nigeria

Background-aim

Evidence of rising incidence of Gestational Diabetes Mellitus (GDM) is well documented and early detection and management of this condition holds the key to reduction of adverse outcomes and preventing

intergenerational transmission of Non-communicable Diseases. We examined the use of the recommended Oral Glucose Tolerance test (OGTT) in screening for GDM in a poor-resource setting to determine the related factors, challenges and opportunities.

Methods

This cross-sectional study involved 122 first line antenatal healthcare providers (AHPs- Doctors, Nurses and community Health) from 60 health care facilities in Jos North Local Government Area, Plateau State Nigeria. The study involved administration of a semi-structured self-administered questionnaire on screening practice for GDM to eligible AHPs.

Result

The mean age of the AHPs was 35.7 + 8.5years and 56 (45.9%) were males. Most (59; 48.4%) worked in private health facilities and 45.9% provided primary health care, 41 (33.6%) were doctors. Only 30 (32.8%) of AHPs use OGTT for diagnosing GDM while majority (99, 78.7%) use either Fasting glucose (FBG) or Random Glucose (RBG). AHPs with good Knowledge of GDM ($p = 0.001$), in Faith-based or Government institutions ($P = 0.001$) and tertiary institutions ($p < 0.001$) were more likely to use OGTT for diagnosing GDM. Doctors compared to other category of AHPs were more likely to use OGTT ($P < 0.001$). AHPs in facilities with availability of automated glucose analyzer were more likely to use OGTT ($P = 0.011$), however AHPs in facilities where dipstick is used for glucose testing were less likely to use OGTT for diagnosing GDM, ($p < 0.005$). Only "working in a tertiary care institution" was an independent predictor for the use of OGTT for diagnosing GDM after multivariate analysis.

Conclusions

Screening for GDM with the recommended OGTT is not widespread and this is related to poor level of knowledge on GDM by AHPs, lower cadre of health care and unavailability of automated glucose analyzer. The prevalent use of fasting and random glucose has implication for increased risk of missed diagnosis of GDM. Increased access to appropriate glucose testing methods and updating knowledge of GDM screening practices among AHPs is crucial.

doi: 10.1016/j.cca.2022.04.961

W203

Melatonin correlate with insulin resistence in women with metabolic syndrome

V. Peneva [b], D. Terzieva [b], M. Mitkov [c], Y. Ronchev [a]

[a] *Clinical Laboratory, University Hospital "Kaspela", Faculty of Medicine, Medical University-Plovdiv, Republic of Bulgaria*
[b] *Department of Clinical Laboratory, Faculty of Pharmacology, Medical University, Plovdiv, Bulgaria*
[c] *Second Department of Internal Medicine, Endocrinology section, Faculty of Medicine, Medical University, Plovdiv, Bulgaria*

Background-aim

Melatonin is a neurohormone mainly produced at night by the pineal gland. The role of melatonin in control body metabolism and insulin release has been controversial. Very different results have been expounded on the relationship between melatonin and metabolic disturbances, including diabetes mellitus, metabolic syndrome (MetS), obesity. The aim of our cross- sectional study was to investigate the association between circadian marker melatonin and MetS parameters in women.

Methods

The study included 32 women with diagnosis MetS according to the consensus criteria of 2009. The relationship between melatonin and parameters of MetS was analyzed. Melatonim samples were taken twice: at night (02:00 h - 03:00 h; light < 10 lux) and in the morning (08:00 h - 09:00 h). Morning venous bloods was drawn after 8 hours of fasting. Glucose, cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels (Olympys AU 480, Beckman Coulter, USA) and insulin levels (Access, Beckman Coulter, USA) were analyzed. Serum melatonin concentrations were measured using ELISA kit (Elabscience Biotechnology Inc, China) and Sirio S microplate reader (SEAC, Italy). Each participant provided informed consent. All data were analyzed by descriptive and nonparametric analysis. Statistical significance was accepted at $P < 0.05$.

Result

The mean age of women with MetS was 32.19 ± 1.8 years. Mean melatonin levels in the morning were 142.76 ± 12.31 pg/ml, and at night – 130.0 ± 10.21 pg/ml, with different variations of the gap between night and day values. Significant negative correlations were found between the night serum melatonin levels and body mass index ($r = -0.335^*$, $P = 0.049$) and serum glucose ($r = -0.430^*$, $P = 0.014$). The difference in the values of night and morning melatonin correlates negatively with serum glucose ($r = -0.382^*$, $P = 0.023$) and positively with insulin levels ($r = -0.480^*$, $P = 0.007$). Morning serum melatonin positively correlates with HOMA-IR ($r = 0.368^*$, $P = 0.045$).

Conclusions

Our investigation suggests that serum melatonin circadian patterns were associated with some of the MetS components. Fluctuations in melatonin secretion may be related to carbohydrate metabolism.

doi: 10.1016/j.cca.2022.04.962

W204

Glutathione peroxidase in women with type 2 diabetes mellitus

D. Arabadzhiyska [b], D. Terzieva [b], Y. Ronchev [a]

[a] *Clinical Laboratory, University Hospital "Kaspela", Faculty of Medicine, Medical University-Plovdiv, Republic of Bulgaria*
[b] *Department of Clinical laboratory, Faculty of Pharmacology, Medical University, Plovdiv, Bulgaria*

Background-aim

Glutathione peroxidase (GPx) is an important intracellular enzyme that breaks down hydrogen peroxides (H2O2) to water and lipid peroxides to their corresponding alcohols mainly in the mitochondria and sometimes in the cytosol. The enzyme plays an important role of inhibiting lipid peroxidation process, and therefore protects cells from oxidative stress. Some studies report that oxidative conditions play an important role in the development of metabolic syndrome, obesity, systemic arterial hypertension, atherosclerosis, and type 2 diabetes mellitus

(T2DM).The aim of our study is to evaluate the serum levels of GPx in women with T2DM.

Methods

The study includes 20 women with T2DM and 26 clinically healthy women. The fasting serum glucose, triglycerides, total cholesterol, HDL-cholesterol (Olympus AU 480, Beckman Coulter) and insulin (Access, Beckman Coulter) concentrations were analyzed. Serum human glutathione peroxidase 1 was determined by ELISA kit (BioVendor, Czech Republic). GPx1 concentrations were measured with "Sirio S microplate reader", SEAC, Italy. Collected data were analyzed using SPSS software, version 17.0. Continuous variables were expressed as mean ± standard deviation. P value < 0.05 was considered significant.

Result

The mean age of the two groups differs not statistically (33.70 ± 3.14 yrs vs 30.34 ± 5.76 yrs, P = 0.570). There were no differences in total cholesterol (4.67 ± 0.97 mmol/l vs 4.47 ± 0.75 mmol/l, P > 0.05) and LDL-cholesterol (3.07 ± 0.75 mmol/l vs 2.46 ± 0.68 mmol/l, P = 0.09) between groups. Patients have significantly lower serum HDL-cholesterol (1.09 ± 0.46 mmol/l vs 1.68 ± 0.40 mmol/l, P < 0.0001) and significantly higher triglycerides (1.50 ± 0.69 mmol/l vs 0.70 ± 0.24 mmol/l, P < 0.0001) and serum glucose (6.85 ± 0.58 mmol/l vs 4.97 ± 0.43 mmol/l, P < 0.0001) compared to controls. Our results show that the mean GPx1 level of women with T2DM is 4.58 ± 3.20 ng/ml and of controls is 3.96 ± 2.84 ng/ml. The mean difference ± standard error of GPx between two studied groups is not statistically significant (0.62 ± 1.17 ng/ml; P = 1.000).

Conclusions

Our results showed that no significant difference was found between GPx levels in type 2 diabetes mellitus patients and the control women.

doi: 10.1016/j.cca.2022.04.963

W205

Serum uric acid and lipid profile in healthy and obese Bosnian children - sex and age differences

J. Fočo-Solak [a], A. Čaušević [c], S. Tihić-Kapidžić [a], S. Hasanbegović [b], M. Malenica [c], E. Begović [a]

[a] *Clinical Centre University of Sarajevo, Department for Clinical Biochemistry and Immunology*
[b] *Clinical Centre University of Sarajevo, Pediatric Clinic*
[c] *Faculty of Farmacy, Department of biochemystry and clinical analyses, University of Sarajevo*

Background-aim

Obesity in children represents the global health problem. Numerous studies pointed out the role of serum UA levels as indicator of unhealthy obesity in youth, where serum uric acid level was significantly related to several indices, such as body mass index (BMI), waist circumference and dyslipidemia. The aim of this study was to find out the differences in serum uric acid levels an lipid profile in obese and healthy Bosnian children of different age and sex and to find possible associations between measured parameters.

Methods

The study included prepubertal (1-12 years) and pubertal children (13-18 years), 115 obese and 100 non-obese children as a control group. Body mass index (BMI), serum uric acid (SUA), cholesterol (CHOL), triglycerides (TRIG), HDL-cholesterol (HDL-C) were analyzed by standard procedures.

Result

There was no significant difference in cholesterol values between the groups (p = 0.779) while BMI, SUA, TRIG and HDL-C values showed significant differences (p < 0.001) for all four parameters). BMI, SUA and TRIG were significantly lower in non-obese in comparison to obese children with value of HDL-C being significantly higher in the former group. Gender differences in SUA levels were only observed in obese children (Male: 347.57 + /-95.78 μmol/L vs Female: 301.97 + /-62.42 μmol/L, p = 0.009).Significant age differences in BMI were observed in both obese and nonobese children(p < 0.001).,while in the latter group, age differences manifested at the level of SUA (p = 0.001) and TRIG (p = 0.018). A significant positive correlation was observed between SUA and BMI (Spearman's rho = 0.206, p = 0.028) and SUA and TRIG (rho = 0.346, p < 0.001) in both obese and non-obese children. A significant negative correlation between SUA and HDL-C (rho = -0.284, p < 0.004) was observed in non-obese group only.

Conclusions

Uric acid; BMI and lipid profile in obese children differ from non-obese. Age and gender differences are seen at SUA level in both examined groups. Therefore, SUA, lipid profile and BMI evaluations can be useful tools in prevention of hypertension and obesity associated cardio-vascular morbidities.

doi: 10.1016/j.cca.2022.04.964

W206

Comparison of COBAS immunoassay with HPLC methods among Saudi diabetic population

N. Alfadda [a], K. Alrifdah [a], L. Beth [a], F. Alseraye [a], F. Albloui [a], S. Haider [b], A. Alsughyyir [b], W. Tamimi [b]

[a] *Department of Laboratory medicine, Security Forces Hospital, Riyadh, Saudi Arabia*
[b] *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical city, King Saud Bin Abdulaziz University for Health Sciences, King Abdullah International Research Center, Riyadh, Saudi Arabia*

Background-aim

The prevalence of diabetes mellitus in Saudi Arabia is very high and every year the number of diabetic patients is increasing. This in turn increase the number of blood samples received in any laboratory for testing for blood glucose and glycated hemoglobin A (HbA1c) levels, which require to report accurate and fast results. Measurements of HbA1c level in the blood is crucial in aiding in the management of diabetic patients. In this study we compared and evaluated the Roche Cobas 502 immunoassay analyzer connected to the sample track system for accurate and better turn-around-time (TAT) reports.

Methods

A total of 26 blood samples were collected from diabetic patients. The samples were run on HPLC Tosoh G8 analyzer and simultaneously on the Roche Cobas 502 which is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. The results were compared using the Microsoft excel and EP Evaluator software. The significance of P-value of <0.05 was used.

Result

The mean and standard deviation values for Cobas 502 and Tosoh G8 were found to be (6.3 ± 1.7) and (6.8 ± 1.9) respectively with a significant p-value (<0.00001). The mean results of HbA1c on Cobas 502 were lower than those on Tosoh G8. The correlation coefficient was found to be 0.9881with p-value of (<0.00001), however, positive bias ranging from 0.8% to 16.9% was observed with an average of 6.7% but insignificant p-value ($=0.3794$). The linearity range was found to be 3.5 t0 18.0% and 4.2 to 20.1% for G8 and Cobas 502 respectively. The TAT was calculated as 40 and 400 samples per hour for G8 and Cobas 502 respectively.

Conclusions

Roche Cobas 502 immunoassay analyzer connected to the sample track system was comparable to HPLC G8 analyzer with excellent correlation and faster TAT but insignificant lower values.

doi: 10.1016/j.cca.2022.04.965

W207

Correlations between asymmetric dimethylarginine, osteoprotegerin and albuminuria in longstanding type 1 diabetic patients

G. Chausheva [a], Y. Bocheva [a], S. Shefket [a], Y. Yotov [d], V. Iotova [b], M. Boyadzhieva [e], K. Tsochev [b], N. Usheva [c]

[a] *Department of General Medicine and Clinical Laboratory, Faculty of Medicine, Medical University of Varna, Bulgaria*
[b] *Department of Paediatrics, Faculty of Medicine, Medical University of Varna, Bulgaria, Bulgaria*
[c] *Department of Social Medicine and Health Care Organization, Faculty of Public Health, Medical University of Varna, Bulgaria*
[d] *First Department of Internal Diseases, Faculty of Medicine, Medical University of Varna, Bulgaria*
[e] *Second Department of Internal Diseases, Faculty of Medicine, Medical University of Varna, Bulgaria*

Background-aim

Patients with type 1 diabetes mellitus (T1DM) and albuminuria are at increased cardiovascular risk. The endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine (ADMA) is increased in renal failure. ADMA is considered as a marker of endothelial dysfunction, respectively atherosclerotic changes by modylating arterial resistance and structure in renal and systematic circulation. Osteoprotegerin (OPG) represents a link between bone and vascular metabolism and it is also associated with endothelial dysfunction in diabetes. The aim of the study is to estimate the correlation between OPG, albuminuria and ADMA in longstanding type 1 diabetic patients.

Methods

The current study included 49 type 1 diabetic patients (24 women and 25 men, aged 41.04 +/- 11,06 years, duration of diabetes 29,9 +/- 9,7 years) and a control group of 31 healthy people (19 women and 12 men, aged 40,29 +/- 9,65). The Diabetic subjects were divided into a normoalbuminuric (<30mg/24h), a microalbuminuric (30-300mg/24h) and a macroalbuminuric (>300mg/24h) group according to urinary albumin excretion rate (UAER). ADMA and OPG levels were measured by ELISA method.

Result

ADMA was significantly increased in patients with micro- and macroalbuminuria – 0,67 (0,44-1,15) μmol/l and 1,096 (0,96- 1,38) μmol/l, compared with diabetics with normoalbuminuria -0,65 (0,21-0,99) μmol/l) ,both $P < 0.05$. OPG was elevated in micro- and macroalbuminuria -15,22 (9,31–21,53) pmol/l and 16,73 (11,56–23,51) pmol/l, compared with normoalbuminuric subjects -10.64 (7.66 – 21,26) μmol/l; both $P < 0.01$. Serum ADMA and OPG levels were higher in people with T1DM, compared to healthy controls (0,71 to 0,67 μmol/l and 12,94 to 12,32 pmol/l). ADMA correlated significantly with UAER in diabetic patients ($r=0,59$; $p<0,0001$) and OPG ($r=0,26$; $p=0,06$). The serum OPG level had also a positive correlation with UAER ($r=0,34$; $p=0,01$) in people with diabetes.

Conclusions

ADMA levels correlates positively with UAER in longstanding type 1 diabetic patients. We propose that ADMA synthesis may be increased in T1DM and this may explain the mechanism by which diabetic nephropathy increases cardiovascular risk. Furthermore, the independent associations of OPG with UAER and ADMA, suggest a link between OPG and the progression of the vascular damage.

doi: 10.1016/j.cca.2022.04.966

W208

Association of blood cadmium concentration with dyslipidemia in the general population of Korea

C.M. Park [a], J.E. Lee [a], S.M. Lee [b]

[a] *Department of Laboratory Medicine, Dongnam Institute of Radiological and Medical Sciences, Busan*
[b] *Department of Laboratory Medicine, Pusan National University Yangsan Hospital, Yangsan*

Background-aim

Cadmium (Cd) is a heavy metal that is well known for its harmfulness to the human body and is accumulated through occupational and environmental exposure. Whether blood Cd levels are associated with the increase in the prevalence of dyslipidemia which contributes to the risk of cardiovascular diseases is unclear. We analyzed the associations between blood Cd concentration and the dyslipidemia status in the general population in Korea.

Methods

A cross-sectional study comprising participants ($n=1148$) aged 20 years or older from the 2017 Korea National Health and Nutrition Examination Survey was conducted. Blood Cd, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides levels were measured in the survey, whereas low-density lipoprotein cholesterol (LDL-C) concentrations were calculated by Friedewald's formula. Associations between

the blood Cd concentrations and the presence of dyslipidemia were assessed using confounder adjusted multiple and logistic regressions.

Result

The median concentration of cadmium in the study population was 0.89 g/L (interquatile range: 0.57 to 1.26). The prevalence of dyslipidemia in this study population was 33.1%. The median Cd concentration was significantly elevated in high total cholesterol and LDL-C group than low cholesterol, LDL-C groups ($p < 0.01$). However, Cd levels were not elevated in high triglycerides or low HDL-C group. Elevated levels of blood Cd were associated with age, income, male sex and smoking status ($p < 0.05$). Multiple logistic regression revealed that blood Cd was associated with high LDL-C (odds ratio: 1.44, 95% confidence interval: 1.03 to 2.02, $p = 0.03$) after adjustment for risk factors of dyslipidemia.

Conclusions

The data of our study support the association between elevated blood Cd concentration and dyslipidemia, especially high LDL-C level.

doi: 10.1016/j.cca.2022.04.967

W209

The P21-mediated and senescence-associated hyperglycemic memory in diabetic nephropathy is therapeutically amendable

M.M. Al-Dabet [a], K. Shahzad [b], A. Elwakiel [b], S. Zimmermann [b], B. Isermann [b]

[a] *Faculty of Health Sciences, Department of Medical Laboratories, American University of Madaba (AUM), 11821 Amman, Jordan*
[b] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, 04103 Leipzig, Germany*

Background-aim

Diabetic nephropathy (dNP) is a major chronic microvascular complication among diabetic patients and a leading cause of end stage renal disease (ESRD) worldwide. dNP is characterized by albuminuria. Moreover, In dNP, the tubular compartment undergoes a particular pattern of damage characterized by induction of CDKi (mainly p21) leading to proliferative arrest, tubular hypertrophy and senescence-like phenotype. Chronic senescence-state would hamper elimination of damaged cells. The progression of those complications, despite improvement of hyperglycemia can lead to long-lasting renal effect. This phenomenon, which is referred to as “hyperglycemic memory”, is still poorly understood. Evidence suggests a key regulatory role of epigenetic mechanisms (DNA methylation & histone modifications).

Methods

Two mouse models with established dNP (16 weeks after STZ-induced persistent hyperglycemia or 16 weeks old db/db mice) were used. Blood glucose was reduced for 6 weeks using an SGLT2-inhibitor, mimicking therapy in diabetic patients. Furthermore, we investigated the role of p21in human dNP and its relevance as a potential diagnostic marker for dNP.

Result

Despite a marked reduction of blood glucose using SGLT2 inhibition, albuminuria, histomorphological changes and glucose, the expression of p21, a senescence-associated cyclin-dependent kinase inhibitor, remained elevated. Sustained p21 expression was linked with demethylation of its promoter and reduced DNA methyl transferase (DNMT) activity and expression. A role of miR-148a, identified in-silico as a potential regulator of DNMT1, was confirmed in tubular cells.

Reducing miR-148a in addition to normalizing blood glucose reversed sustained tubular p21 expression, senescence and renal damage in mice with dNP. In human renal biopsies, tubular p21 expression and senescence marker were observed in patients with dNP compared to those without dNP.

We analyzed the presence of p21 in urine of controls and diabetic patients with(out) dNP. dNP patients were recruited from a study in which a subgroup of patients was randomly assigned to a diet to lower blood glucose. Expression of p21 was elevated in the urine of dNP patients and remained high despite reduction of blood glucose levels upon intermittent diet. Conversely, no p21 expression was detected in the urine of healthy controls.

Conclusions

Epigenetically sustained p21 expression and associated senescence contribute to the hyperglycemic memory in dNP. In human, renal induction of p21 is a specific hallmark of dNP, indicating that urinary p21 identify patients with dNP and remains high despite improving of blood glucose. This pathogenic mechanism can be targeted by inhibiting miR-148a.

doi: 10.1016/j.cca.2022.04.968

W210

Biochemical and physiological derangement in subjects with metabolic syndrome and the effect of reduction in central adiposity

N. Dhakal [a], A. Adhikari [b], S. Bhandari [a], B. Gautam [a], S. Shrestha [a]

[a] *Department of Biochemistry, Gandaki Medical College Teaching Hospital and Research Center Pvt.Ltd*
[b] *NRL Pokhara Pvt.Ltd, Pokhara*

Background-aim

Metabolic Syndrome (MS) involves array of adverse biochemical alterations. Reduction in adiposity are speculated to promote health but the results are conflicting. In this study, we aimed to evaluate oxidative stress, inflammation, peripheral neuropathy and vitamin deficiency before and after improvement of central obesity (CO). This study is important to test the predicted hypothesis, in our context. In addition, such follow up study is new in Nepali population.

Methods

A total of 146 individuals with MS were selected according to International Diabetes Federation (IDF) guidelines. We measured hs-CRP (an inflammatory marker), Malondialdehyde (MDA, an oxidative stress marker), Total antioxidant capacity (TAC), Peripheral Neuropathy (PN) and vitamin D before and after reduction in Waist Circumference (WC) which is a measure of Central adiposity. In male the cutoff WC was < 90 cm and < 80 cm in female according to IDF. Oxidative stress was defined as increase in MDA and decrease in TAC. Statistical analysis was done with p value less than 0.05 as statistically significant.

Result

The duration of follow up was between 2 to 6 months where only 107 had significant reduction in WC. Before intervention, most of the subjects were overweight and has high CO in both male (96.2 ± 3.20

cm) and female (88.4 ± 2.80 cm). The burden of OS was significantly higher. Of total subjects, 28.2% had elevated hs-CRP (7.01 ± 2.10 mg/L; desired level <1 mg/L), 13.1% had PN (21.2 ± 2.90 volts; normal <15 volts) and 45% had insufficient vitamin D (21.69 ± 4.60 ng/ml; normal 30 – 100 ng/ml). After intervention, significant reduction of WC occurred in male (93.7 ± 2.60 cm) and female (85.5 ± 3.10 cm), as compared to their previous value. There was a considerable decrease in the level of hs-CRP, OS and PN with improvement in CO. There was highly significant positive correlation of hs-CRP ($r=0.68$; $p<0.001$ in male, $r=0.95$; $p<0.001$ in females) and MDA ($r=0.80$; $p<0.001$ in male, $r=0.78$; $p<0.001$ in female) with WC.

Conclusions

Improvement in CO has positive effect in reducing inflammation, OS and PN which is present in MS. Therefore, controlling visceral adiposity could prevent chronic complications. However, long term prospective studies are required to conform the results.

doi: 10.1016/j.cca.2022.04.969

W211

Diagnostic accuracy of HBA1C test in diagnosis of diabetes and prediabetes in a multiethnic population

F. Wathigo, D. Maina

Aga khan University Hospital

Background-aim

Conventionally, diabetes mellitus (DM) diagnosis has been based on plasma glucose levels. In 2010, HbA1c was approved by the American Diabetes Association (ADA) and World Health Organisation (WHO) for diagnosis of diabetes. However, different studies have shown variations in diagnostic performance of HbA1c. Performing HbA1c has several advantages over serum glucose such as avoiding the need for a fasting sample, minimal biologic variation, a more precise assay, and HbA1c reflects glycaemia over a longer preceding period of time.

In Kenya, an estimated 750,000 people are diabetic with more than 50% estimated to be unaware of their status. The undiagnosed population may benefit from the roll out of HbA1c testing for DM diagnosis due to its favourable performance characteristics. We therefore sought to determine the diagnostic accuracy of HbA1c and its correlation to fasting plasma glucose, as well as determine the optimal cut-off for diagnosis of diabetes and prediabetes in the study population.

Methods

A cross-sectional study conducted at the Aga khan university hospital Nairobi in 2017 involving 155 participants newly suspected to have diabetes referred by clinicians for laboratory testing. The participants underwent fasting blood sugar as per the ADA guidelines, and in addition had HbA1c analysed concurrently. All tests were performed on the Cobas C501 (Roche). Sensitivity and specificity of HbA1c using the set cut-off of 6.5 % (47.5 mmol/mol) were calculated based on the ADA criteria. The optimal cut-off was determined by use of receiver operating characteristic (ROC) curve.

Result

Fifteen study participants (9.6%) were diagnosed with diabetes based on criteria combining fasting plasma glucose and HbA1c while fifty five (35.5%) had prediabetes. HbA1c showed moderate correlation ($R = 0.72$, $P<0.001$) with fasting plasma glucose.

Maximal diagnostic accuracy was obtained with the HbA1c cut-off of ≥6.5% (sensitivity 93.3%, specificity 98.6%).

ConclusionS

HbA1c had a high diagnostic accuracy for DM diagnosis when compared to the combined testing. A limitation of the study was the omission of glucose tolerance tests as most clinicians shun it in favour of combined fasting glucose and HbA1c testing.

doi: 10.1016/j.cca.2022.04.970

W212

Evaluation of the frequent hemoglobin variants assay in Korea using ultran liquid chromatography-tandem mass spectrometry

L. Chihchiao [a], S. Jongdo [a], N. Youngwon [a], L. Kyunghoon [a], S. Sang Hoon [a], S. Junghan [b]

[a] *Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea*
[b] *Department of Laboratory Medicine1, Seoul National University College of Medicine, Seoul, Korea*

Background-aim

The hemoglobin A1c (HbA1c) level is clinically used to diagnose diabetes mellitus (DM) and to monitor glycemic control in patients. Abnormal high or low HbA1c results in some measurements were caused by more than 1,200 hemoglobin (Hb) variants reported in HbVar database. It is very important to identify the frequent Hb variants in a specific region for DM treatment. Then we developed an identification method for six common Hb variants in Korea using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

Methods

Residual specimens with warning flags on routine HPLC HbA1c assays were collected at tertiary hospitals. All samples after preparation steps including protein digestion with Trypsin were analyzed on an LC-30A Nexera UPLC system (Shimadzu Co., Kyoto, Japan) and AB Sciex Triple QuadTM 6500 system (AB Sciex Pte., Ltd., Framingham, MA, USA). Assay optimization was performed using samples confirmed by sequence analysis. The precursor of six common Hb variants: 657.8 m/z for Hb G-Coushatta, 536.3 m/z for Hb Queens, 510.6 m/z for Hb Chad, 689.9 m/z for Hb Yamagata, 612.0 m/z for Hb Fort de France, and 687.0 m/z for Hb Hoshida was used.

Result

Six common Hb variants in Korea were generally identified. Among 114 specimens most common Hb variant in Korea was Hb G-Coushatta (40.4%). And the second most common Hb variant in Korea was Hb Queens (36.0%). The others were Hb Chad, Hb Yamagata, Hb Fort de France, and Hb Hoshida. However, a few specimens were not identified clearly.

Conclusions

Our study confirmed the developed assay for six Hb variants could be quickly identified without using molecular methods in clinical laboratories.

doi: 10.1016/j.cca.2022.04.971

W213

Therapeutic interventions of aegeline for insulin resistance and type 2 diabetes: biological application through scientific research data analysis and molecular mechanism

D.K. Patel [a], K. Patel [b]

[a] *Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India*
[b] *Krishnarpit Institute of Pharmacy, Rewa Rd, Prayagraj, Uttar Pradesh 212111, India*

Background-aim

Aegle marmelos have been used in the traditional and modern medicine for the treatment of numerous health complication of Human being since very ancient time. It has been used in the traditional medicine for the treatment of diabetes and related secondary complications.

Methods

To know the biological potential of aegeline on diabetes and related complication, different scientific databases have been searched and analyzed from the literature sources to collect needed information for aegeline in the present investigation. Pharmacological datas have been searched from various literature sources and collected for aegeline against their pharmacological application and therapeutic importance. However molecular mechanism for the anti-diabetic activity has been also analyzed through various literature databases analysis in the present investigation. Effect of aegeline on insulin sensitivity was also studied through literature data analysis of the relevant research work of the scientific field. All the data's have been analyzed in the better way to get the effective information for the medicinal application of aegeline.

Result

Literature databases analysis of the scientific research have proven the biological potential of Aegle marmelos for the treatment of diabetes, inflammation, ulcer, hyperglycemic, dyslipidemic, diarrhoeal, malaria, cancer and asthma. Literature databases revealed that aegeline is an alkaloidal amide, isolated from the leaves of Aegle marmelos and has already been shown to be responsible for antihyperglycemic as well as antidyslipidemic activity in the validated animal models of type 2 diabetes mellitus. Synthetic mimics of aegeline and its beneficial effect in insulin resistance have been investigated in the literature work and found to showed better effect and also improved insulin sensitivity and glucose tolerance in 8 week HFD fed C57BL6 mice. Scientific study signified the biological importance of ®3AR agonist for the treatment of insulin resistance and Type 2 diabetes.

Conclusions

Present investigation analyzes the therapeutic application of aegeline in the medicine which could be useful for the development of effective medicine in the future.

doi: 10.1016/j.cca.2022.04.972

W214

Comparison of suitable additives for the reliable diagnosis of gestational diabetes

S. Griebenow [b], M. Fokkert [a], S. Abbes [a], R. Slingerland [a]

[a] *Departement of Clinical Chemistry Isala, Zwolle*
[b] *Greiner Bio-One GmbH*

Background-aim

The number of subjects with diabetes has risen all over the world during the last years. Women with gestational diabetes (GD) are at an increased risk of complications during pregnancy and at delivery. The stability of glucose is important to reliably detect GD and prevent maternal and fetal complications. The routine process from blood collection until analysis requires transport times to a lab of up to 48h. The aim of the study was to investigate two suitable additives used in commercially available tubes for glucose measurements in view of inhibition of glycolysis.

Methods

The study was done at ISALA Hospital (Netherlands) using VACUETTE NaF/K3EDTA and VACUETTE FC Mix (citrate, EDTA and NaF) blood collection tubes. Pregnant donors who were healthy (n = 19) or diagnosed with GD by 75g-Oral Glucose Tolerance Test (n = 24) were recruited. Informed consent was given and the study was approved by EC Netherlands. Venous blood was drawn from each donor into eight tubes (four tubes each tube type). One tube of each type was spun directly after blood collection. Plasma was measured immediately after centrifugation to obtain initial values (fasting) using the Hexokinase method on a COBAS 8000 (Roche, repeatability VC 1%, total precision VC 1.7%). All other whole blood specimens were kept at room temperature (RT). For evaluation of glucose stability, the second tube of each type was spun and measured at 2h, the third at 24h and the last at 48h. Statistical evaluation was done by STATISTICA 12.

Result

Evaluation of all clinical results for glucose concentration and any deviations was based on the Allowable Total Error Table (for glucose 10%) by Data Innovations. Performance testing revealed a clinically significant difference between stored whole blood specimens spun and measured after 2h, 24h and 48h to initially spun tubes repeatedly measured at the corresponding time point. The highest deviations were -11.5%; -13.4% and -11.1%, respectively. That deviation is due to the incomplete inhibition of glycolysis by the enolase inhibitor NaF alone. Using the FC Mix tube containing citrate, EDTA and NaF leads to more accurate glucose measurements by preventing the initial significant drop of glucose up to 4-6h.

Conclusions

The FC Mix tube is more suitable for reliable determination of blood glucose, one of the most frequently measured analytes and of primary importance in diagnosis, monitoring and therapy of GD. The stability of glucose in whole blood specimens drawn in FC Mix tube and stored up to 48h at RT was demonstrated to be superior to the tube containing NaF alone.

doi: 10.1016/j.cca.2022.04.973

W215

Peripheral blood microrna 181b-5p and its target gene prediction in obese individuals with insulin resistance

D. Roy [a], P. Purohit [a], A. Modi [a], M. Khokhar [a], R. Shukla [b], P. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Jodhpur*
[b] *Department of Endocrinology & Metabolism, All India Institute of Medical Sciences (AIIMS), Jodhpur*

Background-aim

Insulin resistance (IR) is associated with a spectrum of disease conditions, including type 2 diabetes mellitus (T2DM), obesity, and metabolic syndrome. The role of various microRNA (miRNA) is implicated in the development of IR, among which hsa-miR-181-5p is known to be a player in T2DM and diabetes-related complications. The current study aimed to investigate the expression of hsa-miR-181b-5p in peripheral venous blood of insulin-resistant individuals. We further integrated and categorized its target genes which may contribute to IR, leading to T2DM.

Methods

40 individuals were recruited in this study after informed consent. Anthropometric measurements were taken and fasting blood samples were analyzed for blood glucose (FBS), HbA1c, insulin and lipid profile. The study subjects were equally divided into insulin-resistant and insulin-sensitive groups according to the HOMA-IR cut-off of 2.5. The expression of hsa-miR-181b-5p (with RNU6 as housekeeping gene) by real-time PCR was analyzed using SYBR Green chemistry. Target genes for hsa-miR-181b-5p were predicted using miRWalk3.0, based on a cut-off score of 1 and validation in miRTarBase, miRDB and TargetScan. The predicted genes were run through Gene Ontology Enrichment Analysis to identify and categorize the biological processes and reactome pathways associated with IR. Microsoft Excel was used for data analysis and visualization.

Result

The study groups were significantly different in body-mass index (29.24 ± 5.14 vs 21.83 ± 2.57, $p < 0.001$), FBS (100.45 ± 9.09 vs 90.95 ± 7.49, $p < 0.001$), and insulin levels (22.93 ± 18.91 vs 6.69 ± 2.20, $p = 0.001$). The hsa-miR-181b-5p was 2.17 fold upregulated in the IR group. Fifty (50) highly scored target genes of hsa-miR-181b-5p were significantly involved in various biological processes, including regulation of protein phosphorylation, regulation of cellular response to stress, and intrinsic apoptotic signaling pathway. The significant reactome pathways were- Signaling by NTRK1, Cellular Senescence, and Signaling by Receptor Tyrosine Kinases.

Conclusions

The findings advocate hsa-miR-181b-5p may have a role in the pathogenesis of IR in obese individuals and may represent a novel marker for this condition.

doi: 10.1016/j.cca.2022.04.974

W216

A combinatory approach of clinical and interactomics analysis to understand the role of matrix metalloproteinase 2 in type 2 diabetes mellitus

M. Khokhar [a], P. Purohit [a], S. Tomo [b], A. Gadwal [a], D. Roy [a], N.K. Bajpai [a], G.K. Bohra [a], D. Yadav [a], P. Sharma [a]

[a] *All India Institute of Medical Sciences Jodhpur, Rajasthan, India*
[b] *Santosh Medical College Ghaziabad, India*

Background-aim

Type 2 Diabetes mellitus (T2DM) is known to cause a multitude of chronic complications. Matrix metalloproteinase-2 (MMP2), involved in extracellular matrix degradation and tissue repair processes, plays a key role in the pathogenesis of vascular complications in diabetes. This study constructed an in-silico molecular network, functional enrichment, and gene ontology of MMP2 with the major regulating transcription factors (TF) and common targeting MicroRNA. Further, a cross-sectional study was conducted to assess the expression of circulating MMP2 in clinical samples of patients with T2DM, which was validated by comparing it with similar publicly available datasets.

Methods

Several keywords, including “Type 2 Diabetes mellitus”, “T2DM”, “Blood”, and “Homo sapiens”, were searched to identify the GEO datasets in the NCBI GEO database. Bioinformatics analyses were performed for comparison and identification of common TFs and target MicroRNAs. The cross-sectional study recruited 70 participants (35 T2DM, and 35 age and sex-matched healthy controls). Anthropometric measurements and Biochemical profiles and MMP2 expression were assessed.

Result

Three gene expression profiles datasets (GSE1009, GSE21321, and GSE156993) from the GEO database were analyzed with GEO2R packages. Keeping a fold change cut off of 0.85 and 1.20 and p-value < 0.05, we identified MMP2 being a common gene in all three datasets. Our analysis of common TFs identified downregulation of ATF2, RUNX2, TFAP2A, and upregulation of SRF and NR4A1 in all three datasets. Based on the highest degree and betweenness, we identified hsa-miR-21 as the top-ranked MicroRNA targeting MMP2. When compared to healthy controls, the expression of MMP2 was downregulated in T2DM as documented in various studies viz. 0.62 folds (GSE9006), 0.74 folds (GSE21321) and 0.94 folds (GSE156993). From our study, we demonstrated a 0.67 Fold (T2DM) downregulation of MMP2 in circulating blood cells when compared with the healthy control population.

Conclusions

This combinatory clinical and interactomics study demonstrated MMP2 to be downregulated in T2DM and identified major regulating

TFs (ATF2, RUNX2, TFAP2A, SRF, and NR4A1) and MicroRNA (hsa-miR-21) targeting MMP2.

doi: 10.1016/j.cca.2022.04.975

W217

Postprandial inflammation and metabolic dysfunction in adolescents with obesity and insulin resistance

S. Wilson, V. Higgins, K. Adeli

CALIPER Program, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Department of Laboratory Medicine & Pathobiology, Faculty of Medicine, University of Toronto

Background-aim

Postprandial dyslipidemia is an independent risk factor for cardiovascular disease (CVD), and inflammatory and metabolic markers are implicated in its pathogenesis. We sought to characterize the postprandial inflammatory and metabolic profiles of adolescents with obesity and insulin resistance (IR), and to assess their role in the pathogenesis of postprandial dyslipidemia.

Methods

Adolescents with normal weight (NW; N = 15), obesity and mild IR (N = 20), and obesity and severe IR (N = 10) were recruited. All participants (12- < 19 years) underwent a 6-hour oral fat tolerance test (OFTT) in the hospital investigational research unit. Inflammatory and metabolic markers were assessed via various immunoassays and nuclear magnetic resonance (NMR) spectroscopy, respectively. The three study cohorts were compared for all analytes using a two-way ANOVA.

Result

Among inflammatory profiling, levels of fasting and postprandial interleukin (IL)-6 were significantly elevated in both obese groups compared to the NW response (main effect of group: $P < 0.05$). Levels of serum calprotectin demonstrated a significant interaction between study group and OFTT time-points ($P < 0.005$). Correlational analyses revealed strong (-0.5 > Spearman rho > 0.5) and significant ($P < 0.05$) associations between tumour necrosis factor-⟨, IL-6, and IL-8 and an atherogenic lipid/lipoprotein phenotype and adiposity and IR, while IL-1ß, IL-18, and monokine induced by interferon-© were strongly associated with bile acid species. In metabolomic profiling, branched-chain amino acids (BCAAs) and alanine were significantly elevated and their postprandial response to a high-fat meal was blunted in obese groups, particularly among those with severe IR (group*time interaction $P < 0.005$). Furthermore, BCAAs and alanine were positively ($rho > 0.7$, $P < 0.05$) associated with IR.

Conclusions

Adolescents with obesity and IR exhibit significant fasting and postprandial dysregulation of several inflammatory and metabolic markers integral to lipid metabolism. These data may offer novel subclinical biomarkers for early metabolic and cardiovascular diseases, such as postprandial dyslipidemia, in at-risk adolescents. Future research should seek to determine the predictive capacity of the studied biomarkers for postprandial dyslipidemia and future CVD and Type 2 Diabetes.

doi: 10.1016/j.cca.2022.04.996

Clinica Chimica Acta 530 (2022) S386–S392

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Pediatric Medicine

M268

Clinical manifestation and genetic mutations in two boys of dent disease and one boy of fanconi syndrome

N. Duan, H. Li, C. Huang

Peking University First Hospital

Background-aim

Dent disease is an X-linked form of progressive renal disease characterized by hypercalciuria, low molecular weight (LMW) proteinuria and proximal tubular dysfunction, caused by mutations in CLCN5 (Dent disease 1) or OCRL (Dent disease 2) genes, respectively. Fanconi syndrome is a consequence of decreased water and solute resorption in the proximal tubule of the kidney. The appearance of Fanconi syndrome caused by proximal tubular dysfunction such as Dent disease usually occurs in early stage of the disease.

Methods

The clinical and laboratory data of three boys were analyzed retrospectively. Genomic DNA was extracted from the peripheral blood of the patients and genetic analyses of these cases were performed.

Results

Our three cases were boys of 3-year-old, 10-year-old and 14-year-old. Proteinuria was showed as the first impression in all cases. All three boys presented with LMW proteinuria and elevated urine microalbumin. Case 1 revealed mutation in exon 11 of the CLCN5 gene [NM_001127899; c.1444delG (p.G483Vfs*21)] and was diagnosed as Dent disease 1. Case 2 carried the homozygous deletion mutation in exon 3 and 4 in the OCRL gene NM_000276.3, and this boy was diagnosed as Dent disease 2. Genetic analysis of Case 3 showed a p.R63W mutation in the HNF4A gene (c.187C > Tchr20-43034835*1p.R63W) that is responsible for Fanconi syndrome.

Conclusions

Urine protein electrophoresis should be performed for patients with proteinuria. When patients have LMW proteinuria and/or hypercalciuria, definite diagnosis and identification of Dent disease and Fanconi syndrome requires further genetic analyses.

doi: 10.1016/j.cca.2022.04.977

M269

Elevated serial urinary catecholamine creatinine ratio in a congenital neuroblastoma

L. Martínez Figueras, C. Montero Domínguez, A. Ortiz Temprado, A. Mata Fernández, P. Blanco Soto

Hospital General Universitario Gregorio Marañón

Background-aim

Neuroblastoma (NBL) is the most common solid tumor in children under 1 year of age with an incidence of 10 cases per million children. This tumor is derived from neural crest cells and it can arise anywhere along the neuroectodermal sympathetic nervous chain, especially in the adrenal medulla. NBL is diagnosed using a combination of urinary catecholamine analysis, radiographic imaging and pathology. Congenital NBL is uncommon and it is normally diagnosed during the third trimester. Improvements in prenatal imaging and widespread use of fetal ultrasonography have led to an increased rate of prenatal diagnosis of fetal NBL. Most prenatally diagnosed NBL are adrenal tumors (90%) at favorable stages.

Methods

Urinary normetanephrine (NM), Homovanillic acid (HVA) and Vanillymandelic acid (VMA) were measured by HPLC (Agilent® 1100 series, BIO-RAD®).

Results

A 29-year-old (gravida 3, para 2) was evaluated by routine prenatal ultrasound (US) at 37 weeks gestation, which showed a solid right adrenal mass in the fetus. Fetal Magnetic Resonance Imaging confirmed the US data and showed a tumoral 2.8x2.8x3.6 cm mass. Its appearance was highly suggestive of NBL. A 3360-g male infant was delivered vaginally at 40 + 6 weeks gestation. Postnatal US confirmed the prenatal findings. The biochemical analysis revealed an increase in serial urine Normethanephrine (NM), Homovanillic acid (HVA) and Vanillymandelic acid (VMA) creatinine ratio (Day 5, 6 and 7). One month later mass size was increased to 68x57x76 mm and metastatic evaluation was positive. The infant was treated with ⟨-adrenergic blockers. At 2 months of age the infant underwent excision of the adrenal mass. Postoperative levels of urine HVA and VMA were normal. Pathological examination is still pending.

Conclusions

The prenatal diagnosis of NBL may confer the advantages of diagnosis of the tumor at an early stage as well as offering early therapy. A sub-

stantial part of the diagnosis of NBL hinges on the measurement of urine catecholamines and their metabolites. Their excretion is a sensitive, rapidly and non-invasive indicator of sympathetic hyperfunction. Our results also suggest that urinary catecholamines might be linked to prognosis and tumor progression. However, 24-h urine samples may not always be collected reliably, especially in pediatric patients. Measurements on random untimed urine specimens require a correction of urinary output for creatinine excretion.

doi: 10.1016/j.cca.2022.04.978

M270

Harmonization of total bilirubin measurement for improved diagnosis and management of neonatal jaundice

M. Vaubourdolle [c], A. Cortey [a], V. Delatour [f], C. Poupon [d], M. Rota [g], F. Schmitt [e], A. Mailloux [b]

[a] *Centre National de Référence en Hémobiologie Périnatale (CNRHP), DMU OriGyn, Hôpital Armand Trousseau, AP-HP.Sorbonne Université, Paris, France*
[b] *Chair of SFBC-CNBH-CNRHP « Neonatal bilirubin » WG - Centre National de Référence en Hémobiologie Périnatale (CNRHP), DMU BioGeM, Hôpital Saint Antoine, AP-HP.Sorbonne Université, Paris, France*
[c] *Département de Biochimie, Hormonologie et Suivi Thérapeutique, DMU BioGeM, Hôpital Saint-Antoine, AP-HP.Sorbonne Université, Paris, France*
[d] *Laboratoire de Biologie Médicale, Centre Hospitalier de Gonesse, Gonesse, France*
[e] *Laboratoire de Biologie Médicale, Centre Hospitalier de Lorient, Lorient, France*
[f] *Laboratoire National de métrologie et d'Essais, Paris, France*
[g] *Service de Biochimie, Centre Hospitalier Intercommunal de Créteil, Créteil, France*

Background-aim

In pediatrics, accurate measurement of total serum bilirubin (TSB) is of major importance for reliable diagnosis and appropriate management of neonatal jaundice. However, several studies evidenced poor comparability of results obtained with the different available methods. This situation is partly due to the lack of Reference Materials, especially for high bilirubin concentrations. To progress on this issue, we have designed a national multicenter harmonization study using neonatal clinical specimens and commutable frozen secondary standards assigned with a reference method.

Methods

36 French clinical laboratories were included in the study, using the 10 most popular routine assays. Each lab analyzed in a single run 30 pools prepared from adults and neonates leftovers, our secondary standard and 2 trueness verifiers in triplicate. Expected values were determined in CNRHP lab using secondary standard with reference method assigned value. Results were further analyzed in 2 sub-groups: TSB < 150 μM and TSB > 150 μM. Following analytical acceptance limits were proposed at TSB > 150 μM :for imprecision estimation maximum CV 3.2 % (repeatability), 4.2 % (interlaboratory variation - same technique), 10 % (all techniques) and for accuracy approach 10 % limit bias. These limits take into accounts the clinical guidelines for diagnosis and therapeutic monitoring of neonatal jaundice.

Results

Results show that the 10 tests studied gave good results for imprecision studies in more than 90 % of clinical labs. However, the accuracy study demonstrated the presence of mean bias > 10% for tests from 2 manufacturers, respectively Ortho TBIL (+30 μM) and Roche Cobas (-25 μM). In the latter case, a -10% systematic proportional error was evidenced. We have shown that raw results can be corrected by a virtual recalibration using a single point strategy founded on our primary standard with assigned value. Finally, clinical consequences of these accuracy defects have been explored on a clinical case of neonatal jaundice.

Conclusions

Our study allowed us to increase our knowledge about analytical performances of routinely used bilirubin tests. These data could be used to raise awareness of clinical laboratories about the need for harmonization of neonatal bilirubin assays. In some cases, recalibration of assays with commutable calibrators can improve comparability of the different methods. In all cases, the added value for the clinical lab will be an adequate interpretation of results using published bilirubin nomograms for identification and monitoring of neonatal hyperbilirubinemia.

doi: 10.1016/j.cca.2022.04.979

M271

Bilateral adrenal neuroblastoma in a 10 year old child

A. Mata Fernandez, C. Montero Dominguez, A. Ortiz Temprado, A. Cambra Conejero

Department of Laboratory Medicine, Hospital General Universitario Gregorio Marañon, Madrid, Spain

Background-aim

Neuroblastoma (NBL) is the most common solid tumor in childhood with an incidence of 10 cases per million children and 95% of cases are diagnosed by 7 years of age. This tumor is derived from neural crest cells and it may arise anywhere along the sympathetic ganglia, the most common site is the adrenal medulla (50%). Currently, NBL is diagnosed by analysis of urinary catecholamine metabolites, histopathology and imaging techniques.

Bilateral adrenal NBL are rare with few cases presented in medical literature. Its presentation suggests synchronous multifocal primaries rather than metachronous metastases. Bilateral adrenal or multifocal primary NBL tumors have been described in up to 20% of patients with familial NBL. The majority of bilateral NBL carry a favorable prognosis.

Methods

Urinary methanephrine (MN) normetanephrine (NMN), homovanillic acid (HVA) and vanillymandelic acid (VMA) were measured by High Performance Liquid Chromatography (Agilent® 1100 series, BIO-RAD®). Detection of MYCN gene amplification and 11q deletion were analyzed by fluorescence in situ hybridization (FISH) and DNA ploidy was determinated by Cytoscan high-density (HD) SNP-array.

Results

A 10-year-old male presented with 2-month history of abdominal distension. Physical examination revealed a left hypochondrium mass. The biochemical analysis revealed an increase in urine MN, NMN, HVA and VMA acid creatinine ratio. Computed Tomography, Magnetic Resonance Imaging and 123I/131I-metaiodobenzylguanidine scan showed bilateral large masses (18x12x14cm left and 6x8x6cm right) in the paravertebral region, compressing the lower poles of the kidneys. The MYCN gene was

not amplified, the DNA ploidy was 2 (left) and 3 (right) and no 11q deletion was detected. The child was diagnosed with stage LII (International NBL Risk Group Staging System (INRGSS)). After 3 cycles of chemotherapy, the boy underwent bilateral adrenalectomy. Postoperatively he received hormone replacement and his catecholamine metabolite excretion was negative. The patient is being controlled.

Conclusions

Four major prognostic factors have been identified which contribute to the severity of the disease according to the INRGSS: age at diagnosis, tumor extension, presence of image-defined risk factors and the molecular data. In our case, bilateral NBL was neither associated with familial cases nor MYCN gene amplification nor chromosomal aberrations. It is recommended that individuals with NBL undergo biochemical and radiographic supervision for detection as well as the staging and follow-up of this tumor.

doi: 10.1016/j.cca.2022.04.980

M272

Which formula for calculated osmolality is the best for newborns and infants?

A. Wąsik [a], J. Bugajska [b], J. Berska [b], K. Sztefko [b]

[a] *Department of Clinical Biochemistry, Children's University Hospital, Krakow, Poland*
[b] *Department of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University Medical College, Krakow, Poland*

Background-aim

In small children serum osmolarity is frequently measured to assess water balance disorders. There are two options for determining osmolality in serum: direct measurement by osmometer or calculation using different formulas. The aim of this study was to compare measured osmolality in children with osmolality calculated by five most commonly used formulas.

Methods

114 serum random samples were collected from children (aged from 1 day to 18 years). Concentrations of sodium (Na), glucose (Glu), potassium (K) and urea nitrogen (Urea) were determined by routine dry chemistry analyzer. Plasma osmolality was measured directly using a vapor pressure osmometer (Osm-m) and was also calculated (Osm-c) by five formulas: 1) Osm-c1 = 1.89(Na) + 1.38(K) + 1.08(Glu) + 1.03(Urea); 2) Osm-c2 = 1.86(Na + K) + Glu + Urea + 10; 3) Osm c3 = 1.86(Na + K) + 1,15(Glu) + Urea + 14; 4) Osm-c4 = 1.09[(1.86 (Na) + Glu + Urea)]; and 5) Osm-c5 = 2(Na) + Glu + Urea. Comparison of measured and calculated osmolarity were performed in children less than one month of age (group 1; n = 31); in children aged > one to three months (group 2; n = 16); in children aged > three months to two years (group 3; n = 51) and in children above 2 years (group 4; n = 16). Typical statistics for methods comparison was used (descriptive statistics, Dunnetta test, Pearson's correlation, Bland-Altman analysis).

Results

The mean values of Osm-c obtained for each formula were significantly lower compared to the mean Osm-m in group 1 and 2. There was no significant statistical difference between the mean value of Osm-m and Osm-c for formula two in group 3 and between Osm-m and Osm-c for all analyzed formulas in group 4. Osm-m and Osm-c results correlated only in groups 1 and 4. The coefficients of correlation between the Osm-m and the Osm-c calculated by different formulas were lower in group 1 ($r = 0.45$–$r = 0.53$; $p < 0.05$) than in group 4 ($r = 0.79$–$r = 0.84$; $p < 0.05$). Bland-Altman plots showed much higher confidence intervals for 95% limits of agreement for the differences between Osm-m and Osm-c estimated by formulas for group 1 and 2 compared with group 3 and 4.

Conclusions

Using formulas for calculation of serum osmolarity in not recommended in newborns and infants.

doi: 10.1016/j.cca.2022.04.981

M273

The physiological jaundice in newborn children

N. Serdarevic [b], A. Muminovic [a]

[a] *Faculty of health sciences Sarajevo, University of Sarajevo*
[b] *Institute for clinical biochemistry and imunology, Clinical center University of Sarajevo, Faculty of health sciences*

Background-aim

The neonatal hyperbilirubinemia is a multifactorial disorder encountered during the neonatal period, especially in the first week of life. When the total serum bilirubin (TSB) rises above the 95th percentile for age (high-risk zone) during the first week of life, it will be considered as hyperbilirubinemia. Jaundice attributable to physiological immaturity which usually appears between 24–72 h of age and between 4th and -5th days can be considered as its peak in term neonates and in preterm at 7th day, it disappears by 10–14 days of life.

Methods

In our investigation, we determined serum concentrations of total bilirubin (TSB), indirect (unconjugated) bilirubin and hemoglobin in children with hyperbilirubinemia and control group. We compare obtained concentrations of total bilirubin, and hemoglobin levels in order to determine the significance of the determination in hyperbilirubinemia. The analysis of 100 patients was carried out on the analyzer Vitros 350 of the company Ortho Clinical Diagnostic.

Results

Our study showed that the mean values of examined parameters are significantly higher in the serum of patients with hyperbilirubinemia. The hyperbilirubinemia was treated with a minimal adverse effect with phototherapy. The efficacy of phototherapy depends on surface area exposed to phototherapy: Double surface phototherapy may be more effective than single surface phototherapy The group with neonatal hyperbilirubinemia patients have a value of neonatal bilirubin on the first day was 201.26 (± 59.48) mol/L, the second day 154.16 mol/L, the third day of measurement was 119.04 mol/L. The mean value for the first day of hemoglobin was 17.4 (± 1.70) g/dL, the second day was 16.4g/dL and the third day the mean value was 15.9 g/dL. A statistically significant correlation of neonatal bilirubin of the first day with hemoglobin ($p = 0.031$, $r = -0.306$), neonatal bilirubin of the second

day with hemoglobin (p = 0.029, r = -0.309) and neonatal bilirubin on the third day with hemoglobin (p = 0.429, r = -0114).

Conclusions

The hyperbilirubinemia is more severe in newborns. The reduction of total bilirubin is treatment for prevention of hyperbilirubinemia.

doi: 10.1016/j.cca.2022.04.982

M274

Cobalamin deficiency detected through expanded newborn screening in Spain

I. Sadik [a], R. Yahyaoui [d], C. Benito [c], M. Rodríguez-Espinosa [b], L. Sánchez-Godoy [b]

[a] *Clinical Laboratory. Hospital La Línea de la Concepción. Cádiz, Spain*
[b] *Clinical Laboratory. Málaga Regional University Hospital. Málaga, Spain*
[c] *Department of Genetics. Málaga Regional University Hospital. Málaga, Spain*
[d] *Laboratory of Metabolic Disorders. Málaga Regional University Hospital. Málaga, Spain*

Background-aim

Undiagnosed, infant vitamin B12 deficiency can result in anemia, failure to thrive, developmental regression, and neurological deficits. Maternal vitamin B12 deficiency is the most common cause. Biochemically, vitamin B12 deficiency leads to total homocysteine (tHcy), methylmalonic acid (MMA), and propionylcarnitine (C3) accumulation. Vitamin B12 deficiency is not a primary target of expanded newborn screening (NBS) programs, but methylmalonic and propionic acidemias (C3 and C3/C2) markers may identify vitamin B12-deficient newborns.

Methods

Amino acid and acylcarnitine levels were determined from 368,152 newborns' single dried blood-spot samples between April 2010 and December 2018 via tandem mass spectrometry (MS/MS) and a commercial reagent kit (MassChrom, Chromsystems, Germany). A cutoff point of p99.9 was set for the healthy population (C3 < 3.87 μmol/L, C3/C2 < 0.17). Another sample was requested if there was increased C3 and/or C3/C2. All cases with persistently high levels were studied further, evaluating mother and child's CBC; plasma acylcarnitine, homocysteine, and vitamin B12 levels; and urine organic acids. Mothers were also tested for gastric parietal cells (GPC) and intrinsic factor (IF) serum antibodies.

Results

C3 and/or C3/C2 levels were persistently high in 84 cases. Further biochemical tests showed 69 vitamin B12 deficiencies, 3 inborn errors of vitamin B12 metabolism (1 TCblR defect and 2 TCN1/CUBN defects), and 12 false positives. The C3/C2 ratio (64/69) was a more sensitive marker than C3 (31/69) for detecting vitamin B12 deficiency.

Most newborns were exclusively breastfed at diagnosis (50/69). One mother had had a partial gastrectomy and one mother was a strict vegetarian. 24 cases of probable maternal pernicious anemia were found (anti-GPC titer over 1:80 and/or anti-IF antibody positive). Newborns of mothers with pernicious anemia had a much more severe deficit than other newborns (tHcy: 40.6 μmol/L vs 16.6 μmol, p < 0.0001; urine MMA: 321 mmol/mol Crea vs 68 mmol/mol Crea, p = 0.01).

Conclusions

Identification of newborns with nutritional vitamin B12 deficiency (very frequent in our population: 1:5,335) is an added benefit of NBS programs. Sensitivity of MS/MS for vitamin B12 deficiency in NBS is still unknown, but including the C3/C2 ratio as a primary marker increases a program's sensitivity. If a vitamin B12 deficiency is suspected, mother and infant should be promptly evaluated. Maternal pernicious anemia screening is cost-effective as it identifies the cause in 35% of cases, which are also the most serious cases. NBS programs should consider newborns diagnosed with confirmed vitamin B12 deficiency as true-positive cases.

doi: 10.1016/j.cca.2022.04.983

M275

New approaches for early diagnosis for sepsis and predict antimicrobial resistence in pediatric patients

O. Jordanova, A. Sofijanova, S. Timovska Naunova, M. Kimovska, H. Mangjukovska, T. Voinovska, S. Neskova, A. Janchevska

University Children hospital-Skopje

Background-aim

Sepsis is a complex life-threatening condition with still high morbidity and mortality in pediatric patients in the University Children hospital-Skopje, whose outcome depends on a early diagnosis and treatment. Timely and appropriate antibiotic treatment improves the outcomes of the disease, reduces the length of hospital stay and costs of hospital treatment. The aim of the study was to determine the mostly isolated causative pathogens from blood culture and to predict antimicrobial resistence in pediatric patients with sepsis.

Methods

The study was designed as a prospective epidemiological investigation conducted in the period of one year (from January 2019 to December 2019) and included pediatric patients with proven sepsis hospitalized in University Children's Hospital in Skopje. We used Film array BCID panel for qualitative detection of multiple bacterial and fungal nucleic acids in positive blood culture samples. Medical data records of admitted children with sepsis were analyzed.

Results

The estimated prevalence of positive blood culture in children was 14.76% with female predominance. The highest number of positive isolates were in the Intensive Care Unit (40.5%). The most common microbe isolation from blood culture was Staphylococcus in 49.4%, 65.4% of Staphylococcus were mecA-methicillin resistant. The other microbe isolates were Enterobacteriae in 25.9% of cases, Seratia marcescens 10.8%, Klebsiella pneumoniae in 8.2%, Streptococcus pneumonia in 7.6%, Candina in 5,1% and Acinetobacter baumannii in 2.5%.

Conclusions

Successful treatment of infectious diseases is ensured by the use of effective and safe antimicrobial drugs that are quality assured, used responsibly and accessible to all who need them. Infections with resistant microbes can be serious diseases with increased mortality rate.

doi: 10.1016/j.cca.2022.04.984

M276

Gasometric and hematological chances in use gentamic 20 mg and ampicilin 500 mg by patients in a neonatal intense care

V. Parreira [a], L. Peder [b]

[a] *Academic in Centro Universitário Assis Gurgacz*
[b] *Doctor teacher in Centro Universitário Assis Gurgacz*

Background-aim

Abstract

Background and Objectives: This study aimed to define the main changes in arterial blood gases and hemogram tests in patients who used ampicillin 500 mg associated with gentamicin 20 mg, in a Neonatal Intensive Care Unit of a private hospital in Cascavel, Paraná, in the period from July to October 2020, classifying the acid-basic disorder of blood gases and changes in the blood count. Methods: Using the Devenport diagram, the Winter equation and the Handerson-Hasselbalch equation, the primary disorder and the secondary compensation in arterial blood gas tests were classified. In the hemogram, the alterations were classified according to the literature and laboratory values. Results: From a total of 34 patients analyzed, 27 made the association of aminoglycosides and penicillins, these being represented by gentamicin and ampicillin, respectively. Of those. 11 had arterial blood gases and blood counts collected either on the same day or within a maximum of 3 days between exams. A total of 19 blood gases and 21 blood counts were analyzed. Of the blood gases that had a primary acid disorder, 6 corresponded to mixed acidosis, 5 were metabolic acidosis with adequate respiratory compensation, and 1 metabolic acidosis associated with respiratory alkalosis. Of the alkalosis as the primary disorder, there were 2 mixed alkalosis, 4 respiratory alkalosis with adequate metabolic compensation, and 1 respiratory alkalosis associated with metabolic acidosis. Of the hemograms, 17 had anisocytosis, 12 had macrocytosis, 12 had hyperchromia. Conclusion: The present study suggests that patients who used the association of 500 mg ampicillin and 20 mg gentamicin present changes in blood gas analysis and blood count that relate.

Keywords: Antimicrobials. Arterial Gasometric. Blood Count. Intensive Care Unit.

Methods

The study was conducted in a private hospital located in the municipality of Cascavel - PR. Data collection was performed in July, August and September 2021. The project was approved by the Ethics Committee on Human Research of the Assis Gurgacz Foundation University Center (FAG), under protocol number CAAE: 44966921.0.0000.5219, opinion number 4.779.475, approved on June 14, 2021.

A quantitative descriptive research was carried out, starting with the printing of a report of antibiotic consumption in the neonatal ICU during the period from July to October 2020, showing which antibiotic was released, the quantity, patient and care, provided by the Tasy® health management system. The information was then tabulated in Microsoft Office Excel ® 2013. After the tabulation, the report was compared with the quantity in the patients' electronic prescriptions, and each visit was accessed to confirm the quantity dispensed.

In Tasy®, the "samy" was accessed to access the blood gas and blood count tests of the patients. Only patients who used the combination of ampicillin 500 mg and gentamicin 20 mg were selected. To delimit the study, only patients who had blood gas and CBC either collected on the same day or at a maximum interval of 3 days were selected.

For the interpretation of the CBC, during the use of the antimicrobial, the laboratory reference values for erythrogammogram, leukogram and platelets were considered, scoring possible changes such as thrombocytopenia, anisocytosis, leukocytosis, anemia, neutrophilia, and if possible analyzing the clinical evolution of patients during the therapeutic cycle of the antimicrobial.

For arterial blood gas analysis, the laboratory reference values for Pco2 (partial pressure of co2) and HCO3 - (bicarbonate) were considered, and the results were interpreted using the Davenport diagram, the Handerson-Hasselbalch equation, and the Winter equation, which allow us to assess the primary disturbance and analyze secondary compensation.

Metabolic acidoses as the primary disorder: Expected Pco2 = (1.5* patient's HCO3 - + 8) ± 2, where HCO3 - is given in mEq/L and Pco2 is given in mmHg. The equation returns an expected value for the patient's Pco2. After, the actual value is compared with the expected value, one of the following situations occurs:

The two values are similar: Adequate respiratory compensation.

Actual Pco2 is higher than expected: Associated respiratory acidosis.

Actual Pco2 is lower than expected: associated respiratory alkalosis.

In metabolic alkalosis as the primary disorder for the secondary disorder evaluation, the following will be used: The expected value of the patient's Pco2 is Expected Pco2 = (patient's HCO3 - + 15 ± 2) and follow the same classifications of metabolic acidoses.

The classification of respiratory acidoses as a primary disorder, it is observed that for every 10 that the Pco2 goes up the HCO3 - goes up 4, if the midpoint of the Pco2 is 40 in the laboratory reference value and the patient's was 60, he varied 20 above the midpoint. What would be the expected value of the HCO3 -, transcribing it would be:

10 Pco2 ———————— 4 HCO3

20 Pco2 ———————— X

X= 8 which added to the midpoint of the laboratory 24 reference value of HCO3 - we would have the patient's expected hco3 of 32 ± 2, thus defining the secondary disorder with a classification of:

The two values are similar: Adequate metabolic compensation.

HCO3 - actual is higher than expected: Associated metabolic alkalemia.

HCO3 - actual is lower than expected: associated metabolic acidemia.

For respiratory alkalosis as a primary disorder, at the end of the rule of 3 of the above example, the result will be decreased by the midpoint of the reference values of hCO3, leaving 16 ± 2 for the previous example, following the same classifications to define the secondary disorder.

Results

The association of gentamicin 20 mg and ampicillin 500 mg presented more metabolic or mixed acid disturbances, when compared to those primarily classified as respiratory. No metabolic alkalosis was classified. Suggesting that the association of these drugs benefits the non-retention of Pco2 by the lungs avoiding the classification of respiratory acidosis in the primary disorder, it is also suggested, that it avoids the increase of HCO3 - resulting in metabolic alkalosis in the primary classification of the blood gas analysis, and that despite fluctuations in blood gas analysis, we obtained 1 death from the patients analyzed.

Of the acidoses defined as primary disturbance, of the mixed ones, the first patient with weight of 2819 g and gestational age of 34 weeks and 6 days, had his CBC collected 3 days after the gasometry, and presented macrocytosis, hyperchromia, anisocytosis, leukocytosis, eosinocytosis and monocytosis that evolved in 2 days, with the same alterations plus a lymphocytosis. The second, weighing 2690 g and gestational age of 35 weeks and 2 days, had his CBC collected 2 days before the blood gas analysis and presented erythrocytosis, macrocytosis, hyperchromia, neutrophilia, anisocytosis, and lymphocytosis. The third patient, weighing 4120 g and with a gestational age of 40 weeks and 2 days, had a complete blood count (CBC) collected on the same day as the blood gas analysis and showed macrocytosis, hyperchromia, anisocytosis, leukocytosis, neutrophilia, and thrombocytopenia, and within two days evolved to macrocytic anemia, leukocytosis with left

deviation, thrombocytopenia, toxic granulations 2+, and mild polychromasia.

Of the metabolic acidoses with adequate respiratory compensation, 4 patients were analyzed. The first with a weight of 1975 g and gestational age of 37 weeks and 2 days, had his CBC collected on the same day as the blood gas and presented an anisocytosis, lymphocytosis, and a monocytosis, which evolved in 2 days to a macrocytosis, hyperchromia, anisocytosis, evolving in 4 days to a macrocytosis, hyperchromia, anisocytosis, leukocytosis, eosinocytosis, and lymphocytosis. The second with a weight of 3280 g and gestational age of 39 weeks and 5 days, collected the CBC on the same day as the blood gas and presented with a leukocytosis, macrocytosis and had rod present on blood extension slide. That progressed the next day to anisocytosis and leukocytosis. The third with a weight of 3370 g and gestational age of 39 semans and 5 days, collected blood gas 2 days after the blood gas and had a macrocytosis, hyperchromia and eosinocytosis. The fourth patient will be described as the first patient of the mixed alkaloses, as he had many fluctuations in the basic acid disturbance during therapy.

The single metabolic acidosis with respiratory alkalosis closes 11 of the 12 primary disorders classified as acidemia. The patient with a weight of 1925 g and gestational age of 33 weeks and 2 days had his CBC collected on the same day as the blood gas and showed one erythrocytosis and one leukocytosis.

Of the alkalosis as a primary disorder, 2 patients presented mixed alkalosis as a primary disorder , the first with weight of 1320 g and gestational age of 32 weeks, which by fluctuation in blood gas will be portrayed here by the disorder of his first blood gas, which presented a mixed alkalosis and had the CBC collected on the same day that presented with an erythrocytosis, After 3 days he had metabolic acidosis with adequate respiratory compensation with no CBC on this date and after 37 days he had respiratory alkalosis with adequate metabolic compensation and had his CBC collected on the same day as the blood gas and showed macrocytosis, hyperchromia, anisocytosis, and lymphocytosis. The second, weighing 1915 g and with a gestational age of 35 weeks and 2 days, had his CBC collected on the same day as the blood gas analysis, which had no fluctuations and showed mixed alkalosis. The CBC showed a macrocytosis, hyperchromia, anisocytosis, thrombocytopenia, and a lymphocytosis.

Of the respiratory alkalosis with adequate metabolic compensation, there were 2 patients, where the first, weighing 3600 g and gestational age of 40 weeks and 2 days, presented respiratory alkalosis associated with metabolic acidosis and in the hemogram collected on the same day of the gasometry with an erythropenia, anisocytosis, leukocytosis, neutrophilia and was also observed erythoblasts 3% with a discrete polychromasia. That evolved after 3 days to an erythrocytosis and an anisocytosis with a respiratory alkalosis blood gas with adequate metabolic compensation. The second with weight of 2365 g and gestational age of 35 weeks and 2 days, presented a respiratory alkalosis with adequate metabolic compensation and had the hemogram collected on the same day of the gasometry, presenting an anisocytosis, eosinocytosis and a lymphocytosis that after 3 days presented a leukocytosis maintaining the basic acid disturbance.

In the mixed disorders, the patients that presented mixed acidosis all presented a leukocytosis in the CBC, and one of them evolved to an anemic picture with macrocytosis, left deviation, thrombocytopenia, and toxic granulations, dying after 2 days. Of the mixed and respiratory alkalosis with adequate metabolic compensation, all the patients presented a lymphocytosis.

It is suggested that, the alkaline environments represented by the respiratory alkalosis and mixed alkalosis in the present study, favored the lymphocyte proliferative process and in the mixed and metabolic acidoses the CBCs had in common a leukocytosis. It is important to report that one patient confirms the suggestion, had the blood gas and CBC collected on the same day and showed a leukocytosis and a lymphocytosis on the CBC when the disorder present was a respiratory alkalosis associated with a metabolic acidosis.

Of the 11 patients analyzed, 6 had gestational age less than 37 weeks, categorizing them as premature. Presenting mainly early sepsis. We cannot affirm that the use of antibiotics caused the alterations described in the study, because the patients already presented pathological clinical conditions that alter the analyzed exams.

It is suggested then, that the mixed disorder causes more alterations in the CBC, being the mixed acidosis the one with greater alterations, followed by the alkalosis. Finally, the present study opens discussion for the influence of the basic acid disorder in the alteration of the CBC.

Conclusions

Despite the small group of patients analyzed, the present study described all the changes in the CBC and blood gas analysis during the use of Ampicillin 500 mg and Gentamicin 20 mg, suggesting that the acid-base disorder has an influence on the changes presented in the CBC. The limitation of the research did not allow calculation of the anion gap in a disorder primarily classified as metabolic acidosis. This suggests a metabolic acidosis with elevated anion gap due to the increased positive charges present in the aminoglycosides. It was also not possible to perform the correction of the gap anion for albumin in those patients with gestational age less than 37 weeks.

doi: 10.1016/j.cca.2022.04.985

M277

Transient hyperphosphatasemia of infancy: A case report and implementation of a decision algorithm

D. Rodríguez Cano, M. Muñoz Calero, S. Castañeda Nieto, F. Rodríguez Cantalejo

Hospital Universitario Reina Sofía, Córdoba

Background-aim

Transient hyperphosphatasemia (TH) of infancy is characterized by a notable elevation of serum alkaline phosphatase (ALP) in the absence of detectable liver or bone disease, returning to normal levels within weeks/months. It is a benign disorder, especially found in patients aged 6-24 months. ALP values >4 normal value (NV) are found in 2.8-6.2% of cases according to different studies. ALP values >1000U/L are less common.

Methods

17-month-old patient followed up by Primary Care because of a several days diarrhoeal process, with fever. Hospitalised for observation and perfused due to dehydration. No other clinical data of interest.

Results

Tests requested to laboratory: ALP value of 5312U/L (NV: 46-116U/L). Other liver markers were normal. Creatinine was 0.28mg/dL (NV: 0.7-1.3mg/dL).

Bone markers were amplified. All were normal, except phosphorus 5.4mg/dL (NV: 2.4-5.1mg/dL).

In our healthcare area, the prevalence of ALP >4 NV is 3.53% and ALP >1000 NV is 0.31% in paediatric patients.

Conclusions

Due to significant increment of ALP and no alteration of other liver markers, liver disease was discarded. Owing to the increase in serum

phosphorus and the normality of the rest of the bone markers, TH of infancy was suspected.

Repeating bone markers were recommended within 6-8 weeks, with evidence of normalisation of ALP and phosphorus.

A decision algorithm was generated, in which if ALP values are >4 NV in children under 24 months, bone and liver profiles are automatically implemented. If both are normal, a comment to ALP is included: "possible TH, repeat in 6-8 weeks to check evolution".

Such significant increments of ALP may cause alarm in the applicant doctor. The inclusion of this algorithm avoids the progression of unnecessary tests, as it is a benign pathology, closing the diagnosis in a single act.

doi: 10.1016/j.cca.2022.04.986

M278

Serological antibody response to SARS-COV-2 vaccination in a large cohort of Canadian children, adolescents, and adults

M.K. Bohn, S. Wilson, K. Adeli

The Hospital for Sick Children, Toronto, ON, Canada

Background-aim

Monitoring immune protection post-administration of an mRNA SARS-CoV-2 vaccine is essential to inform public health policy. The potential utility of quantitative antibody assays to indicate risk of breakthrough infection is of interest. Significant evidence gaps exist in our understanding of antibody response in children and adolescents relative to adults post-vaccination. The current study aims to evaluate age-specific differences in antibody response to SARS-CoV-2 vaccination in a large cohort of Canadian children, adolescents, and adults.

Methods

Quantitative serological antibody response following administration of one, two, or three doses of an mRNA SARS-CoV-2 vaccine were evaluated in a prospective cohort of 454 participants (age range: 6-79y, male: 34%, female: 66%). A subset of participants were longitudinally monitored during a five month period (Aug–Dec 2021). A control cohort of individuals with no history of SARS-CoV-2 infection or vaccination were evaluated to assess specificity. Antibody levels were measured using two immunoassays: DiaSorin LIAISON SARS-CoV-2 TrimericS IgG and Abbott AdviseDx SARS-CoV-2 IgG II assays.

Results

Antibody assay sensitivity in study participants post-second and third dose were 96% and 98%, respectively. Antibody titres varied significantly depending on days since vaccine administration. Participants post-third dose reached maximum titres of 25,000 BAU/mL and ten-fold relative increase in longitudinal cohort. A statistically significant difference was observed between pediatric (mean ± SD = 2037 ± 1515) and adult 1444 ± 1277) antibody titres. A specificity of 98% was observed for participants with no history of SARS-CoV-2 infection or vaccination. A strong correlation between titres on the AdviseDx and TrimericS assays were observed (Pearson R: 0.92).

Conclusions

This is the largest evaluation of commercially available quantitative SARS-CoV-2 antibody assays in a cohort of Canadian children, adolescents, and adults. Findings suggest children have higher antibody titres as compared to adults post-administration of an mRNA vaccine. However, significant variation was observed. Future work is needed to relate antibody presence to functional immune response as well as risk of breakthrough infections.

doi: 10.1016/j.cca.2022.04.987

M279

Pediatric reference interval verification for special chemistry, immunoassay, and cancer markers on the Abbott Alinity CI system

M.K. Bohn, S. Wilson, K. Adeli

The Hospital for Sick Children, Toronto, ON, Canada

Background-aim

The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has developed an extensive database of reference intervals (RIs) for several biomarkers on various analytical systems, including special chemistry, immunoassay and cancer markers used to inform clinical decisions. In this study, pediatric RIs were verified for 13 assays on the Abbott Alinity system based on the analysis of samples collected from healthy children and adolescents (birth-18 years) and comparison to comprehensive RIs previously established for Abbott ARCHITECT assays.

Methods

Analytical performance of Alinity chemistry and immunoassays was first assessed through precision, linearity, and method comparison. Subsequently, 100 serum samples from healthy children recruited with informed consent were analyzed for 13 Alinity assays (i.e. cancer antigen 19-9, cancer embryonic antigen, C3, C4, cortisol, C-peptide, DHEA-S, glucose, immunoglobulin A, G, M, lactic acid, total prostate specific antigen). The percentage of test results falling within published CALIPER ARCHITECT reference and confidence limits was determined. Reference intervals were considered verified if ε90% of laboratory test results fell within previously established confidence limits.

Results

All assays demonstrated acceptable performance on the Alinity ci system. Of 13 assays assessed, 12 met the criteria for verification with ε95% of laboratory test results falling within previously established ARCHITECT limits for most assays. Several pediatric reference values were below the limit of detection for cancer markers (i.e. cancer embryonic antigen, cancer antigen 19-9, total prostate specific antigen). Only 54% of pediatric samples fell within the recommended Abbott ARCHITECT for lactic acid, and thus a new Alinity-specific reference interval is needed.

Conclusions

These data demonstrate marked concordance between ARCHITECT and Alinity systems for 13 assays, as well as the robustness of previously established CALIPER RIs in healthy children and adolescents. Expanding the utility of the CALIPER database (www.caliperdatabase.org) to include Alinity assays for special chemistry and cancer markers will assist clinical laboratories using this new platform and contribute to improved clinical decision-making in pediatric populations.

doi: 10.1016/j.cca.2022.04.988

Clinica Chimica Acta 530 (2022) S393

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Personalized Medicine,Pharmacogenetics

W218

Diagnostic pitfalls of sarcoidosis due to angiotensin-converting enzyme (ACE) inhibitor treatment

A.Á. Szabó *, E.E. Enyedi, I.T. Altorjay, Z. Papp, A. Tóth, M. Fagyas

Division of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Background-aim

Sarcoidosis is a granulomatous inflammatory disease, which can lead to serious pulmonary and cardiac complications. Determination of the usually elevated angiotensin-converting enzyme (ACE) activity is important in establishing the diagnosis and in assessing the success of treatment. However, ACE inhibitor drugs (ACEI) can significantly reduce ACE activity, influencing diagnostic and treatment decisions.

The aim of the study was to set up a measurement method that can detect the presence of any ACEI in the sample, and to investigate to what extent the requested ACE activity measurements were influenced by taken medication, and how this affected clinical decision making.

Methods

In this study, the results were analysed of patients who had diagnostic ACE activity measurements between 2014 and 2021 in Debrecen, Hungary. Serum ACE activity was measured in 4-, 35-, 400-fold dilutions using a fluorescent kinetic method.

Results

A total of 853 diagnostic measurements were performed during the study period, of which 30 patients' results were not evaluated due to missing data. In 302 (17%) cases ACEI effect (>80%) could be observed, resulting a significant decrease in serum ACE activity compared to patients not taking ACEI (median [interquartile range], respectively: 4.42 [2.93-6.75] U/L;1.32 [8.79-13.92] U/L; $p<0.01$). Eighty-three percent of patients with results below the reference range (RR) would fall at least within the normal range, while 43% of patients with results in the RR would have a value above RR if they were not taking ACEI. Thus, sarcoidosis in at least 61 patients may not have been detected in time or at all due to ACEI treatment during the study period. Physicians associated low ACE activity with ACEI treatment in only 3 cases.

Conclusions

With the adjusted method, the misleading presence of ACEI can be highlighted to the physician, helping to ensure proper interpretation of results and decision making. This can significantly reduce the time and cost, as well as increase the efficiency of establishing the diagnosis.

Funding

This project was supported by the János Bolyai Research Scholarship (BO/00069/21/5) of the Hungarian Academy of Sciences and by the ÚNKP-21-5-DE-458 New Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

doi: 10.1016/j.cca.2022.04.851

Clinica Chimica Acta 530 (2022) S394–S402

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Quality Assurance, Accreditation

M216

Use of sigma metrics in the analysis of pre-analytical and post-analytical quality indicators in a tertiary care hospital clinical biochemistry laboratory

S.M. Abbas

Father Muller Medical College Hospital, India

Background-aim

Quality indicators (QI's) are fundamental tools for enabling users to quantify the quality of all operational process. Studies have reported that majority of the errors occur in the pre-analytical and post-analytical phases.

Methods

Eleven quality indicators for pre-analytical and post-analytical phases were identified and analyzed for a period of one year in Clinical Biochemistry Laboratory, as specified by International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine Working Group on Laboratory Errors and Patient Safety (WG-LEPS). The sigma value was calculated for each quality indicator.

Results

A total of 2,71,647 samples and 14,92,485 test requests were screened in clinical biochemistry laboratory. Among the total pre-analytical errors, hemolyzed samples accounted for maximum number of errors (63.58% of the total pre-analytical errors), followed by errors in patient identification (25.05%), quantity not sufficient samples (3.68%), clotted samples (2.99%), inappropriate container (2.62%), lost samples (1.64%), sample mismatch (0.30%) and samples with inappropriate transport system (0.14%). Out of the total number of post analytical errors, urgent test requests with delayed TAT accounted for 84.25%, and reporting errors accounted for 15.75%. Best sigma performance was shown in inappropriate transport of samples and sample mismatch.

Conclusions

Quality indicators should not remain as data capturing mechanisms of academic interest, but also as tools of quality improvement. Errors in pre-analytical and post analytical phases are caused due to inadequacy of training, non compliance's of result entry and review, and lack of automation in these phases.

doi: 10.1016/j.cca.2022.04.404

M217

Six sigma metric evaluations of routine biochemistry parameters and glycated hemoglobin in a fully automated clinical chemistry laboratory

R. Pokhrel, N. Pokhrel, S. Pokhrel, A. Chaudhary, N.B. Khadka

Modern Diagnostic Laboratory and Research Center, India

Background-aim

Quality assurance is an integral part of the clinical chemistry laboratory for assuring the reliability of the results reported .Six sigma is an effective tool for assessment of the overall quality control of the laboratory. Six sigma calculations take into account the total allowable error, method bias and coefficient of variation. Sigma metric calculations are important for assessment of method quality and also for optimizing the quality control procedure by defining the frequencies of internal quality control runs required for different test parameters.

Methods

A retrospective study was conducted in a fully automated clinical chemistry laboratory in Kathmandu, Nepal from the period of June 2019 to August 2019. The laboratory uses RANDOX accusera for internal quality control and has been participating in the CMC (Christian Medical Collage, India) EQUAS program since 3 years. Internal quality control and EQUAS data of the 3 months were used for calculating the sigma metric of the 16 routine Biochemistry parameters including glycated hemoglobin.

Results

Out of 16 parameters included in this study TG (7.36), ALP (6.16) uric acid (6.28), HDL (6.55) and HbA1C (6.55) were excellent on sigma scale with sigma score >6. SGPT, glucose, creatinine and BUN were satisfactory on sigma scale with the score between 3 and 6. Calcium SGOT, total protein, albumin and cholesterol performed poorly on the sigma scale (score <3).

Conclusions

Our study showed highest sigma metric score for TG (7.35) and lowest for total protein (1.54) . Five parameters showed poor sigma score (<3) for which the corrective actions on the analytical procedures are to be taken for bringing them within acceptable sigma scale.

doi: 10.1016/j.cca.2022.04.405

M218

A 10 month evaluation of the routine application of patient moving average for real-time quality control in a hospital setting

H. Van Rossum [b], D. Van Den Broek [a]

[a] *The Netherlands Cancer Institute, Amsterdam, The Netherlands*
[b] *The Netherlands Cancer Institute, Amsterdam, The Netherlands & Huvaros, The Netherlands*

Background-aim

Recently a renewed interest in patient based real-time quality control (PBRTQC) techniques has emerged. This is triggered by the development and availability of new optimization and validation methods. Limited research focused on structurally investigating the true operational value. Therefore we evaluated the routine performance and value of recently implemented moving average quality control (MA QC) procedures.

Methods

MA QC settings were obtained as follows; A flow chart was used to determine when internal QC itself was considered insufficient to ensure proper analytical quality assurance. Next, for these tests MA QC was studied using the online application MA Generator (www.huvaros.com) to obtain settings of optimal MA QC and obtain validated MA QC procedures. MA was operated and managed using GLIMS laboratory information system software and MA QC alarm protocol included; running internal QC, comparison of 4 recently analyzed samples with duplicate analyzer system and reviewing of patient results. Finally MA QC was implemented for 10 chemistry and 6 hematological tests all operated on two analyzer systems for real-time continuous QC.

All MA QC alarms that occurred during the first 10 months of routine clinical application were investigated for the assay specific alarm rate and occurrence in time. Furthermore the causes of MA QC alarms were investigated and alarm relevance was determined based on total allowable bias (TBa) and error (TEa) derived from biological variations.

Results

During the 10 month period, 202 individual MA QC alarms occurred resulting in an overall MA QC alarm rate of 0.030% and a frequency of 4.67 per week. Most alarms were triggered by sodium MA QC. Based on all fully executed and documented MA QC alarm work-ups available, MA QC detected error that in 26.0% exceeded the TBa and in 13.7% the TEa. In 9.2% of the alarms, MA QC alarming triggered instant (technical) corrections. Furthermore, one alarm triggered immediate discontinuation of using a hematological analyzer.

Conclusions

Routine clinical application of PBRTQC or MA QC is feasible with maintaining a manageable number of alarms and enabling detection of relevant analytical error.

doi: 10.1016/j.cca.2022.04.406

M219

An approach to select limit checks and validate their performance independently for pre-analytical and analytical error detection

H. Van Rossum

The Netherlands Cancer Institute, Amsterdam, The Netherlands & Huvaros, The Netherlands

Background-aim

Limit checks are widely used to support the quality assurance of laboratory testing. In general, laboratories have no objective insights in the true error detection performance of limit checks applied. An approach is presented to validate the limit check performance in a laboratory specific manner, independently for pre-analytical and analytical error detection.

Methods

6 months of historical consecutive sodium, calcium and hemoglobin results were used to demonstrate the approach. The MA Generator application (www.huvaros.com) was used to run error detection simulations using different upper limit checks (ULC) and lower limit checks (LLC) using results needed for error detection as readout. Pre-analytical error detection was defined as detecting error in one result and analytical error detection was defined as detecting error within the scheduled internal QC measurement interval, both with a ε97.5% probability. Furthermore the limit check alarm rates were obtained and all were compared with moving average quality control (MA QC) already applied.

Results

For all studied analytes, the pre-analytical error detection and rapid detection of very large analytical error by limit check outperformed the MA QC at the cost of a significant larger number of alarms; 15/week versus 0.038/week for hemoglobin, 10/week versus 0.93/week for sodium, and 2.75/week versus 0.19/week for calcium. Pre-analytical error detection for [LLC/ULC] was [ε-55%/ > 60%], [ε-10%/ε20%] and [ε-40%/ε50%] for hemoglobin, sodium and calcium respectively. Respectively their analytical error detection was [ε-4%/ε15%], [ε-3%/ε4%] and [ε-30%/ε25%].

Conclusions

The obtained ULC and LLC alarm rate, error detection performance together with the performance of the already available MA QC, enabled a substantiated selection of optimal limit checks, validation thereof and integration with MA QC.

doi: 10.1016/j.cca.2022.04.407

M220

The application of quality control circle to improve the quality of samples: A squire-compliant quality-improving study

L. Kangsheng

Department of Clinical Laboratory, Women's Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, Jiangsu 210029, China

Background-aim

Since its application in medical institutions in China, quality control circle (QCC) has gained achievements in medical care and thus earned more attention from the administrative department of health. In order to improve the quality of laboratory specimen, we launched a QCC activity to solve the problems and evaluate the effect of it.

Methods

The data of 30105 unqualified specimens in our hospital were collected from February to June 2017. After the QCC activity, the data of 43125 specimens from July to December 2017 were collected.

Results

Before the QCC activity, the defect rate of the specimens was 0.98% (297/30105), and after QCC activity, the defect rate was 0.45% (193/43125), indicating a significant statistical difference ($p < 0.05$) between them. The achievement rate and improvement rate were 108.2% and 54.1%, respectively

Conclusions

After the implementation of QCC, the defect rate of specimens in clinical laboratories was significantly decreased, and the intangible factors were also improved, which demonstrated the positive effects of QCC on the quality control of specimens.

doi: 10.1016/j.cca.2022.04.408

M221

Is the quality control of the laboratory testing in medical institutions enough? – Analysis of participation rate for external quality assessment in Korea

S. Kim [a], Y. Yun [b], J. Chang [a], T. Um [a], S. Chun [c], K.S. Lee [d], H. Kim [d]

[a] *Department of Laboratory Medicine, Inje University, Ilsan Paik Hospital, Goyang 10380, Republic of Korea*
[b] *Department of Laboratory Medicine, School of Medicine, Konkuk University, Seoul 05030, Republic of Korea*
[c] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul 05505, Republic of Korea*
[d] *Department of Preventive Medicine, School of Medicine, Konkuk University, Seoul 05030, Republic of Korea*

Background-aim

Poor quality of laboratory test results can lead to misdiagnosis and wrong treatment. Therefore, investments for quality testing will yield future benefits. Proficiency Testing (PT) is a widely used tool for monitoring and improving tests in clinical laboratories. In Europe and USA, participation in PT programs is mandatory. However, in Korea, it is optional, and even basic data about PT participation are not available.

Methods

We analyzed the number of clinical laboratories using the National Health Insurance Database according to the medical institution types; clinics, convalescent hospitals, hospitals, general hospitals, and upper general hospitals. The number of PT-participating laboratories was analyzed using the Korean Association of External Quality Assessment Service (KEQAS) database. PT-participating rates of each medical institution types were calculated

Results

The proportion of institution which claims to perform laboratory testing (including handy test) throughout 10 years were 73.9-76% for clinics, 91.9-97.5% for convalescent hospitals, 97.9-99.5% for hospitals, 99.6-100% for general hospitals, and 100% for upper general hospitals. The proportion of institution claiming moderate- or high- complexity laboratory testing were 62.7-66.2% for clinics, 75-93.4% for convalescent hospitals, 96.7-97.9% for hospitals, 99.6-100% for general hospitals, and 100% for upper general hospitals. Among the institutions claiming moderate- or high- complexity laboratory testing, the PT participation rate of the upper general hospital was 100% for 10 years consistently. The mean PT participation rate for 10 years was 2.2% for clinics, 3.4% for convalescent hospitals, 28.2% for hospitals, and 96.6% for general hospitals.

Conclusions

This result would be basic data for making health policies to improve the quality of clinical laboratories.

doi: 10.1016/j.cca.2022.04.409

M222

Evaluation of commutability of external quality assessment material for accuracy based survey of lipid tests

H. Kim, S. Yoon, Y. Yun, M. Hur, H. Moon

Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, South Korea

Background-aim

The Korean Association of External Quality Assessment Service (KEQAS) produced external quality assessment (EQA) materials for use in EQA programs for total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), and triglyceride (TG) measurements. The commutability of EQA materials and commercial general chemistry materials (GCs) was evaluated to investigate their suitability for use in EQA programs.

Methods

From September 18 to 25 2019, 11 EQA materials (five were made according to the Clinical and Laboratory Standards Institute (CLSI) guideline C37-A and six were made according to the modified CLSI guideline), 21 GCs (Lyphochek assayed chemistry control, Bio-Rad Laboratories), 100 frozen individual human samples were analyzed by five routine lipid assays (CHOL HiCo Gen2, HDL-C Gen.4, LDL-C Gen.3, and TRIGL on COBAS c702 [Roche Diagnostics]; Atellica CH Cholesterol 2, Direct HDL, Direct LDL, and Triglyceride on Atellica CH 930 [Siemens Healthcare Diagnostics]; AU cholesterol, HDL-cholesterol, LDL-cholesterol, and Triglyceride on AU5800 [Beckman Coulter]; Kyowa TC, HDL-C, LDL-C, and TG [Hitachi Chemical] on TBA FX-8 [Canon]; and Qualigent CHO, HDL, LDL, and TG [Sekisui Medical Co.] on Hitachi Labospect 008 AS [Hitachi High-Technology Corporation]). The commutability was analyzed according to CLSI EP14-A3 and C53A protocol.

Results

All 11 EQA materials were commutable for all assays. All 21 GCs were commutable for total cholesterol measurement. However, 13 of 21 GCs were noncommutable for HDL-C, LDL-C, and TG measurements and were out of 95% prediction intervals of Deming regression according to EP14-A3 and C53A protocol.

Conclusions

The EQA materials produced by the KEQAS according to the CLSI guideline and according to the modified CLSI guideline were commutable, whichever evaluation approach was applied.

doi: 10.1016/j.cca.2022.04.410

M223

Implementation of a quality management system in toxicological laboratories of health-care institutions of the Republic of Belarus

I. Staseva, N. Kaliadka, Y. Pakhadnia, S. Beliaev

National Antidoping Laboratory, Belarus

Background-aim

There are 14 toxicological laboratories (hereinafter - TL) in the Republic of Belarus that need to implement a quality system in accordance with ISO 15189. Our goal is to go through the accreditation process for one laboratory and implement the quality management system (hereinafter - QMS) in all TL.

Methods

The method of gas chromatography-mass spectrometry (hereinafter - GC-MS) is used for screening narcotics, psychotropics, their precursors and markers and other substances that have a toxic effect (hereinafter - narcotics). Because of the fact that the list of prohibited substances is constantly changing, the methods of analysis are difficult to develop, and therefore the sample preparation of biological matrix (blood, urine) should be as universal as possible for different classes of compounds. The methods should be validated, also their efficiency should have been confirmed through external quality control. It was also necessary to develop and implement the following elements of QMS: staff recruitment and training, development of standard operation procedures, preparation of equipment and rooms, audit performance and other.

Results

The laboratory received accreditation for ISO 15189 (certificate BY/112 8.0001 dated 12.28.2018). In November 2019, as part of the periodic competency assessment, a peer assessment of the National Accreditation System of the Republic of Belarus by the European Accreditation Organization (EA) was carried out. The laboratory is accredited to the following methods: SOP LM 062 Qualitative determination of narcotics in blood samples by GC-MS, SOP GC 016 Qualitative determination narcotics in human urine by GC-MS.

Conclusions

Since 2018, the laboratory has been providing proficiency testing programs for determination of narcotics in urine for all TL in accordance with ISO/IEC 17043. Two workshops were conducted for TL on the development of the QMS. On the basis of the accredited laboratory, a partial centralization of toxicological studies was carried out. For the future, the laboratory sets the following goals:

1. implementation of the QMS in TL;
2. introducing screening methods into the accreditation area using the liquid chromatography-mass spectrometry.

doi: 10.1016/j.cca.2022.04.411

M224

SERVPERF model for measuring laboratory service quality through patient satisfaction

I. Celap [a], D. Marijancevic [c], I. Vukasovic [a], V. Skare [b], T. Komarac [b], V. Cerfalvi [d], D. Ozretic Dosen [b]

[a] *Department of Clinical Chemistry, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*
[b] *Department of Marketing, University of Zagreb Faculty of Economics and Business, Zagreb, Croatia*
[c] *Endocrinology laboratory, Department of Nuclear Medicine and Oncology, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*
[d] *Office for Assurance and Quality Improvement of Health Care, University Hospital Center Sestre milosrdnice, Zagreb, Croatia*

Background-aim

Great contribution to the quality improvement of laboratory service is review of patient feedbacks, which is also one of the ISO 15189 requirements. Measurement of service quality is variable, intangible and heterogeneous, so it is necessary to use models for measuring quality of service, which establish dimensions that affect patients' satisfaction. The aim of the study was to evaluate appropriateness of SERVPERF (Service Performance) model for measuring patient satisfaction with laboratory service in outpatient unit.

Methods

Modified SERVPERF questionnaire included 26 questions covering demographic data, overall satisfaction and following dimensions: reliability, tangibility, empathy, assurance and responsiveness. Participants were asked to rank their satisfaction with the Likert 5-point scale from very dissatisfied (1) to very satisfied (5). One open-ended question were added for additional comments. The questionnaire was sent via Survey Monkey© platform to the patients who requested delivery of laboratory report by an e-mail. Data were analyzed using Microsoft Excel.

Results

Totally, 843 patients have answered on the questionnaire, out of which 78 % were women. The majority of the respondents have university education (53 %) and already have used laboratory service in our hospital (73 %). Overall satisfaction of the respondents was highly ranked 4.1 (satisfied). The respondents were dissatisfied with the tangible dimension such as appearance and size of laboratory space (2.6 and 2.9), while the most satisfied were with the responsiveness such as professionalism and courtesy (4.5) and reliability (4.5). Further, respondents were very satisfied with the laboratory reports delivery options (4.6) as a part of empathy dimension. Open-ended question have shown great dissatisfaction with tangible dimension.

Conclusions

Although the response rate have not been known, the study have shown that the patient satisfaction with laboratory service mainly depends on the reliability and responsiveness dimension. In our case, further improvement of laboratory service should be focused on arranging and extension of waiting room and blood collection spaces.

doi: 10.1016/j.cca.2022.04.412

M225

External quality assesment of medical laboratories: requirement of MKS EN ISO 15189:2013

K. Tosheska-Trajkovska, I. Kostovska, S. Cekovska, D. Labudovic, J. Bogdanska, J. Brezovska, S. Topuzovska

Institute of Medical and Experimental Biochemistry, Medical Faculty-Skopje, Macedonia

Background-aim

One of the main aspects of laboratory quality improvement is participation in external quality assessment schemes (EQAS), proficiency testing (PT) or interlaboratory comparisons (ILC). According to the requirements of the standard ISO EN 15189:2012, participation in EQAS is mandatory for each accredited laboratory.

Aim of the study: Assessment of the results of participation in EQAS for Biochemical Analyses Laboratory (BAL).

Methods

BAL, within the Institute of Medical and Experimental Biochemistry, Medical Faculty-Skopje, is a medical laboratory accredited according to the International Standard MKS ISO EN 15189:2013.

BAL participates in EQAS organized by Instand eV, Dusseldorf, Germany, since 2011. Our laboratory participates in surveys for: haematology (differential blood count), clinical chemistry (conventional analyses) and urine chemistry.Testing samples from each panel are sent to the BAL 3-4 times/year. Acceptance criteria is mean +/- 2SD.

Results

There is variation in performance, with a best annual average performance in 2016 and 2017.

Except for haematology, annual performances for enrolled panels varied from year to year, indicating some difficulty in maintaining consistency in quality. Overall successful rate in EQAS participation varies from 98.4 % for urine chemistry, 96.5-100% for clinical chemistry panel to 100 % for haematology.

The main challenges of the EQAS program observed between 2011 to 2019 were funding, sourcing, and safe transportation of quality panels to our laboratory. Corrective and preventive actions are issued for outlying EQA results. By directing corrective measures at root causes, we had realized that the likelihood of problem recurrence was minimized.

Key benefit of participation in EQAS is improvement of the laboratory performance through: discovering of sources of error; systematic errors; demonstration of effectiveness of changes; common understanding of method differences; discovery of method sensitivities, etc.

Conclusions

We recommend all laboratories to participate in the EQAS program. Successful performance in an EQA programme reflects the effectiveness of the laboratory's quality management. EQA is important for improvement of the laboratory quality management system, as it is a measure of laboratory performance.

Keywords
EQAS
ISO 15189
accreditation

doi: 10.1016/j.cca.2022.04.413

M226

New external quality assessment scheme for fecal occult blood in Thailand

D. Suvagandha, S. Nantarat, P. Soisangwan

Bureau of Laboratory Quality Standards, Department of Medical Sciences, Ministry of Public Health, Thailand

Background-aim

Fecal occult blood test (FOBT) by fecal immunochemical technique (FIT) has been approved and widely used for colorectal cancer screening before confirmation by colonoscopy in Thailand. Because the sensitivity and specificity for human hemoglobin (HHb) of FIT is better than quaiac test. The laboratories that provide the FIT need to participate the external quality assessment scheme for fecal occult blood test (EQAS-FOBT) while the scheme has not been provided and complied with ISO/IEC 17043. In 2018 the EQAS-FOBT has been established as a new pilot survey of EQAS-FOBT in Thailand.

Methods

Totally expectation number of participants were 155 laboratories. This survey was conducted one round. The criteria for selection the laboratory to participate this survey was governmental and private hospitals that can provide confirmation of colorectal cancer by colonoscopy. Two EQA samples including positive in semi-solid form (HHb > 300 ng) and negative in liquid form (HHb = 0 ng) were sent to participants.

Results

A total number of 155 laboratories reported the results. The participant 154 laboratories used qualitative FIT while one laboratory used quantitative FIT. The survey results from all laboratories for positive and negative samples consistent with assign value. Feedback from all participants were 100% satisfied of the EQAS-FOBT and need to continue participate next survey.

Conclusions

In summary, the pilot survey did not found any problems and no negative comment so the new EQAS-FOBT can support the laboratories in Thailand to comply with quality assurance requirement. Furthermore, the EQAS-FOB will not limit service to the laboratories in the hospital

that can provide confirmation by colonoscopy while will accept for participation from all laboratories in Thailand.

doi: 10.1016/j.cca.2022.04.414

M227

Development research on the evaluation and management standards for NGS clinical laboratory accreditation

M. Han [f], J. Kim [j], J.Y. Seo [c], J. Jang [k], K. Lee [d], W.I. Lee [h], W. Lee [a], J. K. Lee [g], J. Song [i], G.C. Kwon [b], Y.K. Lee [e]

[a] *Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, Chungnam National University School of Medicine, Chungnam National University Hospital, Daejeon, Republic of Korea*
[c] *Department of Laboratory Medicine, Gachon University Gil Medical Center, Incheon, Republic of Korea*
[d] *Department of Laboratory Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea*
[e] *Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang, Republic of Korea*
[f] *Department of Laboratory Medicine, Kangdong Sacred Heart Hospital, Seoul, Republic of Korea*
[g] *Department of Laboratory Medicine, Korea Cancer Center Hospital, Korea Institute of Radiological And Medical Sciences, Seoul, Republic of Korea*
[h] *Department of Laboratory Medicine, School of Medicine, Kyung Hee University, Seoul, Republic of Korea*
[i] *Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Republic of Korea*
[j] *Department of Laboratory Medicine, Soonchunhyang University School of Medicine, Seoul, Republic of Korea*
[k] *Genome Research Center, Green Cross Genome, Yongin, Republic of Korea*

Background-aim

In order to implement laboratory developed tests (LDT) like Next Generation Sequencing (NGS) successfully, well-designed laboratory accreditation programs are required as a top priority for the proper evaluation and management of the tests, including instrumentation and reagents. The current NGS clinical laboratory accreditation program (CLAP) by the Ministry of Food and Drug Safety (MFDS) of Korea, which started recently, has several points to be improved in many aspects. The aim of this study was to establish detailed standards and a robust workflow to improve the current accreditation program.

Methods

Firstly, we investigated and analyzed the current NGS CLAP managed by MFDS to find out its drawbacks and the points of improvement. Further, we conducted a survey of the institutions participating in the program and collected their suggestions and comments. Next, we referred to checklists and evaluation standards of other domestic and international accreditation programs, such as Laboratory Medicine Foundation of Korea, Korean Institute of Genetic Testing Evaluation, College of American Pathologists (CAP), and ISO15189, as well as the mandatory regulations on effective accreditation programs. In addition, the industry-university-institute collaboration advisory committee made consultations. Based on our research of many aspects of the current status, we made suggestions on common standards or checklists to evaluate quality management system and proficiency of NGS clinical laboratories as well as operating protocols on managing programs.

Results

Participants of the program and the advisory committee provided informative suggestions and comments for improving the accreditation program: 1) duplicated accreditation with other programs, 2) efficient workflow issue, 3) outsourcing parts of workflow. The research team made overall suggestions regarding detailed standards and checklists to evaluate quality management and proficiency of NGS clinical laboratories, and program management protocols as well. More importantly, outsourcing the on-site evaluation to an expert organization was suggested.

Conclusions

The demand for the proper and safe implementation of cutting-edge technology like NGS in clinical laboratories is increasing. The suggestions from this study will be useful and essential in improving the NGS CLAP by MFDS of Korea.

doi: 10.1016/j.cca.2022.04.415

M228

Use of EQA to help resolve laboratory performance issues

S. Jones, G.J. Davies, A. Thomas

Weqas, Cardiff & Vale UHB, Cardiff, UK

Background-aim

Weqas has over 50 years' experience providing Quality Assurance in Laboratory Medicine, delivering services in Laboratory External Quality Assessment (EQA), Point of Care (PoCT) EQA, Reference Method Development, Internal Quality Control (IQC) and Education & Training.

Programmes are underpinned by commutable, metrological, traceable samples and informative reports. Our team of experienced Scientists provide a unique troubleshooting and problem solving service.

Methods

Troubleshooting tools provided as part of the EQA programme:

- Dedicated helplines with an experienced team of scientists to assist with performance interpretation and troubleshooting advice.
- Support Literature: Comprehensive Report Interpretation guides including a bespoke troubleshooting algorithm to lead participants through the steps to confidently identify and correct analytical errors.
- Repeat samples for re-assay during performance investigation; performance scores can be re-calculated to confirm improved performance.
- Report Interpretation Training Sessions to aid with continued competency.
- Online Resource Library with Interpretive Case Studies.

Results

Troubleshooting Algorithm:
Is it Imprecision?
Errors of imprecision should be corrected first.
Check for causes of imprecision in the following order:

- Exclude apparent imprecision due to curvilinear data
- Exclude clerical errors
- Check for causes of imprecision

Is it Inaccuracy?

Systematic errors can be eliminated by appropriate improvement in methodology.

Inaccuracy can be due to:

- Curvilinear data: Reagent or standard deterioration
- Systematic constant: Usually blank due to reagent, serum or instrument zero
- Systematic proportional: Usually due to calibration, standards
- Mixed systematic: On one point calibration with a cross-over at or near a calibration point (pivoting about calibration point), check zero calibration point. For a two point multi calibration with cross-over at or near one point, check other calibrators and/or zero point

Conclusions

EQA should not be seen as a 'tick box exercise' and the introduction of educational elements, with a robust troubleshooting and problem solving service, has resulted in improved performance.

doi: 10.1016/j.cca.2022.04.416

M229

What do laboratories need from an external quality assessment (EQA) programme – Is it time to redefine the aims of EQA?

M.A. Thomas, S. Jones, G. Davies

Weqas, Cardiff and Vale University Health Board, Cardiff, UK

Background-aim

EQA is defined as a system designed to objectively assess the quality of results by an external agency. The aims were defined by the IFCC in 1977 in that they should: Provide a measure of the quality of a test, a measure of the "state of the art" of a test, obtain consensus values when true values are unknown and to investigate factors in performance.

Methods

In its basic form, EQA should provide a peer review assessment of the quality of the performance and an assessment of the state of the art of methods. However, by using dedicated designs and samples, EQA can be used for long term performance assessment, robustness of methods, sensitivity to interferences, linearity, recovery, specificity, pre- and post-analytical factors, assess trueness and intralaboratory variation.

In the design a number of factors need to be considered such as: number, frequency and type of samples, target value, statistical analysis and the analytical performance specification (APS). The use of material as close as possible to the patient sample minimizes any matrix effect and allows the assessment of accuracy. Other factors to consider include: stability, homogeneity, clinically relevant concentrations at clinical decision limits and use of challenging samples.

Results

The target value varies greatly and is often dependant on the matrix and availability of higher order reference methods. The advantages of the latter is that it provides a true assessment of accuracy , establishes metrological traceability, a requirement of ISO 15189, an independent assessment of manufacturer traceability claim, is not influenced by the number of devices, and is useful in the post market vigilance.

Laboratories should ensure that the quality is appropriate for the needs of the clinical service. It is therefore essential that EQA evaluation criteria should also reflect clinical need. A hierarchical strategy for APS proposed at the European Federation of Laboratory Medicine in Milan is suggested. EQA Programmes play an important role in the continuous education of laboratory staff and should include educational elements relating to: Pre-analytical effects, performance of methods, susceptibility of methods to interference, and the interpretation of the results.

Conclusions

The assessment of quality is no longer confined to the analytical phase and EQA providers need to consider assessment of the whole testing process. It is proposed that the definition should be amended to include assessment of the pre and post analytical phase, providing an educational role, a post market vigilance service and evidence for harmonisation strategies.

doi: 10.1016/j.cca.2022.04.417

M230

Comparison of sigma metrics for thyroid hormones on two automated immunoassay machines

P. Wanjiru [a], A. Amayo [b]

[a] *The Nairobi Hospital, Kenya*
[b] *University of Nairobi, Kenya*

Background-aim

Six sigma applications improve the quality of outputs by abolishing the source of defects and reducing variability in the manufacturing industry. Sigma metrics can serve as a guide for clinical laboratories to make or review quality control frequency and strategy. They can also be used to compare performance of different analytical platforms. Tests with low sigma values (< 3l) indicate that the analytical quality of the laboratory needs to be improved. There are several reports of sigma metrics for general chemistry analytes on automated platforms. There is however little data on application of sigma metrics in hormonal assays using different automated platforms.

The aim of this study was to evaluate and compare thyroid hormone analytical performance for two immunoassay platforms using six sigma metrics.

Methods

Thyroid hormone quality data for Abbott Architect Ci8200 and Roche Cobas e601 were analyzed at The Nairobi Hospital Laboratory, Kenya. Third party commercial normal (Level 2) and pathological high (Level 3) levels of QC materials were assayed before reporting patient samples daily. The instruments were calibrated regularly as required.

Thyroid stimulating hormone (TSH), Free T4 (FT4), and Free T33 (FT3) for the two equipment were studied over a period of 6 months (November 2018 to April 2019). Data from IQC and EQA participation was used.

Sigma metrics were calculated using total allowable error as per CLIA recommendations. Bias was calculated from RIQAS EQA participation while coefficient of variation was calculated from IQC data collected during the study period.

Results

TSH on the Abbott Architect platform had sigma metrics above 3(3.7 and 3.5 at control levels 2 and 3 respectively). The lowest sigma metrics on both platforms were obtained for Free T4. There was significant difference in TSH and FT3 sigma values when the two platforms were compared, with Cobas e601 showing relatively better sigma metrics than Architect for FT3; whereas Architect had relatively better metrics for TSH.

Conclusions

Sigma reports for general clinical chemistry analytes have shown variable performance with several parameters having sigma levels less than 3. In this study, the sigma metrics for FT4 indicated a need to apply more stringent IQC measures to improve analytical performance.

doi: 10.1016/j.cca.2022.04.418

M231

Application trial of moving average as a tool of realtime quality control of clinical chemistry

S.M. Lee [b], Y. Dong-Won [b], J. Cheon [b], S. Lee [c], H. Cho [c], I. Kim [a]

[a] *Department of Laboratory Medicine, Pusan National University School of Medicine, South Korea*
[b] *Department of Laboratory Medicine, Pusan National University Yangsan Hospital, Yangsan, South Korea*
[c] *Ortho Clinical Diagnostics Korea, Seoul, South Korea*

Background-aim

Moving average (MA) was proposed as continuous analytical quality control (QC) for chemistry. Lack of informatics system allows of MA and complexity of optimizing MA is not used in the most clinical laboratories in the practice. As several middleware systems begin to support the MA applications, clinical laboratories must determine the criteria to including assay results, algorithms used to calculate average value, and the limits for out-of-control alarming. We will show a simple example of applying MA to middleware on the day-time chemistry analysis in a tertiary hospital in Korea.

Methods

After the installation of MA option in the middleware system, Instrument Manager (Data Innovations, VT, USA), two laboratory staffs monitored MA graphs of 26 assay results of AU5800 (Beckman Coulter, CA, USA). For three electrolyte assays – sodium (Na), potassium (K), chloride (Cl), we had tried to check the performance of MA QC by artificial error situation that was made by changing the correlation factor (slope value) from 1 to 0.8. Using changed calibration, 40 residual patient serum specimen were tested and MA was calculated of the last 20 assay results by two calculation algorithms, simple mean (SM) and the exponentially weighted moving average (EWMA) containing weighting factor 0.05, without truncation limits. Cumulated MA target mean and standard deviation (SD) values, test number before warning and error alarming were evaluated and compared for two calculation algorithm.

Results

Before making error, MA values of last 20 results showed smooth curves in the 1SD range of MA target automatically calculated after re-starting during one month except one outlier event for K. Automatically established MA target were similar by two algorithms - 137.04 ± 5.07, 4.245 ± 0.545, 102.47 ± 3.88, and 4.322 ± 0.433, as target mean ± SD by SM, 137.61 ± 5.02, 4.336 ± 0.562, 103.01 ± 3.98, and 4.146 ± 0.449, by EWMA, sequentially of Na, K, Cl and albumin. After changing correlation factor, by decreased test results of electrolytes, MA values were decreased and exceeded 2SD warning limits after 7 results for Na and Cl, 35 results for K by SM, after 7 and 6 results for Na and Cl, and after 50 results for K by EWMA. For 3SD error limits MA exceeded 3SD error limits after 11 results by SM, after 13 and 14 results by EWMA only for the Na and Cl. After fixation of factor the returning rate was faster by EWMA algorithm.

Conclusions

With middleware software, MA was easy to apply and can monitor systemic error as a continuous QC tool. In the artificial error situation, EWMA and 3SD limit showed lower error detection performance to detect error than SM and 2SD. Through this practical appilication and simulation case, the clinical laboratories who consider MA application could be understand the concept of MA and refer to the optimization and validation methods.

doi: 10.1016/j.cca.2022.04.419

M232

Use of EQA to help resolve laboratory performance issues

M.A. Thomas, S. Jones, G. Davies

Weqas, Unit 6, Parc Ty Glas, Llanishen, Cardiff CF14 5DU, UK

Background-aim

EQA in medical laboratories has evolved over the past 50 years to provide more sophisticated systems compared with the simple analytical performance evaluation of earlier years, providing pre- and postanalytical elements as well as data interpretation, problem solving and education.

Weqas programmes are underpinned by commutable, metrological, traceable samples, informative reports, educational workshops and problem-solving support. These tools are pivotal to help resolve laboratory performance issues and improve quality.

Methods

Education and problem solving

- Dedicated helplines providing performance interpretation and troubleshooting advice.
- Support Literature, Interpretation guides and a bespoke troubleshooting algorithm to help participants identify and correct analytical errors.
- Samples for performance investigation; scores can be re-calculated to confirm improved performance.
- EQA Interpretation workshops with Case Studies.

Results

The algorithm leads the participant through a series of questions using their EQA reports to identify any errors. Imprecision is identified using metrics that measure dispersion of the data about the best fit line

(R value and Sy). Inaccuracy is identified using the linear regression equation, y = mx + c, where y is the lab result, x the target value, m the slope of the line, and c the y-intercept. Deviation from a m of 1.0 suggests systematic proportional error, whilst c gives a measure of the systematic constant error.

Inaccuracy can be due to:

Curvilinear data: Reagent or standard deterioration, m, c and r

Systematic constant: Usually blank due to reagent, serum or instrument zero, c

Systematic proportional: Usually due to calibration, standards, m

Mixed systematic: Usually a cross-over at or near a calibration point, check other calibrators and/or zero, m and c.

Conclusions

The key objective of EQA is in identifying laboratory performance issues and to provide the necessary tools to help resolve those issues. EQA should not be seen as a 'tick box exercise' or a pass/fail exercise but should be used to support laboratories in continuously improving services for the benefit of patients. Feedback from participants using Weqas problem solving tools has supported their effectiveness in improving the laboratory performance.

doi: 10.1016/j.cca.2022.04.420

M233

Patient based real time quality control as a complementary tool to external quality assessment schemes in clinical laboratories

C. Çavuşoğlu [a], A. Coşkun [c], E. Matpan [b], M. Serdar [c], M. Kilercik [c], M. Serteser [d]

[a] *Acıbadem Labmed Clinical Laboratories, Department of Medical Biochemistry, Istanbul, Turkey*
[b] *Acıbadem Mehmet Ali Aydınlar University School of Medicine, Department of Medical Biochemistry, Istanbul, Turkey*
[c] *Acıbadem Mehmet Ali Aydınlar University School of Medicine, Department of Medical Biochemistry, Istanbul, Turkey*
[d] *Acıbadem Labmed Clinical Laboratories, Department of Medical Biochemistry, Istanbul, Turkey*

Background-aim

External quality assessment schemes (EQAS) are independent evaluation programs for the implementation of standardization and harmonization between laboratories. EQAS have significant advantages such as determining laboratory individual performance, monitoring and documenting analytical quality and evaluating test performance depending on different methods and instruments. Real-time monitoring is a technique allowing laboratories to determine the current state of patients' results. However, EQAS cannot monitor the laboratory performance in real time because of having long survey times between scheduled programs and performing only single measurement in each period. In this study, we aimed to use the Patient-Based Real-Time Quality Control (PBRTQC) monitoring program as a complementary tool for EQAS to detect the deviations in patients' tests results.

Methods

Five measurands (ALT, FT4, hsCRP, 1.25-dihydroxyvitamin D, and Tyrosine) that evaluated by different statistical methods (D/Dmax, z score and %bias) and significantly deviated from the target value based on the EQAS assessment were included in the study. For each measurand, the average of normals (AON) in the periods with and without deviations and the relative differences (RD) were calculated. The RDs > relative root mean square (RMS-RiliBAK) were considered significant.

Results

For all meaurands, no significant deviations were observed between the AONs obtained in the entire periods with and without deviations. The highest difference was observed in FT4 (2,6%). However significant deviations were found in short time intervals particularly for FT4.

Conclusions

PBRTQC is a powerful tool to detect particularly short-term deviations in patients test results and can be implemented in medical laboratories easily. Since EQAS cannot detect all deviations during the entire survey period, PBRTQC can be considered as a complementary tool to EQAS in monitoring of patients results.

doi: 10.1016/j.cca.2022.04.421

Clinica Chimica Acta 530 (2022) S403–S405

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Rare Disease

W219

Lysine protein intolerance – Clinicopathological spectrum of a rare disease

S.B. Hashmi, L. Jafri, H. Majid, A.H. Khan, S. Ahmed

Aga Khan University Hospital, Karachi, Pakistan

Background-aim

Lysine protein intolerance (LPI) is a rare metabolic disease resulting from recessive-inherited mutations in the SLC7A7 gene encoding the cationic amino-acids transporter. The disease is characterized by protein-rich food intolerance with secondary urea cycle disorder, but symptoms are heterogeneous ranging from neurological involvement, infiltrative lung disease, kidney failure to auto-immune complications.

Methods

An observational study was conducted at section of Chemical Pathology, Department of Pathology and Laboratory Medicine. Patients tested for urinary and plasma amino acids from January 2013 to October 2018 at the Biochemical Genetic Lab, AKU were included in this study. The plasma amino acids and urine amino acids were quantified by HPLC. Clinical and biochemical data was collected from the structured biochemical genetics laboratory requisition forms. Data was analyzed by Microsoft Excel 2016.

Results

We identified 6 patients. All were male. Mean age was 24 months + 10days. All patients had decreased levels of lysine, ornithine and arginine in plasma whereas elevated levels of these amino acids in urine samples. All were product of consanguineous marriage. In all six patients, vomiting was the most common symptom followed by seizures, developmental delay, and drowsiness. Hepatomegaly was present in all patients. Only one patient is on follow-up.no mutation analysis was done.

Conclusions

Although clinical manifestation of lysine protein intolerance appeared in first two years of life, most of them suffered a delay in diagnosis of even some years after the onset of symptoms, highlighting the difficulty in the diagnosis of LPI.

doi: 10.1016/j.cca.2022.04.423

W220

Mutations of chitotriosidase gene may delay the diagnosis of sarcoidosis

M. Fagyas [b], G.L. Beke [b], A. Csongrádi [b], I.T. Altorjay [b], A. Enyedi [a], Z. Papp [b], A. Tóth [b]

[a] *Department of Surgery, University of Debrecen, Hungary*
[b] *Division of Clinical Physiology, Department of Cardiology, University of Debrecen, Hungary*

Background-aim

Serum chitotriosidase (CTO) can be a useful biomarker either for diagnosing sarcoidosis or for assessing the severity of sarcoidosis. There is a common, 24 bp duplication polymorphism of CTO gene (rs3831317), which influences serum CTO activity, hereby makes the evaluation of results more difficult.

Here we aimed to determine the effect of the 24 bp duplication polymorphism of CTO gene on serum CTO activity, and to reveal other potential CTO mutations with significant activity modifying effects.

Methods

We enrolled 123 healthy individuals into the study. Serum CTO activity was measured with a fluorescent kinetic CTO activity assay, and CTO 24 bp genotype was determined using an allele-specific PCR. Unknown mutations were identified with Sanger sequencing and the encoded CTO protein was analyzed by MALDI-TOF mass spectrometry after immunoprecipitation.

Results

The twenty-four base pair duplication polymorphism significantly influenced serum CTO activity, namely individuals with homozygous wild genotype had the highest CTO activity (651.8 ± 314.1 mU/L, n = 72), individuals with homozygous mutant genotype had no measurable activity (n = 3), while individuals with heterozygous genotype had

intermediate CTO activity (442.3 ± 307.3 mU/L, n = 48). We identified a young man, who was heterozygous for 24 bp duplication polymorphism but he had no serum CTO activity. Sanger sequencing revealed a 29 bp deletion in exon 9 of CTO gene (rs536102546), which results in a premature stop codon and in a truncated protein product. We confirmed that the duplication and deletion mutations of this proband were in trans position. Molecular weight determination with mass spectrometry supported the presence of truncated proteins in the serum of the proband.

Conclusions

Approximately 40% of people have a mutation, which influences serum CTO activity. Beside the common 24 bp duplication polymorphism, there could be several other infrequent mutations which cause alterations in enzyme activity. Since serum CTO activity is used for diagnosing rare diseases (sarcoidosis, Gaucher disease, etc.), sequencing of CTO gene is worth considering when unexpectedly low serum CTO activity is measured.

doi: 10.1016/j.cca.2022.04.424

W221

Lean six sigma analysis of sweat testing against the College of American Pathologists' standards

H.I. Khan

The AGA Khan University Hospital, Pakistan

Background-aim

Sweat testing is the standard method for screening and diagnosing cystic fibrosis (CF) which is a debilitating, chronic, inherited and common life threatening genetic disease. College of American Pathologists (CAP) has twenty-five standards for sweat testing alone including standards related pre-analytical, analytical and post-analytical phase alongside monitoring of sweat production failure rate. On assessing the compliance of all standards, only rejection rate of sweat testing was not persistently meeting the benchmark (10% for < 3 months and 5 % for more than three months of age).The purpose of this research was to study the effect of the Six Sigma DMAIC approach in reducing the sweat production failure rate.

Methods

We examined the hypothesis that lean-based reorganization of sweat testing process flow would improve quality and reduce waste in the system. An audit was performed to improve the sweat production failure rate. Sweat conductivity testing is offered to screen both inpatients and outpatients for CF. This prospective study was conducted in the section of Chemical Pathology, Department of Pathology and Laboratory Medicine Aga Khan University (AKU). Using six sigma process improvement DMAIC methodology the study was conducted between April-2014 to October-2019. Using this Define, Measure, Analyze, Improve, Control approach, interventions were designed and evaluated that addressed the issues identified as barriers to efficiency in sweat testing. All data was entered and analyzed on SPSS version 21 and Microsoft Excel.

Results

The audit finding revealed that the sweat production failure rate was higher when junior technologists performed the procedure. Sweat conductivity testing was catered as walk in procedures with no prior clinical history or evaluation of patients. Evidence of complete training of technologists performing the test was missing. Technologists performing the test were not aware of CAP standards of sweat testing. Based on our findings senior and experienced technologist was made to perform the test only. Instructions for the patients' parents or guardian and for receptionists regarding patient preparation were developed and circulated. Hydration status was assessed before starting the procedure and dehydrated patients were deferred. We switched from Macroduct to Nanoduct® Neonatal Sweat Analysis System as it requires lesser sample quantity (5ul) which made analysis easier and hindrance of non-sufficient sample was overcomed. Competency assessment of all technologists performing the test was documented. For ongoing monitoring, sweat production failure rate was made part of dashboard indicators. Furthermore, in terms of cost saving previously, overall 207 patients were refunded the charges of the test due to non-production of sweat. The laboratory had to bear the excess burden of a total of PKR 1148850. Whereas, after the implementation of audit teams suggestions, in 2019 the re-compensation sum came down significantly to PKR 476000 owing to the substantial decline in failure rate (n = 80).

Conclusions

In conclusion, this quality improvement project led to reduction in failure rate via close monitoring and coordination of the project team. We were able to achieve a significant reduction in sweat failure rates, from 45% to 15% (for patients aged 3 months). Patient's selection, technologist education and training and proper sweat collection systems are critical to achieve a good sweat production rate. We recommend that these factors should be looked into while performing a sweat test procedure.

doi: 10.1016/j.cca.2022.04.425

W222

Metabolomic analysis of HeLa cells deficient in purine de novo synthesis

L. Mádrová [b], R. Brumarová [b], D. Dobešová [b], J. Václavík [b], Š. Kourõil [a], J. De Sousa [a], J. Friedecká [a], D. Friedecký [a], V. Barešová [c], M. Zikánová [c], T. Adam [a]

[a] *Department of Clinical Biochemistry, University Hospital in Olomouc, Olomouc, Czech Republic*

[b] *Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University and University Hospital in Olomouc, Olomouc, Czech Republic*

[c] *Research Unit for Rare Diseases, Department of Pediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic*

Background-aim

Enzyme defects of purine de novo synthesis (PDNS) cause severe conditions in humans. Three genetically determined defects of the pathway have been identified so far: ADSL deficiency (OMIM 103050), AICA-ribosiduria (OMIM 608688) and newly described PAICS deficiency. Clinical signs of these defects are mainly neurological, such as seizures, psychomotor retardation, epilepsy, autistic features and others. The aim

of this work was to describe metabolic changes of CRISPR-Cas9 genome-edited HeLa cells deficient in the individual steps of PDNS, which represent the first human cellular model for both known and potential defects of the pathway.

Methods

Combination of targeted and untargeted metabolomic analysis was applied in the study of the cell lines. High performance liquid chromatography coupled with high resolution tandem mass spectrometry were used for both analyses. Acquired data were evaluated by univariate and multivariate statistical tools. The identity of statistically significant unknown molecules from an untargeted study was confirmed by fragmentation. Data from a targeted analysis were processed in Cytoscape to visualize the most affected metabolic pathways.

Results

The most statistically significant metabolites found in the deficient cell lines compared to control were the intermediates of PDNS preceding particular deficient enzyme. Some of these direct substrates were detected in all phosphorylation forms including triphosphates. Disturbed PDNS resulted in altered pool of adenine and guanine nucleotides, but without shifting the energy status of the cells out of physiological range. Changes were seen also in the levels of pyrimidines, presumably due to a decreased level of ATP in the cells. Increased level of serine biosynthesis metaboliltes and lower level of glycine observed in all deficient cell lines are most probably a result of purine depleted medium used for cultivation and complete inhibition of PDNS resulting in excess of methyl units otherwise consumed by the pathway.

Conclusions

Changes identified in the metabolome of the deficient cells represent a model of analogical changes that might occur in the patients suffering from defects of PDNS and could lead to the improvement of diagnosis and help in the study of prevalence.

doi: 10.1016/j.cca.2022.04.426

Clinica Chimica Acta 530 (2022) S406–S413

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Reproductive Medicine, Pregnancy

W223

Angiogenic profile according sFlt-1/PlGF ratio before and after delivery in patient with preeclampsia

I. Samardjiski, V. Livrinova, A. Atanasova Boshku, I. Todorovska, S. Simeonova Krstevska, V. Gjirevski

University Clinic of Gynecology and Obstetrics, Skopje, The Former Yugolav Republic of Macedonia

Background-aim

Preeclampsia (PE) is a multisystem disorder in pregnancy with a specific collection of signs and symptoms as a result of serious dysfunction of multiple organs. Abnormal placentation in the first trimester, most likely triggers the disbalance of the placental anti-angiogenic factor soluble fms-like tyrosine kinase-1(sFlt-1) and pro-angiogenic placental growth factor (PlGF), which result in systemic endothelial dysfunction (injury) to progressive end-organ damage. According the PROGNOSIS study, the cut-off value of the sFlt-1/PlGF ratio over 85 confirms the suspected PE and proved to be useful in preeclampsia diagnosis. A severely elevated sFlt-1/PlGF ratio is associated closely with the need to deliver within 48hours.

Methods

We determined the serum sFlt-1/PlGF ratio using Elecsys assay /E411-modular system(Roche) before delivery, and 2 days after delivery in pregnant woman with preeclampsia. The study includes 17 patients recruited from hospitalized patients at the University Clinic of Gynecology and Obstetrics in Skopje with suspected preeclampsia, for a period of 3 months. We devided patients in two groups, early-onset PE < 34 gestational weeks (n = 9), and late-onset PE > 34 gestation weeks (n = 7).

Results

The average value of sFlt-1/PlGF ratio in the group of pregnancies with early-onset PE was 568.31 pg/ml (range 227.06 - 1408.6), while in the group of pregnancies with late-onset PE the average value was 243,84 pg/ml (range 89,72 - 813.27). Higher sFlt-1/PlGF ratio in early-onset PE compared with late-onset PE, was accordant to the more antiangiogenic state and progressively worsening clinical course observed in women with early-onset PE.

The values obtained 2 days after delivery in both groups were significantly reduced, in pregnancies with early-onset PE averaging 105.77 pg/ml (28.28 - 137.66) and 74.37 pg/ml (38,18 - 126) in the other group, respectively.

Conclusions

All pregnant patients with confirmed diagnosis of preeclampsia had sFlt-1/PlGF ratio over the cut-off value of 85 before the delivery, and significantly lower sFlt-1/PlGF ratio 2 days after the delivery which likely confirms the role of the placental angiogens in the pathogenesis of PE. The sFlt-1/PlGF ratio confirms its possible relevance as a biomarker in the diagnosis and prognosis of the disease determining the angiogenic profile, and the follow-up test after the delivery. This finding can be applied for further planned clinical trials.

doi: 10.1016/j.cca.2022.04.428

W224

Is there a relationship between seminal zinc concentration and sperm quality in overweight men?

N. Kozopas [a], A. Kost [b], H. Maksymyuk [a]

[a] *Department of Clinical Laboratory Diagnostics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*
[b] *St. Paraskeva Medical Centre, Lviv, Ukraine*

Background-aim

Zinc (Zn) is an important component of the normal functioning male reproductive system. In addition, Zn deficiency has been linked with male infertility.

This study aimed to investigate a correlation between the basic sperm parameters and the concentration of zinc in seminal plasma of normal and overweight men.

Methods

The study included 130 men who were screened at a reproductive clinic during 2019. The participants were divided into two groups according to the calculated body mass index (BMI, kg/m2): control group (CG) with normal value (18.5-24.9), and pre-obesity (25.0-29.9). The main sperm parameters were evaluated according to the World Health Organization Guidelines (2010). We also calculated motile sperm concentration (MSC) and total motile sperm count (TMSC). The seminal Zn concentration (mol/L) was determined by a spectrophotometric method. The study didn't include patients with leukospermia ($>1x10^6$ml) and disorders of the reproductive system (varicocele, epididymitis, prostatitis). The Mann-Whitney U-test was used to compare groups. A p-value below 0.05 was considered statistically significant.

Results

The main sperm parameters did not differ significantly between the experimental groups, except slightly lower normal morphology ($p = 0.017$). Furthermore, pre-obese group showed significantly decreased TMSC ($p < 0.01$) while MSC remained unchanged. The overweight men had higher seminal Zn concentration by 20% compared to CG ($p = 0.002$). Within all patients, a seminal Zn was weakly positively correlated with sperm concentration ($r = 0.18$) and BMI values ($r = 0.25$); and negatively correlated with normal morphology ($r = -0.19$).

Conclusions

Most of the basic sperm parameters didn't change in patients with overweight, except slightly lower normal morphology and significantly decreased total motile sperm count. However, the levels of seminal Zn were increasing in pre-obese patients and were weakly positively correlated with BMI and sperm concentration. We suggest that it may be related to the involvement of Zn in the stabilization processes of cell membrane and chromatin, as well as to the Zn-mediated reduction of oxidative stress in overweight men.

doi: 10.1016/j.cca.2022.04.429

W225

Physiologic dyslipidaemia in preganncy can become pathologic to precipitate preeclampsia in gravid African women; Experience in Ile-Ife, Souwthwest Nigeria

G.O. Adeosun

Department of ChemicalPathology, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Osun State, Nigeria

Background-aim

To evaluate the effect of sequential alterations of plasma lipid and lipoprotein in the etiology of preeclampsia and the gestation age at which dyslipidemia became pathologic and suspicious to precipitate preeclampsia.

Study design: We undertook longitudinal research on pregnancy at the antenatal clinics of a teaching hospital south-west Nigeria in two phases. The semi –longitudinal phase was conducted among 79 pre-eclamptic pregnancy and 80 normotensive healthy pregnant women monitored from 3rd trimester to 3 days post-partum., while the complete longitudinal phase involved 10 preeclamptics and 20 normotensive healthy pregnant women followed up from the first trimester of pregnancy to six weeks post-partum

Methods

Study design: We undertook longitudinal research on pregnancy at the antenatal clinics of a teaching hospital south-west Nigeria in two phases. The semi –longitudinal phase was conducted among 79 pre-eclamptic pregnancy and 80 normotensive healthy pregnant women monitored from 3rd trimester to 3 days post-partum., while the complete longitudinal phase involved 10 preeclamptics and 20 normotensive healthy pregnant women followed up from the first trimester of pregnancy to six weeks post-partum.

Methods: Venous blood samples obtained from the participants at each point of contact (trimester) during the study period were placed into K2 EDTA anti-coagulant specimen bottles for the assay of total cholesterol (TC), high-density lipoprotein (HDL) and triglyceride (TG) using enzymatic/spectrophotometric methods while low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) was calculated using Friedewald's formula.

Results

Lipid fractions were significantly altered ($p < 0.001$) in preeclamptic pregnancies compared with the controls. Triglyceride alterations became pathologic to suspect the development of preeclampsia at 10.9 weeks of gestation (ODR = 29.952, CI = 1.046-857.998@$P < 0.05$. The lipids regressed significantly to almost pre-pregnancy values at 6 weeks postpartum.

Conclusions

Alterations of plasma lipids in pregnancy can be pathologic and is a risk factor of preeclampsia.

doi: 10.1016/j.cca.2022.04.430

W226

NGS-based pipeline for routine non-invasive preimplantation genetic assessment in IVF

K. Gombos [b], B. Galik [d], K. Godony [c], A. Varnagy [c], J. Bodis [c], A. Gyenessei [d], G.L. Kovacs [a]

[a] *Department of Laboratory Medicine and Szentagothai Research Centre, MTA-PTE Human Reproduction Scientific Research Group, University of Pecs, Hungary*
[b] *Department of Laboratory Medicine and Szentagothai Research Centre, University of Pecs, Hungary*
[c] *Department of Obstetrics and Gynecology and MTA-PTE Human Reproduction Scientific Centre, University of Pecs, Hungary*
[d] *Szentagothai Research Centre, University of Pecs, Hungary*

Background-aim

Non-invasive preimplantation genetic testing for aneuploidy (NIPGT-A) facilitates embryo assessment. Using genome-wide analysis of DNA in the culture medium we assume that the detected DNA exclusively arises from DNA leakage from the trophectoderm and inner mass cells. However, there are many resources of contamination (from the polar bodies, cumulus cells and fragmented DNA bound to human albumin). Our aim was to provide a clinical strategy for NIPGT-A that is based on next-generation sequencing (NGS) technology and an optimized bioinformatic pipeline.

Methods

3rd day spent embryonic culture media (SECM) from 542 ICSI fertilized embryos with quality morphology scores were collected together with blank culture media as control. 184 SECM samples were used (miscarriage group = 20; healthy neonates = 83). From the latter group 20 were selected for NGS. MALBAC amplification was carried out and NGS sequencing was performed on Illumina HiSeq 4000 instrument, with 50-bp single-read configuration. After raw data quality control and filtering sequences were mapped to the Homo sapiens GRCh37 genome. Results were summarized using QualiMap bamqc tool v2.2.1. Chromosomal abnormalities were identified based on copy-number variation

algorithms. Sequencing resulted on average 12 M reads (50bp single end) per sample.

Results

OR calculations confirmed statistically significant difference between the culture media droplets of aborted embryos and the control media ones. Evaluation of the CNV results in the 1Mb bins of the autosomes and comparing the results different databases revealed 17 relevant chromosomal alterations, that occurred only in the aborted embryo group and were related to registered chromosomal alterations and major developmental impairments. We identified the DNA fraction related to cultured embryo containing carryover human gDNA contamination. Human embryos that show competence for blastocyst development and successful pregnancy are different in culture media gDNA content compared with embryos that abort after implantation.

Conclusions

Analysis of DNA profiles of day 3 SECM demonstrated that a higher gDNA copy number is associated with impaired intrauterine development and miscarriage. Our workflow can be integrated into clinical practice and can supplement existing NIPGT screening methods.

doi: 10.1016/j.cca.2022.04.431

W227

The role of plasma N-terminal pro-B type natriuretic peptide level in the diagnosis of pregnancy complications and prediction of neonatal outcomes

F. Yu, Y. Jiang

Department of Laboratory Medicine, West China Second University Hospital, Sichuan University, Chengdu, China

Background-aim

Preeclampsia (PE), gestational hypertension (GH) and gestational diabetes mellitus (GDM) are the major pregnancy complications that pose important threats to the health of pregnant women and newborns. The purpose of this study was to investigate the diversity of plasma N-terminal pro-B type natriuretic peptide (NT-proBNP) levels in the pregnancy complications and to clarify its predictive effect of pregnancy outcome.

Methods

Two hundred and eight singleton pregnant women who were executed regular obstetric examinations in West China Second University Hospital from August 2015 to October 2018were selected in this study, including early-onset PE group(n = 52), late-onset PE group(n = 32), GH group(n = 21), GDM group(n = 49) and normal pregnant women as control group(n = 54). The concentrations of NT-proBNP among different groups were measured and the correlation between NT-proBNP levels and pregnancy complications was analyzed.

Results

The plasma NT-proBNP levels in early-onset PE group and late-onset PE group were significantly higher than those in other groups ($P < 0.05$). The Receiver Operator Characteristic (ROC) curve study showed the plasma NT-proBNP levels had excellent diagnostic performance for early-onset PE group and late-onset PE group. The areas under the ROC curve (AUC) were 0.864 and 0.825 when the cut-off value was 142.3pg/ml and 183.5pg/ml for these two groups, respectively. The plasma concentrations of NT-proBNP were positively correlated with neonatal outcomes, the AUC was 0.788 when the cut-off value was 257.5pg/ml.

Conclusions

NT-proBNP was an effective indicator for differential diagnosis of pregnancy complications and prediction of newborn outcomes, and can be used for monitoring of early-onset PE and late-onset PE.

doi: 10.1016/j.cca.2022.04.432

W228

Pregnancy complications in Filipino women

C.A. Durante

University of Perpertual Help – Dr. Jose G. Tamayo Medical University, Philippines

Background-aim

There is an increasing trend of pregnancy complications in the Philippines. However, these are not well documented due to the lack of a national database. This study describes the prevalence of hypertension and lipid abnormalities among Filipino pregnant women, which result to complications during pregnancy.

Methods

Multi-stage stratified random sampling was employed and 6,166 pregnant women were included in the study. Demographic data was gathered through an interview questionnaire. Blood samples were drawn to determine fasting blood sugar (FBS), cholesterol, HDL and LDL, and triglycerides levels.

Results

One in ten pregnant women (n = 661) showed signs of pregnancy complications. High risk pregnancies were identified, ranging from heart disease (2.9%), gestational diabetes (3.2%), asthma (3.51%) and goiter (4.12%). Prevalence of pregnancy-induced hypertension is 18.26%. Dyslipidemia is high among sample population with a prevalence of 39.54% (n = 376). Results showed that patients with hypertension are twice more likely to have pregnancy complications than those with normal BP (OR:1.95, CI 0.47, 6.08).

Conclusions

Based on the data, there is a high prevalence of pregnancy complications in the Philippines which is associated with having hypertension and lipid abnormalities. Health policies should be made to cater to the early detection of disease in pregnant women and widespread campaign for affordable prenatal check-ups.

doi: 10.1016/j.cca.2022.04.433

W229

Association of serum homocysteine, vitamin B12 and folate levels in pre eclamptic women

S. Pradhananga

Institute of Medicine, TUTH, Nepal

Background-aim

Pre-eclampsia is a syndrome that chiefly includes the development of new-onset hypertension and proteinuria after 20 weeks of pregnancy. Improper placentation is mainly responsible for the disease. Hypertension, diabetes mellitus, nulliparity, multiple pregnancies and thrombotic vascular disease contribute as the risk factors for pre-eclampsia. The severity of disease is a consequence of thrombocytopenia, disseminated intravascular coagulation, central nervous system abnormalities, renal failure and hepatocellular damage. Hyperhomocysteinemia may be a cause of the endothelial dysfunction provoked by oxidative stress in pre-eclampsia.

Methods

This is a cross sectional study involving seventy-two subjects with pre-eclampsia who met inclusion and exclusion criteria. Cases were divided into mild and severe as per American College of Obstetricians and Gynecologists. Blood pressure, Urine protein, serum Homocysteine, Vitamin B12 and folate levels were compared in both mild and severe forms of pre-eclampsia. Concentration of Vitamin B12 and folate were measured using Vitros ECI and homocysteine was measured using CLIA. SPSS 23.0 was used to analyze the data. Tests were performed with Mann Whitney Test and Spearman's rank correlation test. A p-value <0.05 is considered statistically significant.

Results

This study shows no significant difference in age and weeks of gestation in both mild and severe forms of pre-eclampsia. Mean concentration of homocysteine is higher (13.1± 6.41micromol/L) in severe Pre-eclampsia as compared to mild cases (7.67 ± 2.86 micromol/L). Mean concentration of folate is lower in severe cases (35.4± 24.18 micromol/L) when compared with mild cases of pre-eclampsia (57± 23.48 micromol/L).

Conclusions

Homocysteine levels are increased in severe Pre-eclampsia when compared with mild pre-eclampsia. Folate levels are decreased in severe Pre-eclampsia as compared to mild pre-eclampsia. Homocysteine levels can be considered as a predictive risk factor of pre- eclampsia but not yet a screening tool.

doi: 10.1016/j.cca.2022.04.434

W230

Association between low serum Vitamin B12 and high folate level in pregnant women with gestational diabetes mellitus

S. Shakya, B.K. Yadav, E.T. Tuladhar, P. Paudyal

Institute of Medicine, TUTH, Nepal

Background-aim

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. There is disturbance in insulin secretion and its utilization.The exact pathophysiology is still unclear. However, obesity, maternal age, family history of Diabetes Mellitus (DM)are the common risk factor associated with GDM. Vitamin status during pregnancy is also important as its requirement is increased during pregnancy. Folate and Vitamin B12 are linked with insulin resistance through its metabolite; homocysteine. Deficiency of any one can lead to increase in homocysteine and shows the effects. Folate is supplemented in early pregnancy however B12 is not given. Thus, deficiency of Vitamin B12 if present during pregnancy will be masked by folate supplementation which will be responsible for the development of insulin resistance in GDM.

Methods

This is a cross-sectional study done in ninety-one GDM women at 24–28 weeks of gestation visiting outpatient department (OPD) or admitted in the ward of Gynecology and Obstetrics, Tribhuvan University Teaching Hospital. Serum Folate, B12 and Insulin were measured with Chemiluminescence assay. SPSS ver. 20.0 was used to analyze the data. Statistical analysis was performed with t-test, Mann–Whitney test, Spearman'srank correlation test and Chi square test. Data were expressed in terms of mean ±SD and median. A p-value <0.05 is considered statistically significant.

Results

High folate and low B12 was found in 59 women with GDM with 87.78±33.83ng/ml and 224.71±96.47 pg/ml respectively. The mean age and BMI of GDM participant was 30.82 years and 25.14 kg/m2. Women with GDM were tended to be multiparous, had positive family history of diabetes mellitus, and supplemented with folate in early gestation period. The median value of HOMA IR was 2.08 which was found to be significantly correlated with fasting sugar, HbA1C and B12 level. Similarly, association of BMI and B12 was found to be significant with p value <0.05. However, no association was found between folate and B12, HOMA IR. Significant difference in mean of HOMA IR was noticed in different group of age, BMI, fasting sugar, HbA1C and B12.

Conclusions

High folate coupled with B12 deficiency was found in GDM women with increased HOMA IR value suggesting its pathological role in GDM development. This finding has potential implications for antenatal supplement recommendations but will require confirmation in future studies.

doi: 10.1016/j.cca.2022.04.435

W231

Evaluation of serum Anti Mullerian Hormone level in expected low prognosis group women treated of in-vitro fertilization/intra cytoplasmic sperm injection

F.R. Marpaung [a], A.S. Priyanto [b], F.A. Kusumawati [b], S. Soehita [a]

[a] *Department of Clinical Pathology, Dr.Soetomo Academic Hospital, Faculty of Medicine, Universitas Airlangga, Indonesia*

[b] *Department of Obstetric and Gynaecology, Morula Clinic, National Hospital Surabaya, Indonesia*

Background-aim

The use of Anti Mullerian Hormone level in predicting outcomes of in-vitro fertilization/ intra-cytoplasmic sperm injection (IVF/ICSI) in poor responder women has been widely used. Since the term "poor responder" of Bologna criteria has been changed to "low prognosis" according to the POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) criteria, which divided low prognosis into 4 groups, therefore these study aim was to evaluate serum AMH levels in the group suspected of low prognosis women treated with IVF/ICSI

Methods

A total of 252 patients of suspected low prognosis groups were assessed retrospectively between 2018 and 2019 in Morula Clinic, National Hospital, Surabaya, Indonesia. Serum AMH cut off values determined for low prognosis groups. Serum AMH levels and pregnancy rate were compared among 4 groups of suspected low prognosis women treated IVF/ICSI

Results

In this study, the serum AMH cut off value for predicting low prognosis women using POSEIDON criteria was 1.7 ng/mL with sensitivity 86.7% and specificity 70%. The serum AMH levels were significant differences in 4 groups of suspected low prognosis women treated IVF/ICSI ($p < 0.05$). Interestingly, there were no differences in pregnancy rates among those groups.

Conclusions

Serum AMH level could predict low prognosis women treated IVF/ICSI. It is important to determine cut-off value to predict the low prognosis women. The pregnancy rate among low prognosis groups was not differences.

doi: 10.1016/j.cca.2022.04.436

W232

The value of measuring NK cell fraction in Korean women with reproductive failure

M. Park [b], E. Pack [a], S.G. Lee [b], S. Park [b], R. Choi [b], Y. Kim [b], M. Min [a]

[a] *Department of obstetrics and gynecology, Bundang Cheil Women's Hospital, Bundang, Republic of Korea*
[b] *Laboratory Medicine, Green Cross Laboratories, Yongin, Republic of Korea*

Background-aim

The Korean Society for Reproductive Immunology recommends immunotherapy in women with reproductive failure such as recurrent miscarriage and/or implantation failure, and show abnormal natural killer (NK) cell levels or cytotoxicity. However, the prognostic value of measuring peripheral NK (pNK) cell parameters still remain uncertain and more studies are needed. The aim of this study is to assess the value of pNK cell measurement in Korean women with reproductive failure.

Methods

We enrolled total 226 women who visit Bundang Cheil Women's Hospital and test NK cell fraction in GC Labs, from June to December, 2018, and analyzed retrospectively. The NK cell fraction and count were tested by flow cytometric analysis, Cytomics FC500 (Beckman-Coulter, France). We reviewed the medical records to investigate obstetrical history, medical history, obstetrical course of the patients. The data was analyzed with independent t-test and Chi square test using MedCalc statistical software (version 19.1.5, MedCalc Software bv, Belgium).

Results

In total 226 patients, the median age was 36 years old, range 25 - 44. Almost patients had infertility (217/266, 81.6%) and 96 patients had recurrent miscarriage (42.5%). The mean peripheral NK cell fraction in lymphocytes was 13.8%, range 3.2% – 41.7%. Sixty-six patients (29.2%) had underline etiology of pregnancy morbidity, such as genetic abnormality, morbidity of uterus or ovary, disease of thyroid, etc. There is no significant difference of NK cell fraction according to recurrent miscarriage or etiology. The 72 patients had recurrent miscarriage or infertility, no underline etiology, and increased NK cell fraction ($> 12\%$). In this population, 51 patients (51/72, 70.8%) received immunotherapy (IVIG or steroid) and they had significantly better pregnancy success (implantation success and no morbidity until 20 weeks gestational age) than those who did not ($P = 0.028$).

Conclusions

In women recurrent miscarriage and higher NK cell fraction than 12%, patients who received immunotherapy had better pregnancy outcome than those who did not. Therefore, measuring peripheral NK cell fraction has value for screening women who may benefit from immunotherapy. Further studies are needed to confirm this result.

doi: 10.1016/j.cca.2022.04.437

W233

Correlation of Pregnancy Associated Plasma Protein A (PAPPA) and free beta human chorionic gonadotrophin (FBHCG) measurements between Roche Cobas e-411 and SNIBE Maglumi 4000 plus analyzers

A. Haliassos [b], A. Kapetanaki [c], Y. Jinyun [d], K. Paidousi [a], D. Rizos [c]

[a] *Diamedica, Athens, Greece*
[b] *ESEAP, Athens, Greece*
[c] *Hormone Laboratory, Aretaieio Hospital, Athens, Greece*
[d] *SNIBE-Shenzhen New Industries Biomedical Engineering Co., Ltd, Shenzhen, PR China*

Background-aim

Pregnancy Associated Plasma Protein A (PAPPA) along with free beta human chorionic gonadotrophin (FBHCG) are the most important biochemical markers used in the prenatal screening for chromosomal abnormalities in the first trimester of pregnancy. The most widely used methods for their determination are enzyme-labeled immunometric automated methods using monoclonal antibodies on Immunochemistry Analyzers.

The object of our study was to evaluate the recently released PAPPA and FBHCG assays by SNIBE Diagnostics (Shenzhen, P.R. China) on SNIBE Maglumi 4000 plus analyser, in comparison to Roche Cobas assays on Cobas e-411 analyser.

Methods

Both methods use two monoclonal antibodies but Maglumi uses chemiluminescent detection while Cobas uses electrochemiluminescent detection. We used fresh serum samples from pregnant women in the first trimester of pregnancy.

Results

Data were analysed using the Microsoft Excel software. Values from both analytical systems, SNIBE Maglumi 4000 plus and Roche Cobas e-411 showed excellent correlation and very good agreement as follows:

PAPPA: n = 618, r = 0.975, Slope = 1.124; 95% CI [1.104 – 1.144], Intercept = -0.116; 95%CI [-0.202 to -0.030].

FBHCG: n = 620, r = 0.965, Slope = 0.946; 95%CI [0.926 – 0.966], Intercept = 4.9; 95% CI [3.7 – 6.1]

As a remark, SNIBE measurements are about 12% higher than Roche for PAPPA. Two samples showed repeatedly extremely different PAPPA measurements between Roche and SNIBE methods and were excluded from the analysis (sample1: 0.992 IU/L Roche, 4.897 IU/L SNIBE; sample2: 1.121 IU/L Roche, 23.33 IU/L SNIBE). We are investigating possible reasons that interfered in these measurements.

Conclusions

Our results showed that the very good correlation between the two analytical systems, for both parameters, permits the use of PAPPA and FBHCG values interchangeably, provided that the above regression equations are used in any case.

doi: 10.1016/j.cca.2022.04.438

W234

Prevalence of dyspermia in infertile African black men

I.L. Uke [c], M. Charles-Davies [c], P. Carrera [d], M. Ferrari [d], O. Amodu [a], O.M. Akinosun [b]

[a] *Molecular Biology Unit, Institute of Child Health, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[b] *Molecular Biology/Toxicology Unit, Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[c] *Reproductive Endocrinology Unit, Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[d] *St Raphael's Hospital, Milan, Italy*

Background-aim

Dyspermia has been associated with reduced life span as men with impaired sperm counts, sperm motility or semen volume have higher mortality rates compared with men with normal semen quality.

The prevalence of dyspermia and its relationship with age were investigated in infertile men in Nigeria, Africa's most populous black nation.

Methods

A total of 445 men >18years attending the infertile clinic of a tertiary hospital in Ibadan, Nigeria were enrolled consecutively into the study. Seminal fluid analysis was doneusing the WHO 2010 criteria for semen analysis. Data obtained were analysed by Student's t-test and Pearson's correlation coefficient were considered significant at $p < 0.05$.

Results

The mean age of men was 38.0 ± 6.3 years. The prevalence of dyspermia was 42.3% (n = 188).52 (11.7%) were azoopermic, 136 (30.6%) were oligozoospermicwhile 257 (57.8%) were normospermic. Comparison between the age of dyspermic group and normospermic group showed no significant difference($p = 0.894$) and correlation between age and sperm count was not significant ($r = 0.017$, $p = 0.720$).

Conclusions

Dyspermia especially oligospermia may be a significant contributor to male factor infertility in Nigeria and affect about 42% of young men of <40years. Interestingly, it is not associated with age in this study.

doi: 10.1016/j.cca.2022.04.439

W235

A 31-year-old woman with complete androgen insensitivity syndrome

C. Ji [a], T. Tan [b]

[a] *Department of Laboratory Medicine, Sichuan Provincial People's Hospital, Chengdu 610072, China*
[b] *Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu 610072, China*

Background-aim

see Results

Methods

see Results

Results

A 37-year-old Tibetan woman with infertility was referred to the outpatient clinic of the obstetrics and gynecology department of our hospital. She had experienced with a dizziness and weakness for three years. The patient said that she had sporadic menstruation and hypomenorrhea. She took no medications and had no known allergies. Now the patient lived with her husband in a mountain farm. Her mother had conceived her easily. The patient's father and two sisters were healthy; both of her sister had their own children.

On examination, the weight was 60.1 kg, the height 160.8 cm, and the body-mass index (the weight in kilograms divided by the square of the height in meters) 23.2. And she has normal female external genitalia and breast development, with sparse pubic hair and axillary hair. The remainder of the physical examination was normal. Ultrasound and pelvic computed tomography (CT) scan showed normal female external genitalia and absence of cervix and uterus with a blind vaginal; presence of dense symmetric, homogeneous enhancement of gonads structure sized 2.3 × 1.4 cm and 3.2 × 1.7 cm located in the anterior pelvis.

On the basis of the medical history and results on examination, the most likely diagnosis in this case is either mullerian aplasia or complete androgen insensitivity syndrome.

Several day later, chromosomal analysis on the peripheral blood lymphocytes revealed a 46,XY karyotype. Serum hormone measurement showed elevated levels of testosterone (17.23 nmol/L; reference range for women: 0.38–1.97 nmol/L) and anti-mullerian hormone (AMH)

(695.88 ng/ml; reference range for women: 0.24–11.78 ng/ml), and inhibin B (275.49 pg/ml; reference range, <139 in premenopausal women during the follicular phase, <92 in premenopausal women during the luteal phase). These results were consistent with the diagnosis of complete androgen insensitivity syndrome. Then the patient has determined to identify her specific androgen-receptor mutation. Sequences generated from patients were compared with the published AR complementary DNA (cDNA) reference sequences from the National Center for Bio-technology Information (http://www.ncbi.nlm.nih.gov/; GenBank accession number: NM_000044.4). The gene sequencing results showed that a DNA-binding domain site mutation (c.1730G > T), resulting in a cystine to valine substitution and a histidine to phenylalanine substitution.

After four weeks of iron treatment, there were significant effects on hemoglobin, and the patient's fatigue disappeared. We recommended surgical removal of the intraabdominal gonads due to the risk of testicular cancer. But the patient would not to undergo further treatment. It is important to pay more attention to patients with extremely high level of AMH.

Conclusions

see Results.

doi: 10.1016/j.cca.2022.04.440

W236

Effect of metformin on levels of androgen in obese women having PCOS with & without Mg supplementation: A randomized control trial

Y. Bansal [b], N. Sharma [a]

[a] *Department of Biochemistry, All India Institute of Medical Sciences, Bhopal, MP, India*
[b] *Department of Biochemistry, Maharishi Markandeshwar University, Solan, HP, India*

Background-aim

Among the most common endocrinal disorders in females of reproductive age group is Polycystic ovary syndrome. According to Rotterdam's criteria, the prevalence of PCOS worldwide is 3% to 10% and 10 million women are globally affected by this condition and with clinical conditions like hirsutism, oligomenorrhoea, and infertility. As a multifactorial and polygenic disease, hyperactivity of protein Cyt p450c17-alpha encoded by CYP17 is responsible for PCOS causing increased luteinizing hormone (LH) release and thus the action of LH on theca cells is amplified and they work more similar to the testicular Leydig cells rather than normal ovarian theca cells. So the present study explored the steroidogenic effect of metformin in PCOS women with and without supplementation of Mg.

Methods

This randomized control trial was conducted among 72 obese women with PCOS aged 25-40 years old fulfilling all 3 diagnostic Rottendam criteria. Subjects were randomly divided into three groups where Group A received 240 mg/ day Mg supplementation, Group B received 1000mg metformin per day and Group C women received both (n = 24 each group) for 12 weeks

Results

Serum androgen-related to PCOS were determined at baseline and after the 12th week of treatment. Statistically, a significant difference was found in levels of androgen among all the three groups. Group C resulted in a significant change on levels of 17 Hydropregnenolone (P = 0.0005), 17 Hydro progesterone (P = 0.0003), estrogen (P = 0.005) LH (P = 0.005) & FSH (P = 0.001) as compare to group A & B. Statistically significant changes were observed in the levels of lipid profile in Group C as compare to A& B.

Conclusions

This study demonstrates for the first time that the combination of Mg supplementation with metformin significantly decreased lipid profile and the male androgen levels in obese women & that of the androgen excess with high lipid profile may play a critical role in the steroidogenic pathway of obese patients of PCOS.

doi: 10.1016/j.cca.2022.04.441

W237

Method comparison study of AMH between SNIBE MAGLUMI® CLIA assay and Roche ECLIA assay

S. Cuesta De Juan, J. Garcia Ayuela, A. Mata Fernadez, R.A. Torrado Carrion, M. Garcia-Alcala Hernandez, C. Dacosta Galan, R. Piqueras Picon, A. Siguin Gomez

Central Laboratory in Eurofins Megalab, Spain

Background-aim

AMH (Anti-Müllerian hormone) is a peptide growth factor of the transforming growth factor-® superfamily that is produced by growing ovarian antral follicles, which is well known for its role in sexual differentiation and is a reliable marker of ovarian reserve.

The clinical use of AMH as a predictive and diagnostic marker of infertility, menopause onset, a diagnostic tool for polycystic ovary syndrome and assessment of ovarian function has recently been extended and emphasized

The aim of this work was to evaluate the concordance between two direct methods for the determination of AMH: Sandwich Chemiluminescence Immunoassay (CLIA) and Sandwich Electrochemiluminescence Immunoassay (ECLIA).

Methods

A total of 184 serum samples were included in this laboratory comparison study, 183 females and 1 male (ages range from 0 days to 50 years old).

The analytical study of quantitative determination of AMH in human serum was measured with kits for CLIA on the SNIBE (Shenzhen New Industries Biomedical Engineering Ltd, China) MAGLUMI 1000 and kits for ECLIA on Roche platform (Roche Diagnostics Ltd, Switzerland) Cobas e601. The results were analyzed with Passing-Bablok regression and Bland-Altman plot.

Precision for MAGLUMI CLIA AMH assay was determined by testing 1 control pool and 3 human serum pools (Low, Middle, High concentration) in three replicates with 30 samples per run at two independent runs per day for 5 testing days.

The statistical test used was the XLSTAT 2018 Software Statistical the Microsoft Excel®

Results

The correlation between these 2 methods showed a coefficient of correlation r = 0.99 (p < 0.001) the intercept was -0.0834 and the slope 0.888 (y = -0.0834 + 0.888x).

The result of the assay for the pool serum 1 was SD 0.029 mg/mL and CV 2.76%, for the pool serum 2 was a SD 0.121 ng/mL and CV 2.40%, pool serum 3 showed a SD 0.577 ng/mL and CV 4.54% and de pool control SD 0.365 ng/mL and CV 2.40%. All de pools serum showed a good repeatability between run and day. The total precision of the assay was 2.40%-4.54%

Conclusions

Our comparison study showed a high concordance and high correlation result of AMH between MAGLUMI CLIA assay and Roche ECLIA assay. The precision of MAGLUMI AMH is good. Thus Maglumi Chemiluminescence Immunoassay platform could be a good option for assessing AMH in laboratory use.

doi: 10.1016/j.cca.2022.04.442

W238

Development of an external quality assessment (EQA) programme for pre-eclampsia markers

G.J. Davies, S. Jones, M.A. Thomas

Weqas, Unit 6, Parc Ty Glas, Llanishen, Cardiff CF14 5DU, United Kingdom

Background-aim

Pre-eclampsia (PE) continues to be a poorly understood complication of pregnancy, affecting 2 to 8% of pregnant women worldwide with a UK rate of approximately 3%. The UK National Institute for Health and Care Excellence guideline on hypertension in pregnancy defines pre eclampsia as new hypertension with significant proteinuria after 20 weeks' gestation. These non-specific symptoms result in a diagnostic accuracy of only 30% in those suspected of having the condition.

Placental growth factor (PlGF) and Soluble fms-like tyrosine kinase-1 (sFlt-1) (as a ratio with PlGF) have emerged as promising markers to help diagnose and rule out Pre-eclampsia.

In 2020, Weqas developed an EQA programme to assess and monitor the performance of these tests.

Methods

Initially spent patient samples were collected and provided by Oxford University Hospitals. These samples were sent out for the first 5 distributions with further samples prepared from EDTA plasma spiked with recombinant sFlt-1 and PlGF. Three samples were distributed every month to approx. 40 participants over a 20 month period.

Results

The Roche Elecsys immunoassay for sFlt-1 and PlGF was used by approx. 25 participants with users reporting sFlt-1/PlGF ratio as a marker of Pre-eclampsia. The Quidel Triage Meter immunoassay for PlGF was used by approximately 15 participants.

CVs of 3-7% were observed for the Roche sFlt-1 assay, 5-18% for the Roche PlGF assay and 4-14% for the Roche sFlt-1/PlGF ratio. The Quidel Triage PlGF assay reported CVs of 10-25%.

For the Roche Pre-eclampsia risk interpretation element the majority of reported results agreed with the Weqas interpretation.

Weqas Interpretation 'Unlikely': 95% reported 'Unlikely' (95% Specificity)

Weqas Interpretation 'Elevated Risk': 89.6% reported 'Elevated Risk' or 'Very High Risk'

Weqas Interpretation 'Very High Risk': only 1.3% reported 'Unlikely'

Conclusions

The Roche Pre-eclampsia risk showed a sensitivity of 94% for 'Non-Negative' Result (only 6% false Negatives). The data suggests this can be used as a rule-out test due to its high sensitivity.

For the Quidel Triage PlGF assay when PlGF < 12 (test Positive, highly abnormal) 100% reported < 12 (highly abnormal), when PlGF > 100 (test Negative, normal) 94% reported > 100 (normal).

doi: 10.1016/j.cca.2022.04.443

Clinica Chimica Acta 530 (2022) S414–S419

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Sampling, Storage, and Physiological Variations

M234

Evaluation of the quick clotting serum separate tube, VQ-tube, a product for clinical chemistry and thyroid hormone assays

S.J. Jo [b,*], H. Chae [b], Y. Lee [a], S.H. Song [c], J. Lee [b]

[a] *Department of Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital, Soonchunhyang University College of Medicine, Bucheon, Republic of Korea*

[b] *Department of Laboratory Medicine, College of Medicine, the Catholic University of Korea, Seoul, Republic of Korea*

[c] *Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea*

Background-aim

Blood collection tube affects specimen quality and laboratory results. Plasma specimens has shorter processing time compared to serum specimen. Serum specimen remains stable after centrifugation and differences in test results between plasma and serum has been reported. Quick clotting serum separate tube (SST) is expected to be useful for shorter turnaround time and accurate test results. A new quick clotting SST VQ-Tube™ (AB Medical, Korea) requires 5 minutes clotting time to obtain serum specimen. We compared test results obtained when blood was collected in VQ-Tube™ and current commerce SST V-Tube™ in clinical chemistry and thyroid hormone assays.

Methods

One hundred volunteers from four hospitals were recruited and the peripheral blood samples were collected in each of the tubes. The collected samples in VQ-Tube™ were processed 5-minute clotting before centrifugation and samples in V-Tube™ were processed 15-minute clotting as manufacturer's recommendation. All samples were visually reviewed for serum clarity after centrifugation. Obtained specimens were used for 16 clinical chemistry assays (protein, albumin, cholesterol, total bilirubin, direct bilirubin, AST, ALT, ALP, GGT, glucose, BUN, creatinine, LD, sodium, potassium, chloride) and 3 thyroid hormone assays (T3, fT4, TSH).

Results

The differences (%) of the test results obtained from the samples in each tube were satisfied with CLIA '88 allowable difference ranges (19 assays). The differences of test results between tubes were satisfied desirable specification for inaccuracy except for glucose (2.75%). The paired t-test revealed significant differences between the results of 6 assays but each set of results showed good correlations (GGT, 0.999; creatinine, 0.988; glucose, 0.989; LD, 0.987; potassium, 0.947; TSH, 0.990). 3 samples showed turbid serum conditions both VQ-Tube™ and V-Tube™ but differences of test result were not significantly different.

Conclusions

The new quick clotting VQ-Tube™ demonstrated reliable test results compared to commonly used SSTs. This quick clotting tube will provide fast test results with adequately separated serum specimens.

doi: 10.1016/j.cca.2022.04.445

M235

Contradiction between separation gel coagulation tube and heparin anticoagulation tube

L. Pang [*], N. Duan, C. Huang, Y. Xing, J. Du, H. Huang, H. Li

Department of Clinical Laboratory, Peking University First Hospital, Beijing, China

Background-aim

A 57-year-old woman presented to the emergency department with a 1 month of weakness and fatigue. The separation gel coagulation tube showed less serum, which indicates the polycythemia vera. However, the heparin anticoagulation tube showed less cells, which is in accordance with the hematocrit.

Methods

Hemoglobin, calcium, immunoglobulin and beta 2-microglobulin were measured. We also performed serum immunofixation electrophoresis, peripheral blood smear, bone marrow aspiration and flow cytometric analysis.

Results

Laboratory studies revealed hemoglobin level of 7.7 g/dL, calcium level of 2.98 mmol/L, IgG level of 78.9 g/L and beta 2-microglobulin level 6.13 g/mL. The immunofixation electrophoresis showed M protein

of IgG lambda. A peripheral blood smear showed rouleaux formation (red cells stacked up like a pile of coins). Bone marrow aspiration was performed and showed 53% proplasmacyte. Flow cytometric immunophenotyping of plasma cells from the bone marrow showed diminished CD19 and CD27 expression, with aberrant expression of CD56, CD20 and monoclonal cytoplasmic lambda light chain expression. The patient was diagnosed as multiple myeloma and treated with bortezomib-melphalan-prednisone (VMP) regimens.

Conclusions

In this case, the gel in the separation gel coagulation tube was mixed with the serum, despite correct centrifugation. This anomalous behavior which we called floating gel is likely attributable to the high density of serum. This irregular distribution of RBC usually occurs when plasma protein concentrations are high, especially M protein in multiple myeloma.

doi: 10.1016/j.cca.2022.04.446

M236

ACTH – Important sample collection, storage and processing requirements

C.H.C. Tan [*], Z. Wong, C.P. Yeo

Clinical Biochemistry Laboratory, Department of Clinical Pathology, Singapore General Hospital, Singapore

Background-aim

Adrenocorticotropic Hormone (ACTH) is used for investigating adrenal and pituitary diseases and ectopic ACTH production. It is notoriously unstable in-vitro due to proteolysis; needs for sample collection in pre-cooled tubes and expedited processing posed logistical challenges. This paper aimed to study the impact of sample tube condition, storage temperature and processing time; and review our laboratory's current protocol.

Methods

ACTH was analyzed on Roche e801 immunoassay analyzer. 4 K2EDTA tubes of blood (3 pre-cooled and 1 non-pre-cooled tubes) were collected from each of 35 healthy subjects recruited with informed consent, centrifuged to obtain plasma for analysis. The first pre-cooled tube and non-pre-cooled tube were analyzed immediately after sample collection. The second pre-cooled tube was kept at room temperature for 4hr then analyzed. The third pre-cooled tube was kept at 2-8˚C for 4hr, centrifuged, 2 aliquots made; one analysed straight after, the other kept at 2-8˚C for additional 2hr then analysed. Percent difference between the first pre-cooled tube and other tubes was generated via difference plots on Analyze-It software.

Results

Difference in ACTH between pre-cooled and non-pre-cooled tubes, when analysis took place immediately after sample collection, was not clinically significant (mean bias 1.5%; 95%CI:0.1-2.8%). ACTH deterioration relative to storage time was evidenced by lowered ACTH when samples in pre-cooled tubes were kept at 2-8˚C for 4 hours before analysis (mean bias -4.4%, 95%CI:-5.8 to -3.0%); and further decrease when plasma was kept for another 2hours before analysis (mean bias -10.2%; 95%CI -14.3 to -6.1%). Storage in cool temperature helped; ACTH decrease in samples taken in pre-cooled tubes then kept at 2-8˚C (mean bias -4.4%, 95%:-5.8 to -3.0%) was less, as compared to ACTH decrease in samples taken in pre-cooled tubes then left standing at room temperature (mean bias -9.8%, 95%CI: -11.5 to -8.1%).

Conclusions

ACTH is stable when samples was collected, kept at 2-8˚C and analysed within 4 hours. Our study did not demonstrate that sample collection in pre-cooled tubes was essential. As this observation may be unique to the tropical climate, laboratories should perform stability studies to assess optimal sample processing procedures.

doi: 10.1016/j.cca.2022.04.447

M237

Sampling time during hemodialysis treatment: Urgent leukocytes count

P. Desoski [a,*], R. Grozdanovski [b], A. Onchevski [c], S. Gelev [b], E. Babalj-Banskolieva [b], B. Gjorgjievska [b], K. Spasovska [b], M. Ilievski [b], I. Nermin [b]

[a] *Biochemical Laboratory, Special Hospital for Nephrology and Dialysis, "Diamed", Skopje, North Macedonia*
[b] *Hemodialysis unit, Special Hospital for Nephrology and Dialysis, "Diamed", Skopje, North Macedonia*
[c] *Vascular accesses, Special Hospital for Nephrology and Dialysis, "Diamed", Skopje, North Macedonia*

Background-aim

Regular sampling for patients on hemodialysis (HD) is done prior the treatment. But in certain circumstances (usually when suspicion for infection is present) sampling is performed during the treatment as well. Many studies regarding leukocytes (WBC) decrease (as a result of dialysis membrane biocompatibility) and their kinetics during HD has been present, but none regarding proper sampling time. This pilot study considered hemoconcetration, membrane biocompatibility and presence of heparin in patient's blood versus most proper time for WBC sampling in ethylenediaminetetraacetic acid (EDTA) containing tubes during HD.

Methods

29 patients (age 61 ± 20) during one HD treatment (4h 30min each, on polyethersulfone biocompatible high flux membrane and continuous unfractionated heparin treatment -3300 IE average) throw arteriovenous fistula were analyzed. Sampling was in K_2EDTA tubes: prior the start of HD (control measurement, all patients with WBC between 4-10 x10^9/L), 5 minutes (min) after each heparin administration (5, 65, 125, 185 and 245 min) and after HD. WBC were analyzed 5-15 min after sampling, on Sysmex XP-300 hematological analyzer, and findings further corrected for hematocrit changes (to exclude the hemoconcetration effect during HD). Comparison of corrected values was done between control and each other measurement. WBC kinetic curves were plotted (inter measurements and intraindividual).

Results

Statistically significant decrease of WBC compared to pre HD measurement was detected only in the sample taken on the 5th min (p-

0.025), most probably induced by initial contact with biomembrane. The kinetics during HD showed steadily stabilization of WBC, but not reaching the starting value (excluding two patients that showed final increase). Even though heparin and EDTA were continuously present in the same sample, insignificant variations between measurements during the rest of the treatment, suggests no important interference.

Conclusions

Sampling for WBC count in the first 45 min of HD should be avoided, and for the rest of the period interpreted with caution (only if urgent). What stays for further investigation, is why the rise of WBC was present in some (6.6%) of the patients.

doi: 10.1016/j.cca.2022.04.448

M238

Urine biobanking: The effect stability of urinalysis in collection for DNA purification on different storing time and temperature of the urine sample

R.I. Ahmad Mulyadi Lai [a,*], H.I. Ahmad Mulyadi Lai [c], S. Ventasamia [b]

[a] *Clinical Biochemistry & Immunology, Geneflux Bioscience Sdn Bhd, Selangor, Malaysia*
[b] *Faculty of Medicine, Universitas Kristen Indonesia, Jakarta, Indonesia*
[c] *Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan*

Background-aim

Urinalysis tests are the cheapest, fasted and easiest source of the specimen, which contain a boundless amount of information on individual health status. In recent years, urine specimens becoming an important source of information for biomarker studies such as bladder cancer. Therefore, it is important to have an optimized procedure for a storage urine specimen to maintain the stability and quality of DNA. In this study, we seek to investigate the effects of urinalysis and DNA quality results test between urine storage at room temperature, urine storage at refrigerated 2˚C-8˚C and frozen at -20˚C.

Methods

This was a pilot study based on a convenience sample from a random urine sample. The urinalysis tests involve the molecular application, physical, chemical and microscopic examination of urine. Chemical tests and microscopic examination results are highly dependent on the type of urine sample, storage time, and storage temperature before the examination. A urine sample collected in sterile collection cups. The sample was divided into 3 storage conditions: room temperature (RT), refrigerated at 2˚C-8˚C and frozen at -20˚C. Urinalysis test was done after collection at intervals of 0, 12 and 24 hours. For chemical examination was a test using dipstick tests, while for microscopic examination viewed under microscope. DNA extraction kits was used to extract DNA and NanoDrop spectrophotometers was used to evaluate the integrity and concentration of DNA.

Results

A significant increase of samples with crystal formation was observed in samples stored at room temperature for up to 4 hours. Some samples stored at room temperature showed an increase in pH and the presence of nitrite and a decrease in the sample's leukocytes and glucose. The results showed that samples stored under refrigerated effectively to maintain the stability of samples compared samples stored at room temperature but DNA concentration is decreased compared to frozen urine specimens. Our results comprised of optimum high DNA concentration for different storage, it shown that in room temperature the maximum storage for good DNA quality is not more than 6 hours, refrigerated at 2˚C-8˚C not exceed than 24 hours while frozen concentration well preserve DNA quality up to 20 days.

Conclusions

In this study, we optimize the suitable temperature and maximum storage time for DNA extraction in a urine specimen. Suggested that urine specimens should be stored at refrigerated at 2˚C-8˚C to preserved and maintain the physical and chemical characteristics and frozen at -20˚C for molecular diagnosis. Furthermore, our study highlight that prolongs the storage of urine in room temperature can enhance more precipitation of crystal.

doi: 10.1016/j.cca.2022.04.449

M239

False elevation of tumor markers: Chromogranin a and gastrin due to use of proton pump inhibitors

V. Vidranski [*], D. Krilic, B. Ladika Davidovic, J. Perisa, I. Mlakar Pedisic, I. Samija

University Hospital Centar Sestre milosrdnice, Zagreb, Croatia, EU

Background-aim

Importance of preanalytics as the most vulnerable part of the total testing process is well known in laboratory medicine. Gastrin (G) is the main hormone stimulating gastric acid secretion, but it exerts proliferative and anti-apoptotic actions on various cancer cell types. Chromogranin A (CgA) is small glycoprotein located in the core vesicles of many neuroendocrine cells. Most frequent cause of false elevation of CgA and gastrin is use of H2 blockers or proton pump inhibitors (PPI). It is still uncommon to ask the patient before sampling about taking PPI as well as it's not writen how many days patient must stop taking PPI to avoid false positive values. High efficacy of PPI make them most frequently prescribed classes of drugs worldwide (studies-more than 30% of patients). The plasma elimination as half-life is approximately 1-1.5 hours when 80% of an oral dose is excreted as inactive metabolites in the urine and feces.

Methods

41 year old female healthy volunteer took blood day before (blood 1) taken PPI as esomeprazole (Emanera, Krka, Slovenia, EU), 20mg /every 12h; 48 days after continuously taking medicine (blood 2) and then 5th (blood 3) and 13th (blood 4) day after stopping with medicine. Blood samples (red cap, 6mL, sera samples) (Greiner Bio-One, Austria) were collected between 8 – 10 am, after overnight total fast (no water taken). Tubes were immediately centrifuged 4^0C /10min/2150G at Rotina32A (Hettich, Germany). Gastrin was done on Immulite2000xpi (Siemens, Germany) and CgA by ELISA (MRX Dynatech, USA). Normal ranges: gastin 13-115 ng/L, CgA <100 g/L

Results

CgA = 31 and G = 29 in blood 1 were within normal ranges, in blood 2 highly elevated CgA = 199 and G = 395, in blood 3 within normal ranges CgA = 37 and G = 33 and in blood 4 normal CgA = 33 and G = 31.

Conclusions

We have confirmed that patients on PPI therapy had significantly higher fasting serum gastrin and CgA. False positive elevation of markers can lead to misinterpretation of laboratory test results and thus to misdiagnosis or course of oncology monitoring. Patients should be asked before blood sampling blood for CgA and gastrin, if they are taking PPI and, in collaboration with clinicians, have their blood sampled 4 days after stopping the drug.

doi: 10.1016/j.cca.2022.04.450

M240

Evaluation of utility of BD vacutainer barricor for clinical routine biochemical analytes in hemodialysis patients receiving anticoagulant therapy

J. Shin *, H. Choi, S. Kee, M. Shin, J. Shin

Department of Laboratory Medicine, Chonnam National University Medical School and Hospital, South Korea

Background-aim

The plasma has benefits for shortening turnaround time, minimizing the interference of microfibrin, and not requiring additional time to clot completely especially from patients receiving anticoagulant therapy. BD Vacutainer Barricor Tube (Barricor) (Becton Dickinson (BD), Sparks, MD, USA) was recently introduced to provide a more stable lithium heparin plasma by using mechanical separator. We aimed to evaluate the comparability and applicability of Barricor for hemodialysis patients with anticoagulant therapy compared to standard serum or plasma gel tubes.

Methods

Samples were collected from a total of 40 patients undergoing hemodialysis for measuring a total of 28 routine biochemical analytes using BD vacutainer SST II Advance Tube (SST), BD Vacutainer PST Lithium Heparin Tube (LiH), and Barricor. After centrifugation at 3,200 g for 5 minutes for Barricor and at 2,000 g for 10 minutes for SST and LiH, analysis was performed on AU5800 series (Beckman Coulter, Brea, CA, USA), Nova CRT (Diamond Diagnostics, Holliston, MA, USA) and ADVIA Centaur XPT (Siemens, München, Germany) in duplicate.

Results

Barricor was statistically comparable to LiH for 13 parameters. Barricor and LiH were clinically comparable except for ionized calcium and sodium. Moreover, the difference for ionized calcium and sodium was not seemed to have a clinically meaningful impact on diagnosis as the bias was less than 2%. Although Barricor was statistically different from SST for 20 parameters, Barricor and SST were clinically equivalent except for total protein, ionized calcium, and potassium. The significant biases of total protein and potassium were attributed to matrix difference as LiH exhibited clinically substantial difference from SST, being comparable to Barricor. In the correlation analysis, the Pearson correlation coefficients between three tubes were over 0.8993 except for direct bilirubin. The existence of fibrin mass or strand was detected in SST for 21 patients of total 40 patients.

Conclusions

Barricor provided clinically comparable results except for total protein, ionized calcium, and potassium when compared with SST or LiH. Moreover, Barricor had no risk of interference resulted from gel or microfibrin, which would be beneficial for hemodialysis patients with anticoagulant therapy.

doi: 10.1016/j.cca.2022.04.451

M241

Do phlebotomists in emergency departments reduce haemolysis in clinical chemistry samples?

J.L.V. Reeve *, H. Madden, P.J. Twomey

Department of Clinical Chemistry, St. Vincent's University Hospital, Elm Park, Dublin 4, Ireland

Background-aim

Haemolysis is an interferent common to specimens originating from Emergency Departments (ED). This is due to the acute setting, the frequency of phlebotomy and the heterogeneity of staff performing venepuncture. Haemolysis falsely elevates intracellular analytes and may cause spectral/chemical interference in susceptible Clinical Chemistry methods. To improve the quality of the blood specimens taken, Phlebotomists were established in ED in December 2019. We wished to determine if this initiative reduced haemolysed samples received into the Clinical Chemistry laboratory.

Methods

Haemolysis-index (H-index) results measured in ED specimens were retrospectively collected from the laboratory information system over two eight week periods; from December 9th 2018 (no Phlebotomist present) and from December 9th 2019 (Phlebotomist present). Patient demographics were not retrieved. Roche assay H-index cut-offs for potassium (54 mol/L), iron (125 mol/L), amylase (310 mol/L) and creatinine (497 mol/L) were applied to the H-indices to clinically contextualise the degree of haemolysis. Comparison of 2018 and 2019 data which exceeded each of the four H-index cut-offs was conducted using the Chi-squared test for independent, categorical variables; statistical significance was set at a P-value of 0.05.

Results

The H-index cut-offs for all four tests were exceeded more frequently in 2018 compared to 2019; no Phlebotomist v Phlebotomist. In relative terms, the H-index for potassium was exceeded in 8.54% of ED specimens in 2018 compared to 6.25% in 2019, iron 3.98% v 2.60%, amylase 1.02% v 0.66% and creatinine 0.41% v 0.26%. Chi-squared values (and associated p-values) for the H-index cut-offs for potassium, iron, amylase and creatinine were 29.54 (<0.001), 23.51 (<0.001), 6.09 (0.01) and 2.41 (0.12), respectively. The difference in the creatinine H-index cut-

off data between the years may have been insufficiently powered to reach statistical significance.

Conclusions

The introduction of trained phlebotomists to ED demonstrated a significant reduction in haemolysis across all but grossly haemolysed specimens. The quality of phlebotomy will make a difference particularly to moderately haemolysed samples which are those most frequently encountered by the lab.

doi: 10.1016/j.cca.2022.04.452

M242

Biotin prevalence among health check-up individuals from Asian countries

Y. Jeon [b], T. Pichayayothin [a], C. Chen [c,*]

[a] *Bangkok R.I.A. Lab, Thailand*
[b] *Green Cross Laboratories, South Korea*
[c] *Scientific Affairs, Core Diagnostics, Abbott Laboratories, Singapore*

Background-aim

Biotin is widely available over the counter (OTC) and increasingly used for nutritional or therapeutic purposes. The excess amount of biotin in patients' blood may cause interference with certain immunoassays that utilize a biotin-streptavidin free capture methodology. The prevalence of biotin interference in patients presenting to hospitals or having routine blood tests is still largely unknown, especially in Asian countries. The objective of this study is to evaluate the biotin prevalence in random health check-up population in 3 Asian countries, i.e., Korea, Singapore and Thailand.

Methods

A total of 3000 surplus serum specimens, randomly collected from health check-up individuals presenting to the clinical laboratories in the 3 Asian countries, were screened for biotin using an Abbott ARCHITECT biotin Research Use Only assay. Samples with biotin screening result ε 10 ng/mL were further tested by LC-MS/MS confirmatory assay for biotin and its primary metabolites. The overall and site-specific prevalence of detectable biotin was determined using the screening assay while the biotin metabolite profile was determined using the LC-MS/MS method.

Results

Of the 3000 samples screened, 82 (2.7%) had biotin concentration ε 10 ng/mL (considered as positive) and 22 (0.7%) had biotin concentration ε 20 ng/mL. No age and gender dependence was observed. Korean site has the highest rate of positive screens (3.5%) whereas Thailand site has the lowest (2.1%). Of the 82 positive screens, 31 (38%) showed detectable (ε 5 ng/mL) biotin and/or metabolites by LC-MS/MS confirmatory testing. Specifically, biotin, bisnorbiotin, and biotinsulfoxide were detected in 29/31 (93.5%), 23/31 (74.2%), and 17/31 (54.8%) samples, respectively; biotin alone (without detectable metabolites) was detected in 7/31 (22.6%) samples.

Conclusions

Using a screening assay, 2.7% (82/3000) of the random health check-up samples from the 3 Asian countries were found to be potentially susceptible to biotin interference. Thirty-eight percent of them (31/82) were confirmed by LC-MS/MS, majority of which contained biotin metabolites that may also play a potential role in interfering with immunoassays.

doi: 10.1016/j.cca.2022.04.453

M243

Establishing in-house thresholds for lipaemia interference and verification of a high speed centrifugation lipaemia removal protocol for common chemistry tests

M.H. Lim *, C.P. Yeo

Clinical Biochemistry Section, Department of Clinical Pathology, Singapore General Hospital, Singapore

Background-aim

Lipaemia is a commonly encountered interference in the laboratory that affects approximately 1% of samples. Lipaemia interference can occur via several mechanisms including light absorbance by lipoproteins, volume displacement effect, and differential partitioning between the hydrophilic and hydrophobic phases. Elimination of lipaemia may not always be possible with a repeat collection and it is necessary to identify the interference threshold for each assay to avoid reporting spurious results. We aim to establish optimised thresholds for lipaemia interference for urea, creatinine, glucose, sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphate, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase on the Beckman AU 5800 and creatine kinase MB and high sensitivity troponin T on the Roche Cobas e801.

While ultracentrifugation is considered the gold standard for lipaemia removal, it is not widely available and high-speed centrifugation has been reported to be a suitable alternative. In the second part of our study, we sought to determine if a high-speed centrifugation protocol of 15 minutes at 20000 xg provides similar efficacy in lipaemia removal when compared against a simulated ultracentrifugation protocol.

Methods

Patient samples were pooled to obtain two concentrations for each analyte. Increasing amounts of Intralipid were added to create samples with up to Intralipid concentrations of 2000 mg/dL. Controls spiked with deionised water were prepared to account for dilution effects introduced by Intralipid addition. All samples were analysed in triplicates and their averages computed. Differences against controls were assessed using the Royal College of Pathologists of Australasia (RCPA) allowable limits to determine the optimal threshold for each assay.

Using the equation, RCF1 x time1 = RCF2 x time2, whereby RCF is the relative centrifugal force of each rotor and time is the duration of centrifugation, an ultracentrifugation protocol was approximately simulated by 60 minutes of centrifugation at 20000 xg. Intralipid-spiked samples were split into two aliquots and centrifuged for 15 minutes or 60 minutes at 20000 xg. Post-centrifugation, infranatant were collected,

analysed in triplicates, and compared against control results. RCPA allowable limits were used as acceptance criteria.

Lipaemic index was analysed for all samples in the study. All statistical analyses were done using Microsoft Excel.

Results

Magnesium was most susceptible to lipaemia interference with significance observed at 300 mg/dL of Intralipid, while calcium, phosphate, albumin, alanine aminotransferase and aspartate aminotransferase only demonstrated significant lipaemia interference beyond 500 mg/dL of Intralipid. The remaining analytes were unaffected by lipaemia up to an Intralipid concentration of 1000 mg/dL.

Post centrifugation, all samples demonstrated a significant reduction in lipaemic index and the infranatant appeared clear on visual inspection. When compared to controls, samples from both centrifugation protocols did not exhibit significant differences.

Conclusions

In-house optimised thresholds for lipaemia interference were established for 20 common chemistry tests. Beyond these thresholds, samples should undergo a lipaemia removal process before analysis to minimise the interference. Centrifugation of samples for 15 minutes at 20000 x g demonstrated similar efficacy in lipaemia removal when compared to a simulated ultracentrifugation protocol and can be used as an alternative in the absence of an ultracentrifuge. Caveats should be applied when interpreting native lipaemic samples as no synthetic lipid preparation can precisely replicate the heterogeneity of lipoproteins in humans.

doi: 10.1016/j.cca.2022.04.454

Clinica Chimica Acta 530 (2022) S420–S422

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Sports Medicine

W239

Plasma expression of circ_ZNF609 and miR-615 in endurance runners

M. Montagnana [a], M. Benati [a], E. Danese [a], G.L. Salvagno [a], C. Tarperi [b], E. Paviati [a], M. Gelati [a], F. Schena [b], G. Lippi [a]

[a] *Dep. Neurosciences, Biomedicine and Movement Sciences, Clinical Biochemistry Section, University of Verona, Italy*
[b] *Dep. Neurosciences, Biomedicine and Movement Sciences, School of Sport and Exercise Sciences, University of Verona, Verona, Italy*

Background-aim

Circular RNAs (circRNAs) are highly stable non-coding RNAs acting as gene regulators. These small single-stranded RNA molecules function as microRNAs (miRNAs) sponges, thereby upregulating or downregulating miRNA target gene expression. circ_ZNF609 is expressed in human myoblasts, regulates myoblast proliferation and acts as a sponge for miR-615. Therefore, this preliminary study was aimed to evaluate plasma expression of circ_ZNF609 and miR-615 in 30 endurance runners before and after a half-marathon run.

Methods

The study population consisted of 30 male (52 ± 14 years) Caucasian endurance runners, engaged in a 21.1 km run under competitive conditions. Blood samples were collected before the run (T0), immediately afterwards (T1), and 3 hours after the trial (T2). Total RNA was isolated from plasma samples with Trizol LS Reagent. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to quantify circ_ZNF609 and miR-615 expression levels. GAPDH and U6 snRNA were chosen as reference for normalizing expression levels. The difference of values obtained at the three different time points was evaluated with Wilcoxon's test for paired samples. The level of statistical significance was set at $p < 0.05$.

Result

At T1 the plasma circ_ZNF609 expression levels were lower than those measured at T0 ($p = 0.0002$). Three hours after the run (T2), the values of circ_ZNF609 remained significantly lower than those measured at T0 ($p < 0.0001$). Plasma miR-615 expression levels were significantly increased ($p < 0.0001$) immediately after the run (T1), but returned to baseline at T2 ($p = 0.79$).

Conclusions

These results suggest that middle-distance competitive running may be associated with substantial changes in circ_ZNF609 and miR-615 plasma expression levels in middle-aged athletes. Further studies are needed to confirm this observation and for understanding how these findings interplay with muscle pathway during endurance exercise.

doi: 10.1016/j.cca.2022.04.456

W240

Effect of moderate intensity aerobic exercise on fertility hormones in male obese subjects in Nnewi

S. Meludu [b], I. Onuora [b], E. Dioka [b], C. Obi-Ezeani [a]

[a] *Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria*
[b] *Nnamdi Azikiwe University, Awka, Anambra State, Nigeria*

Background-aim

Obesity is a major public health issue worldwide, contributing to increased fertility hormone alteration. The aim of this research was to find out effect of moderate Intensity exercise on fertility hormones of different gonadal status in obese individuals.

Methods

Eighteen obese male (Class I) were subjected to moderate intensity aerobic exercise three times a week for 12 weeks. 18 non obese subjects were used as control for the baseline determination. Fasting blood samples were collected before the intervention (baseline), at 6weeks and 12weeks of intervention and were analyzed by standard method Enzyme Linked immunoassay. Statistical analyses were performed using SPSS version 23.0.

Result

The mean levels of testosterone and FSH were significantly lower in class I obese when compared with control group while oestradiol was significantly higher ($p < 0.05$). There were significant increases in FSH and testosterone and decrease in oestradiol after exercising for 12wks when compared with baseline ($p < 0.05$). Oestradiol level decreased significantly in eugonadic obese male participants after 12weeks moderate intensity exercise when compared with baseline ($p > 0.05$). For obese male participants with compensated hypogonadism, there were no significant changes in all the hormones after 12 weeks of moderate intensity exercise ($p > 0.05$). Serum LH and oestradiol in Obese with Hypergonadotropic hypogonadism were significantly decreased 6 weeks and 12weeks after commencement of moderate intensity exercise ($p < 0.05$) respectively on comparison with the baseline serum levels, Furthermore, testosterone level also was significantly increased after 12weeks only and was within the reference range ($p < 0.05$).

Conclusions

Obesity can lead to fertility hormone alteration. However moderate intensity exercise could be beneficial in ameliorating the changes in fertility hormones observed in obese male individuals.

doi: 10.1016/j.cca.2022.04.457

W241

Correlation between the content of lipid peroxidation products in the saliva and indices of heart rate variability in qualified athletes

A. Ovchinnikov, A. Deryugina

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russian Federation

Background-aim

The aim of this investigation was to show the efficiency of non-invasive diagnostics of oxidative stress in athletes through the search of correlation between the level of lipid peroxidation (LPO) products in the saliva and indicators of heart rate variability (HRV).

Methods

40 first-class swimmers and 30 qualified runners at the age of 16-20 years took part in the study. Sportsmen were tested the control exercise which consisted of the series of 4×50 m by the main swimming style with a 45 s rest between them for the swimmers, and 3×100 m by a flat race with a rest between the distances also for 45 s for the runners. Salivary samples were collected for the measurement of conjugated dienes (CD), conjugated trienes (CT) and Schiff bases (SB) using the spectrophotometer «SF-2000» according to a Volchegorsky technique. Cardiorhythmogram recording was carried out with the use of the hardware-software complex «Poly-spectrum-rhythm» and was tested by means of variation pulsometry, statistical method to estimate HRV and spectral analysis of cardiointervals.

Result

The Pearson test showed that the indices of HRV characterizing the enhancement of sympathetic activity statistically significantly ($p < 0.001$) positively correlated with the content of LPO products in the saliva. So, the Pearson correlation coefficient (R) between stress index (SI) and CD was 0.62, R for SI and SB was 0.51. At the same time, R for the index of centralization (IC) and CT was 0.44, R between IC and SB was 0.45. Moreover, statistically significant ($p < 0.001$) R was obtained for the spectral indicators of HRV and the analyzed markers of oxidative stress in the saliva. So, R between the total power of HRV (TP) and CD was -0.43. R between the relative power in high frequency range of HRV (HF%) and CD was -0.55, R for HF% and SB was -0.57. R for the relative power in very low frequency range of HRV (VLF%) and CT was 0.63, R between VLF% and SB was 0.58.

Conclusions

Stimulation of sympathoadrenal system via the intensive physical activity induces an excessive formation of reactive oxygen species with the following growth of free radical and peroxide processes in the saliva. Thus, the saliva can be used as a highly informative biological substrate for non-invasive analysis of oxidative stress in the practice of sports medicine.

doi: 10.1016/j.cca.2022.04.458

W242

Effect of three months physical exercise on some oxidative stress markers in premenopausal university students in Nnewi, Nigeria

C.M. Njoku[a], S.C. Meludu[b], C.E. Dioka[a], J.A. Onuegbu[a], J.E. Okwara[a], W.S. Nnaemeka[b], O.A. Onyegbule[a]

[a] *Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*
[b] *Department of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria*

Background-aim

Consistent physical exercise has several health benefits including reduced risk of cancer, diabetes and cardiovascular disease. Ironically, contracting skeletal muscles generate free radicals. Prolonged and intense exercise can result in oxidative damage to cellular constituents.The aim of the study was to evaluate the effect of physical exercise for three months on some oxidative stress markers in premenopausal women.

Methods

This was a prospective comparative study. A total of 60 participants were recruited. They were divided into 2 groups, 30 subjects that had physical exercise and 30 controls that did not exercise. Baseline blood samples were collected from the two groups. The exercise group did moderate -to- vigorous intensity physical exercise for three months. Blood samples were later collected from both exercise and control subjects on the first, second and the third month. Total antioxidant capacity (TAC), Superoxide dismutase (SOD) and Malondialdehyde (MDA) were measured using spectrophotometric methods.

Result

After three months of exercise, there were significant reductions in weight and BMI of the exercise group compared to the corresponding baseline and control group ($P < 0.05$). MDA significantly increased after one month of exercise and significantly decreased at the second and third months of exercise compared to the baseline and control group ($P < 0.05$).There were significant reductions of TAC and SOD after one month of exercise and significant increases after the second and third months of exercise compared to the baseline and control group ($P < 0.05$).

Conclusions

The exercise group showed adaptive response to oxidative stress markers at the second and third months of moderate-to-vigorous exercise. Premenopausal women can take advantage of consistent physical exercise in order to favor further rapid recovery of the oxidation caused by exercise.

doi: 10.1016/j.cca.2022.04.459

W243

Changes of erythrocyte metabolism in response to the [ARing] strand submaximal test in elite athletes

A. Ovchinnikov

Department of Sports Medicine and Psychology, Lobachevsky University, Nizhny Novgorod, Russia

Background-aim

Given that red blood cells (RBCs) play a crucial role in oxygen transport to the tissues, which is the primary factor determining aerobic capacity of athletes, the effects of continuous aerobic exercise - during which severe development of metabolic acidosis does not occur but a hypoxic stimulus does - on intracellular RBC metabolism need to be carefully considered and examined. The aim of this study was to assess how the strand submaximal test (-test) affects erythrocyte metabolism in athletes.

Methods

Twenty highly qualified national level athletes, aged 18-22 years, were recruited for this study. The Å-test was performed on a mechanically braked cycle ergometer. Blood samples were collected before (pre-exercise) and immediately after the exercise (postexercise). The following variables were measured: RBC 2,3-Diphosphoglycerate (2,3-DPG), RBC ATP, thiobarbituric acid reactive substances (TBARS) in erythrocytes, erythrocyte catalase activity, RBC count, RBC electrophoretic mobilities, and hemoglobin (Hb) level. Assumption of normality was verified using the Shapiro-Wilk test. Since all data were normally distributed, comparisons were made using paired Student's t-test. All values are shown as mean ± standard deviation.

Result

The Å-test caused a significant decrease in RBC 2,3-DPG levels (7.16 ± 0.69 vs. 5.24 ± 0.60 mmol/L RBC, $p < 0.001$). RBC ATP concentration (5.61 ± 0.84 vs. 6.06 ± 0.82 mmol/L RBC, $p < 0.001$) was elevated in response to exercise, as were RBC electrophoretic mobilities (0.80 ± 0.18 vs. 1.15 ± 0.25 μm × cm/V/s, $p < 0.001$). RBC count (5.22 ± 0.34 vs. 5.38 ± 0.29 million cells per microliter, $p < 0.001$) and Hb content (138.60 ± 5.77 vs. 144.25 ± 6.70 g/L, $p < 0.001$) were also significantly higher at postexercise compared to pre-exercise. Regarding TBARS level (0.45 ± 0.21 vs. 0.49 ± 0.22 nmol (mg Hb)$^{-1}$, $p = 0.3$) in RBCs and erythrocyte catalase activity (241.05 ± 23.80 vs. 246.01 ± 27.01 kU/g Hb, $p = 0.4$), there were no statistically significant differences.

Conclusions

In the erythrocytes of elite athletes, 2,3-DPG levels decreased in response to the Å-test, while ATP concentration, RBC electrophoretic mobilities, RBC count, and Hb content increased. No differences were observed in RBC TBARS level and erythrocyte catalase activity at postexercise compared to pre-exercise.

doi: 10.1016/j.cca.2022.04.460

W244

Measurement of different salivary biomarkers in young cross-country skiers at rest and following the Bruce protocol exercise test

A. Ovchinnikov [c], A. Deryugina [b], S. Shchurov [a]

[a] *Department of Physical Education, Lobachevsky University, Nizhny Novgorod, Russia*
[b] *Department of Physiology and Anatomy, Lobachevsky University, Nizhny Novgorod, Russia*
[c] *Department of Sports Medicine and Psychology, Lobachevsky University, Nizhny Novgorod, Russia*

Background-aim

An athlete's ability to maintain skilled performance and/or optimal muscle function during exercise can be compromised as a result of several homeostatic perturbations. Many types of muscular exercise induce metabolic disturbances, as evidenced by changes in different biomarkers, including in saliva. The objective of this study was to evaluate how the Bruce protocol exercise test affects salivary biomarkers related to biochemical homeostasis.

Methods

Sixteen young cross-country skiers, aged 14-16 years, participated in the study. Athletes performed the Bruce protocol exercise test. Saliva samples were collected before (pre-exercise) and immediately after the exercise (postexercise). Diene conjugates (DC), triene conjugates (TC), Schiff bases (SB), thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), alanine aminotransferase (ALT), aspartate transaminase (AST), total protein, glucose, creatinine and urea were measured. Assumption of normality was verified using the Shapiro-Wilk test. Since not all data were normally distributed, comparisons were made using Wilcoxon signed-rank test. Data are expressed as median and interquartile range (Me[IQR]).

Result

Salivary DC levels were elevated (0.19[0.07] vs. 0.23[0.07] a.u., $p < 0.01$) in response to exercise, as were TC (0.12[0.06] vs. 0.22 [0.24] a.u., $p < 0.001$), SB (20.9[9.1] vs. 59.1[84.7] a.u., $p < 0.001$), and TBARS (0.8[0.4] vs. 1.2[0.5] μmol/L, $p < 0.001$) concentrations. Total protein (1.0[0.8] vs. 1.4[2.1] g/L, $p < 0.05$), creatinine (22.4 [10.9] vs. 32.0[23.9] μmol/L, $p < 0.001$) and urea (23.3[24.3] vs. 29.8 [19.3] mmol/L, $p < 0.01$) levels were also significantly higher at postexercise compared to pre-exercise. Regarding SOD (1.1[1.0] vs. 1.1[0.6] a.u., $p = 0.5$), AST (3.0[3.3] vs. 3.3[3.6] U/L, $p = 0.3$) and ALT (3.0[2.3] vs. 3.0[2.2] U/L, $p = 0.4$) activities in saliva, there was no a statistically significant change. No differences were also observed in salivary glucose (0.04[0.1] vs. 0.05[0.1] mmol/L, $p = 0.3$) concentration at postexercise compared to pre-exercise.

Conclusions

These findings suggest that saliva may be considered as an attractive option for the assessment of exercise-induced metabolic disturbances in athletes.

doi: 10.1016/j.cca.2022.04.461

Clinica Chimica Acta 530 (2022) S423–S434

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Standardization, Harmonization

M244

Use of reference change value in the harmonization of the report of interferences caused by hemolysis

D.H. Heredero Jung, S. Elena Pérez, F. Marqués García, C. Agulló Roca, A. Alonso Prada, S. Obregón Muñoz

Clinical Laboratory Service, University Hospital of Salamanca, Salamanca, Spain

Background-aim

Interferences caused by in vitro hemolysis constitute an important source of variability. It is mandatory to create and implement laboratory protocols to report, with the highest precision, the potential alterations it may be generating. It is necessary to establish individual cut-off points for each parameter according to their grade of disturbance. Reference Change Value (RCV) takes both intraindividual biological coefficient of variation (CV_i) and analyitical imprecision (CV_a) into account, so it is useful for values-based decision making when handling these interferences.

The aim is to establish cut-off points to report hemolysis values in 5 parameters: potassium (K), lactate dehydrogenase (LDH), folate, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), with the use of RCV.

Methods

Starting from an experimental pilot study about analytical interferences of haemoylsis done in our laboratory, RCV was calculated for each analyte using the formula RCV= $2*1.96*\sqrt{(CV_i^2+CV_A^2)}$. CV_A was obtained from the internal quality control data in a period of 3 months. Results of the analytical variability study were represented by means of interferograms, and regression lines and linear regression coefficients were obtained for each of them. Replacing the RCV values in the regression line equation, corresponding hemoglobin concentrations were determined.

Results

Hemolysis index (HI) cut-off points were established. The first cut-off matches the Systematic Error (SE) from the biological variation database of $SEQC^{ML}$. The second cut-off was designated according to the RCV. 4 HI intervals were made for each parameter: K (0-9.8; 9.8-264.61; 264.61-1000; >1000), LDH (0-11.05; 11.05-55.91; 55.91-1000; >1000), Folate (0-91.99; 91.99-384.6; 384.6-1000; >1000), ALT (0-45.6; 45.6-264.93; 264.93-1000; >1000), AST (0-165.01; 165.01-904.81; 904.81-1000; >1000).

Expert rules were programmed in the Laboratory Information System (LIS). When HI is in the first interval, results are given. In interval 2 the results are informed, but a commentary is added in order to warn against possible under or overestimation. In interval 3 results are not given, specifying that hemolysis is affecting the tests. If HI is over 1000, all results are deleted from the petition.

Conclusions

RCV corresponding HI references de cut-off point from which the increase of variability has clinical relevance. An HI exceeding this value may provoke an interference which will not reflect the clinical state of the patient with accuracy, affecting the therapeutic decisions. This strategy let us automatize the report of hemolysis with criteria and build an appropriate management of hemolyzed samples, giving an added value to the handling of patients. Ultimately, a more personalized control of hemolysis for each parameter is achieved.

doi: 10.1016/j.cca.2022.04.463

M245

Medicinal importance of gymnema sylvestre in traditional and complementary medicine for musculoskeletal disorders with their chemical and biological standardization

D.K. Patel, K. Patel

Department of Pharmaceutical Science, Sam Higginbottom University of Agriculture, Technology and Sciences, Payagraj 211007, Uttar Pradesh, India

Background-aim

Gymnema sylvestre belonging to family asclepiadaceae is widely distributed in the tropical Africa, southern China, Deccan peninsula of western India, Vietnam, Malaysia, Japan, Srilanka, Germany and USA. In traditional medicine, Gymnema sylvestre is used as a stomachic, anti-microbial, anti-hypercholesterolemic, hepatoprotective, antieruodonic, antiviral, anti-diabetic and diuretic effect. Water extract inhibit absorption of glucose and act against glucosyltransferase. The plant extracts are used in folk, ayurvedic and homeopathic systems of medicine. In order to develop better standardization tools, chemical and biological standardization have been done for Gymnema sylvestre using different markers.

Methods

Extraction of Gymnema sylvestre has been done through hot extraction techniques with ethyl alcohol. Phytochemical test were carried out to know the different phytoconstituents which was also checked through TLC methods. Total phenol and flavonoid content was also determined to support their medicinal uses. Parameters such as loss on drying, pH value and solubility study were also carried out to know their physical constant. Gymnemic acid content was determined through gravimetric techniques. Fingerprint analysis and quantitative analysis of quercetin in the extract were also performed through HPTLC techniques.

Results

Phytochemical analysis revealed the presence of various phytoconstituents such as tannin, alkaloid, triterpenoid, saponin and flavonoid. Solubility in water and alcohol was found to be 86.36% and 88.24% where as moisture content was found to be 4.20%. Gymnemic acid content was found to be 26.24% w/w. Total phenol and flavonoid content was found to be 0.80% and 1.90%. E. coli and salmonella were found to be absent in the extract, however total bacterial count and yeast and moulds contents were found to be 650 and 60 cfu/g. Quantitative analysis through HPTLC revealed the presence of 2.95% w/w of quercetin.

Conclusions

This study will be helpful for the standardization and traditional uses of Gymnema sylvestre in traditional and complementary medicine.

doi: 10.1016/j.cca.2022.04.464

M246

Estimated mean recovery of two LDL cholesterol homogeneous methods by non-HDL particle size distribution

Y.S. Shin [a], E.S. Shin [a], J. Hong [b], H. Kim [b], W. Lee [b], S. Chun [b], W. Min [b]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

Conventionally, the Friedewald method is used for LDL-cholesterol (LDL-C) measurement. However, more recently, homogeneous methods that directly measure LDL-C are being used. We investigated the differences in measurement and evaluated the mean estimated recovery rate between two homogeneous methods for each non-HDL fraction separated by particle size.

Methods

Using the Lipoprint LDL system, 355 specimens ordered for LDL subfraction profile from April to July 2017 at Asan Medical Center were separated into 12 subfractions (VLDL, IDLC, IDLB, IDLA, LDL 1-7, and HDL) by lipoprotein particle size. Total cholesterol, triglycerides, LDL-C, and HDL cholesterol of leftover specimens were measured with AU5800 (Wako method below) and Cobas8000 (Roche method below). The mean estimated recovery rate of each fraction by the homogeneous methods, defined as the slope of a regression line between cholesterol percent (by Lipoprint) and percent estimated cholesterol value (by homogeneous methods), was calculated.

Results

As Lipoprint LDL-C concentration increased, Wako method LDL-C also increased, while that by Roche method decreased. Comparing Wako and Roche methods showed that higher Wako method LDL-C concentration occurred at low LDL-C concentrations, whereas higher Roche method LDL-C concentration occurred with increasing concentration. Comparison of the mean estimated recovery rate by Roche and Wako methods revealed significantly lower rate of IDL fraction (79.6% vs. 92.1%; $P < 0.01$); IDLC fraction (53.7% vs. 89.7%; $P < 0.01$); and IDLB fraction (55.5% vs. 100.9%; $P < 0.001$). Significantly higher mean estimated recovery rate occurred in LDL fractions (112.4% vs. 90.1%; $P < 0.001$) and large LDL fractions (104.9% vs. 88.6%). However, no significant difference was observed in mean estimated recovery rate with IDLA fraction (94.9% vs. 92.8%; $P = 0.880$) and the small LDL fraction (106.3%; 101.2%; $P = 0.511$) between the two methods.

Conclusions

The findings showing that different mean estimated recovery rates occurred depending on the lipoprotein fraction. Relatively easy evaluation of the recovery rates of homogeneous methods, utilizing Lipoprint may help standardization of homogeneous methods.

doi: 10.1016/j.cca.2022.04.465

M247

Stability of brain damage markers NSE and S100B in cerebrospinal fluid samples

J. Periša, V. Vidranski, M. Širanović, V. Hostić, I. Šamija

University Hospital Center Sestre milosrdnice, Croatia

Background-aim

Harmonisation of sample stability studies is required to upgrade stability databases and obtain various information. General guidelines for handling of specific analytes/proteins in cerebrospinal fluid (CSF) samples still need to be specified, since stability for specific analytes is often deficiently described in manufacturer's package inserts. For this reason we decided to run a sample stability pilot study on two biomarkers for brain damage: neuron specific enolase (NSE) and S100B protein in CSF.

Methods

CSF was obtained from extra-ventricular drainage systems from patients situated in intensive care unit. Total number of NSE and S100B determinations was 45 and 23 respectively. Upon centrifugation CSF sample was divided into two aliquots: first was stored at -20°C, and second was used for determination of NSE and S100 on Cobas e411 (Roche Diagnostics, Mannheim, Germany) analyser. Stored aliquots were thawed and reanalysed in single batch. Samples were divided in 4 groups according to duration of storage (1st group: 0-6 months, 2nd: 7-12 months, 3rd: 13-18 months and 4th: 19-24 months).

Results

Wilcoxon test showed statistically significant difference for all 4 NSE sample groups: 1st - P = 0,002, 2nd - P < 0,001, 3rd - P = 0,016 and 4th - P = 0,016. Median for NSE measurements from fresh CSF was 63,32 μg/L (95% CI = 41,50 to 92,23) and 3,59 μg/L (95% CI = 2,08 to 10,13) for stored samples. Under same storing conditions, S100B didn't show statistically significant difference between groups: 1st - P = 0,063, 2nd - P = 0,110, 3rd - P = 0,625 and 4th - P = 0,063. S100B median for fresh CSF samples was 8,10 μg/L (95%CI = 2,99 to 13,24) and didn't vary form median for stored and thawed S100B samples: 8,35 μg/L (95%CI = 2,66 to 10,98).

Conclusions

This study proved NSE to be instable compared to S100B protein while stored under same conditions. Lack of information on storage time and temperature might have repercussions in obtaining accurate results when immediate sample processing is not available. Therefore we suggest CSF sample guidelines should obtain and more precisely specify stability of various analytes/proteins stored at different temperatures.

doi: 10.1016/j.cca.2022.04.466

M248

Six sigma approach to enhance acute patient pathways by minimising variation; commercial partnership to maximise precious resources

A. Stretton, G. Barwegen, R. Postigo, A. Malpass, T. Ellison

BD Life Sciences, USA

Background-aim

Six Sigma is a well-known manufacturing methodology which is employed to reduce variation in process output. BD worked in partnership with an eminent European Hospital to identify areas for process improvement, to reduce variation in acute patient pathways using a Six Sigma approach. The value stream of an end-to-end patient pathway was mapped to identify areas of variation that may well not be visible by the timestamps that are routinely captured.

Methods

Value stream end-to-end patient pathway mapping, process mapping and spaghetti mapping were used along with a Six Sigma methodology to understand and capture the current as-is process. The attributes with the greatest impact and variation to the process were identified using a Boruta analysis and data is presented in whisker box plot format. In machine learning and statistics, attribute selection is the process of selecting a subset of relevant attributes (variables and/or predictors) for use in model construction. We used Boruta analysis to provide focus on the activities with the greatest variation that negatively impacted patient pathways. The process was mapped from arrival at a busy emergency department to result availability from clinical chemistry.

Results

11,806 acute patient pathways were mapped (month of October 2019). 57.5% (6,789) were female.

The mean length of stay was 6.24 hours (median 3.21). The Boruta analysis identified the greatest variation in process steps related to sample transport time and laboratory processing. It was of note that most variation was experienced when there is less work in process (at quieter times of the day).

Conclusions

Process standardisation methodologies that are used in manufacturing environments (such as in vitro diagnostics manufacturing) are not commonly applied by healthcare professionals (HCPs) to managing patient pathways. By working in partnership with HCPs, we can leverage the industry leading approaches to deliver improvements in processes which result in process outputs being on time, every time, in a way that is capacity matched across a patient pathways enabling flow and reducing bottle necks which should result in improved patient care and outcomes.

doi: 10.1016/j.cca.2022.04.467

M249

Establishment of reference intervals (RIS) for common biochemical parameters in healthy Nepalese adults

R.V. Mahato [b], M. Lamsal [a], K. Ichihara [c]

[a] *B.P Koirala Institute of Health Science, Department of Biochemistry, Dharan, Nepal*
[b] *Tribhuvan University, Institute of Medicine (TU,IOM), Ayurveda Campus and Teaching Hospital, Kathmandu, Nepal*
[c] *Yamaguchi University,Faculty of Health Sciences, Department of Laboratory Sciences, Ube, Japan*

Background-aim

Despite immense importance of reference interval (RIs) for clinical diagnosis, there have been no reliable RIs available for Nepalese. Therefore we organized a nationwide study to establish reference intervals for commonly tested biochemical parameters as a part of the worldwide RI study coordinated by IFCC Committee on Reference Intervals and Decision Limits (C-RIDL).

Methods

This study was conducted according to the C-RIDL's harmonized protocol with recruitment of apparently healthy volunteers nationwide from five major cities. 555 age and sex matched subjects (54% Males and 46% Females) were chosen as reference individuals. All serum specimens were transported to Kathmandu and measured collectively by use of Beckman-Coulter/Olympus AU480 chemistry analyzer. The sources of variations of reference values (RVs) were evaluated by use of multiple regression analysis (MRA) and nested ANOVA. RIs were derived by parametric method. Latent abnormal values exclusion (LAVE) method was applied to reduce possible influence of metabolic syndrome. RIs were standardized with reference to value-assigned serum panel provided by C-RIDL.

Results

By ANOVA, sex-related changes were typically noted for urate, creatinine, iron, gamma glutamyltransferase (GGT), immunoglobulin M, and transferrin, but not for HDL-cholesterol. Age related changes were noted

for total cholesterol (TC), triglyceride, LDL-cholesterol, and CRP. RIs were established accordingly. The effect of LAVE was noted just for AST, ALT, GGT and CRP. In comparison with other collaborating countries, Nepalese RIs were found low for urea, cholesterol and ALT, and high for triglyceride and GGT.

Conclusions

As a part of global multicenter collaborative project for derivation of RIs, this study is considered to be the first study for the Nepalese population to establish RIs for the majority of general biochemistry parameters under a standardized protocol. Partitioning of RIs by gender and age was necessary for many analytes. RI derived can be used for Nepalese people.

doi: 10.1016/j.cca.2022.04.468

M250

A blue print for implementation of a regional reference laboratory using total laboratory automation with hub and spoke model

A.J. Bhuiyan

Department of Laboratory Medicine and Pathology, Hamad Medical Corporation, Qatar

Background-aim

Transformation from multiple routine laboratories into a centralized fully automated facility serving a large patient population requires bold measures, careful planning and teamwork. There are many considerations such as an effective business plan, cost analysis, significant investment in automation with lean processes, electromechanical design, alterations of space and function, logistical issues. specimen transportation, consolidation, standardization, altered workflows. storage capabilities and education/training. These all necessitate a structured approach to avoid delays and costly mistakes that can jeopardize expected outcomes. The whole process of automating chemistry, serology and immunology from concept to final implementation is explained for anyone considering a similar approach.

Methods

The subject of this proposal is to describe the journey taken and the practical steps involved in achieving a state-of-the art laboratory, fit for purpose and capable to meet the needs of a fast and growing population in the State of Qatar, which stands at 2.8 million. Hamad Medical Corporation (HMC) manages thirteen hospitals including the national ambulance service and a home healthcare service and provides 90% of health service in Qatar. The Department of Laboratory Medicine performs over 19 million tests per year. Majority of these tests (over 70%) are coming from chemistry (routine and esoteric immunochemistry), viral serology and high-volume immunology. The objective was to instigate a centralized (CCL, central clinical lab) service to perform the majority of testing mentioned above in a single platform with additional network of limited services rapid response core laboratories (RRLs) close to each critical care unit in the 13 hospitals (”Hub and Spoke”). This meant replacing twenty-seven different business contracts made with various diagnostic companies with a single contract having guaranteed price per reportable result (GPPRR) for supplying all the necessary TLA systems, reagents, consumables and maintenance for both central and rapid response urgent laboratories.

Results

1. Develop an analytical and methodical approach for consolidation and harmonization of their laboratory services to consolidate multiple laboratory sites into a centralized high volume reference laboratory with first-in first-out principle that ensuring better quality and turn around time (RRL less than 1 hour and CCL less than 4 hours)

2) Streamlining processes for specimen collection, transportation, receiving and processing to maintain the integrity and stability of specimens for analysis, reducing significantly error-prone preanalytical mistakes

3) Optimized IT (information technology) system to manage scope of operations from CPOE (Computerized Physician Order Entry) to laboratory result reporting

4) Developed a highly and effective systematic approach to reducing cost, removing redundancies, centralizing logistic supports for greater efficiency and productivity

5. Producing an outcome-based productivity, patient safety/satisfaction and fulfilling the internationally accredited KPIs (Key Performance Indicators)

Conclusions

After years of inefficiencies, duplication, redundancies and overutilization of laboratory services that resulted in increasing costs and misuse of services. Serving as the National laboratory the leadership of Department of Laboratory Medicine and Pathology, a central part of Hamad Medical Corporation, three years ago took a bold strategic decision to consolidate and standardize services, apply LEAN practices.

Cost savings were ensured using a single vendor through extensive international tender processes. Other significant benefits included: integration of all sites for a standardized service, reduction in number of blood collections and tubes, optimal utilization of staff, improving TAT by combining stat and routine tests, first-in first-out (Lean). A network of rapid response laboratories was also introduced in major facilities. This further enabled consolidation, harmonization and standardization with same methodologies and instruments to improve quality, productivity and efficiency and a seamless operation with efficient logistics and minimum downtime.

Preanalytical process improvements from sample collection, autologging to timely analysis, result reporting (auto-verification) and auto-archiving/retrieving were coordinated in a unified manner. One of the major challenges of this enormous project relied on the integration of hospital information (HIS), laboratory information system (LIS) and vendor’s middleware to provide an unified nerve center in order to manage the entire network of stakeholders. We are sharing these experiences in this proposal.

doi: 10.1016/j.cca.2022.04.469

M251

Potential reference measurement procedure— Determination of c-reactive protein in serum by isotope dilution mass spectrometry based on enzyme digestion

D. Song [b], W. Zhu [a], H. Li [b]

[a] *Nanjing Institute of Measurment and Testing Technology, China*
[b] *National INstitute of Metrology, China*

Background-aim

C-reactive protein(CRP) is one of the most important biomarkers for monitoring cardiovascular disease and systemic inflammation. The routine methods are enzyme-linked immunosorbent assay, immunoturbidimetry and immunoluminescence. Because of the lack of appropriate reference standards, the test results of manufacturers vary greatly, so it is urgent to develop a reference measurement method for CRP.

Methods

In this study, the monoclonal antibody of CRP was combined with magnetic beads, and the CRP in serum was extracted and purified by immunoadsorption. After trypsin digestion, the CRP was quantitatively analyzed by isotope liquid chromatography tandem mass spectrometry (IDMS). The ratio of the peak area of the enzyme cut peptide to the labeled peptide was calculated, and the pure CRP reference material was added to the blank serum as the control. 29 clinical samples with different concentrations covering 0-100mg/L were used to compare the IDMS method with 8 routine methods. ERM-DA 474 was used to verify the method.

Results

The validity of this method is verified by detection limit (LOD) and quantitative limit (LOQ) . The results of 29 serum samples and 8 routine methods were within 95 confidence intervals. The measured value of ERM-DA 474 was 41.87 mg / L, which was within the uncertainty range.

Conclusions

This study established a method for the determination of CRP in serum by using three signature peptides. This method can eliminate the uncertainty factors caused by protein extraction rate, enzyme cutting efficiency and instrument stability. The experimental results can be traced to the pure CRP Certified reference material GBW09227 produced by National Institute of Metrology,China, and the measurement results can be traced to SI units.The method is fast, simple and economical. It can be used as a potential reference method for the measurement of CRP in serum.

doi: 10.1016/j.cca.2022.04.470

M252

Routine serum creatinine measurement state of accuracy and inter-laboratory harmonization in China

X. Guo, L. Hou, L. Qiu

Department of Clinical Lab, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

Background-aim

Creatinine (Cr) is crucial for renal function and is the most commonly used index of renal function in patients for both treatment monitoring and prognosis. Small analytic changes in Scr can create major shifts in the distributions of eGFR, which then cause large differences in the eGFR-based chronic kidney disease (CKD) classification of patients. However, the status of creatinine testing in Chinese clinical laboratories is complex because many laboratories use open systems to detect creatinine. The aim of the study was to investigate the accuracy and intra-laboratory variation of serum creatinine measurements in clinical laboratories in China.

Methods

The study included 27routine creatinine methods from hospitals in different regions of China, representing different creatinine assays from 14 manufacturers and covering 3 Jaffe and 24 enzymatic methods. The imprecision study was performed according to the Clinical and Laboratory Standards Institute (CLSI) EP15-A guideline using two levels of serum pools (about 80 μmol/L and 300μmol/L). Forty fresh frozen serum pools which creatinine concentrations were assigned by isotope dilution mass spectrometry (ID-MS/MS) procedures were used for the accuracy study.

Results

The within-laboratory CV% for 27 creatinine assays varied from 0.76% to 5.48% for low concentration serum pool and 0.41%~3.20% for high concentration serum pool, respectively. The Passing-Bablok regression shows an excellent linear correlation between ID-MS/MS method and the 27 routine creatinine methods ($R2 > 0.999$). However, the average bias of 40 serum pools using 27 detection systems varied-18% to 22%.Bias (%) from the target values were higher for low creatinine concentrations, especially the picric acid method.

Conclusions

Although most creatinine assays claim to be traceable to the gold standard method (ID-MS/MS) or reference material, large inter-assay differences and inter-laboratory differences still exist in China. Further research to improve harmonization between methods is required.

doi: 10.1016/j.cca.2022.04.471

M253

Comparison between two measurement systems for triglyceride in Korea

J. Lim, G.C. Kwon

Department of Laboratory Medicine, Chungnam National University Hospital, Daejeon, South Korea

Background-aim

Triglyceride (TG) is tri-esters consisting of glycerol bound to three fatty acid molecules. There are two measurement systems for triglyceride; the one is an enzymatic method without free glycerol blanking (nonblanking method) measuring total glyceride and the other is an enzymatic method with free glycerol blanking (blanking method) measuring net triglyceride. The aim of this study is to identify the measurement performance of the two methods and to determine the difference between the two methods in Korea.

Methods

From 2016 to 2019, commutable frozen sera were distributed to routine laboratories as a part of lipid standardization program in Korea and for each trial, three levels were used. The participating laboratories were required to measure the samples three times a day for two days. The tar-

get value of total glyceride was measured at Korea Centers for Disease Control & Prevention, and the target value of net triglyceride was determined by total glyceride subtracted by free glycerol measured at Reference Material Institute for Clinical Chemistry Standards (ReCCS) in Japan.

Results

The percent bias for nonblanking method ranged -7.57% to 2.67% and that of blanking method ranged from -7.62% to 4.89%. The mean absolute bias for nonblanking and blanking methods were 1.7% and 2.1% respectively. The interlaboratory coefficient of variations (CVs) for nonblanking and blanking methods were 2.8% and 3.4% respectively. In comparison with total glyceride, the measurement results of blanking method showed difference of -5.7 mg/dL and -5.5% bias on average. The percent bias between two methods was within within acceptable National Cholesterol Education Program (NCEP) criteria of 5%, however, it was over significantly high when the target value was less than 114.0 mg/dL.

Conclusions

For triglyceride measurement, the two routine measurement systems were well standardized by meeting the NCEP criteria with excellent interlaboratory CVs. However, these two systems showed a significant difference when the TG value was low. Therefore, further research is needed to determine the clinical impact of these differences.

doi: 10.1016/j.cca.2022.04.472

M254

Improving the accuracy and reliability of free thyroxine (FT4) measurements through the CDC clinical standardization programs (CSP) using the IFCC reference system for FT4

A. Ribera, K. Poynter, O. Sugahara, L. Zhang, U. Danilenko, H.W. Vesper

Centers for Disease Control and Prevention, United States

Background-aim

Reliable free thyroxine measurements are essential for assessing thyroid function and correctly diagnosing and treating thyroid disorders. Standardization of FT4 measurements is critical to improving and maintaining the accuracy and reliability of FT4 assays. This will improve diagnosis, treatment and prevention of thyroidal illnesses.

Currently, there are no serum-based reference materials available for FT4 to assess the accuracy and reliability of FT4 assays. CDC CSP is collaborating with the IFCC to address this need by optimizing and implementing an accurate and sensitive higher-order Reference Measurement Procedure (RMP) for FT4 that will be used to assign target value to serum-based materials.

Methods

The CDC FT4 reference method is using equilibrium dialysis in combination with liquid chromatography tandem mass spectrometry (LC-MS/MS) as developed and described by the IFCC C-STFT. FT4 in serum is isolated from the binding proteins in 1 mL equilibrium dialysis cells for 4 hours at 37 oC. FT4 is further isolated by extractions prior to LC-MS/MS analysis. To determine the concentration of FT4 in serum, certified primary reference materials are used to prepare calibration materials. Chromatographic separation is achieved using a C18 reverse phase column with a gradient of methanol and water with 0.1% formic acid. Quantification by selective reaction monitoring is performed in the positive mode using electrospray ionization. Two transitions are monitored for each analyte and internal standard.

Results

The within- and between-day imprecision for the CDC RMP are 2.2–3.9% and 1.8–2.6%, respectively. By comparisons with the RMPs at Ghent University in Belgium, Radboud UMC in the Netherlands, and the Reference Material Institute for Clinical Chemistry Standards in Japan, the CDC RMP reported a bias within +2.5% of the mean for all labs. Factors affecting measurement accuracy were investigated, to maximize recovery for optimum performance of the method. Using this method, FT4 was detectable in all samples, and thus, suitable for analysis of hypo-, eu-, and hyperthyroid patients.

Conclusions

This candidate reference method for FT4 in serum demonstrates appropriate accuracy and precision, and will be used to develop reference materials and for comparison studies with routine FT4 methods.

doi: 10.1016/j.cca.2022.04.473

M255

The implementation of metrology in clinical diagnostics: Successes and challenges

M. Quaglia [a], C. Hopley [b]

[a] *LGC Ltd, NML Teddington, UK*
[b] *LGC Ltd, NML, Teddington, UK*

Background-aim

While progress were made over the past decade towards harmonisation and metrological standardisation of clinical laboratories results, the large amount of clinical targets and their complexity require joint efforts and continuous technical development.

The aim of this presentation is to discuss our metrology activities alongside clinical laboratories, NHS England and EQA scheme providers. Examples will include: i. the harmonisation of new born screening tests; ii. the value assignment of certified reference materials (CRM); iii. the development of more challenging reference measurement procedures (RMP) targeting protein biomarkers such human growth hormone, brain natriuretic peptides (BNP) and ⟨-synuclein. For the latter a combination of conventional methods and more emerging mass spectrometry based techniques for protein structural analysis measurements are beneficial.

Methods

Double exact matching isotope dilution mass spectrometry was utilised for quantification of primary calibrators and the development of RMP. qNMR, mass spectrometry and mass balance techniques were applied to define the purity of the primary calibrators. Hydrogen deuterium exchange (HDX-MS) and ion mobility spectrometry-mass spectrometry (IMS-MS) were implemented to provide higher order structural information of protein biomarkers in solution and their binding properties.

Results

A study was undertaken with a number of NHS (National Health Service, UK) newborn screening laboratories, in order to compare different calibration strategy and methods. Harmonisation and standardisation of the results was achieved by implementing improved methods and using SI traceable calibrators.

A RMP was developed to underpin the validation and implementation into clinical laboratories of routine methods for quantification of ⟨-synuclein, the major component in Lewis'bodies, Furthermore, RMPs are under development to provide SI traceable reference values for quantification NT-proBNP and 1-32 BNP.

The benefits of applying HDX-MS and IMS-MS techniques alongside quantification methods during the development of RMP will be shown.

Conclusions

Strategies for the development of RMP and commutable reference materials for small organic and biological targets are in development and important progress were made in different areas. However continuous research and development is required to better define the target measurand and address the measurement challenges of complex analytes such as olygomers or complex proteins. Metrology in the field of higher order protein structure is still in its infancy, but has great potential in underpinning the development of RMP in the field.

doi: 10.1016/j.cca.2022.04.474

M256

Ring trial data of a harmonized mass spectrometry based method for mutiplexed quantitation of serum apolipoproteins

R. Ruhaak [a], I. Begcevic [c], C. Toth [b], S. Kuklenyik [b], U. Ceglarek [c], C. Cobbaert [a] O.N Behalf Of The Ifcc Wg Apo-Ms

[a] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center (LUMC), Leiden, The Netherlands*
[b] *Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*
[c] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig (UKL), Germany*

Background-aim

To ensure test accuracy in time and space, serum apolipoprotein test results should ideally be SI-traceable with measurement uncertainties within predefined performance specifications. Worldwide standardization efforts are put into place to achieve such accuracy. An international working group from the International Federation for Clinical Chemistry (IFCC) aims to develop a Reference Measurement System for serum/-plasma apoA-I, apo(a), apoB, apoC-I, apoC-II, apoC-III and apoE. Mass spectrometry (MS) using bottom-up proteomics is believed to be highly suitable for standardization efforts, as the technique is antibody-independent. Here we assess the interlaboratory variation among three candidate reference laboratories using a harmonized procedure.

Methods

Three candidate Reference Laboratories (CDC, UKL and LUMC), each with their own MS instrumentation, developed a harmonized candidate MS-based Reference Measurement Procedure using a, predefined multi-step approach. The labs evaluated the performance of their instrument, developed common transitions, system suitability testing and sample preparation. To assess the interlaboratory variation in this network, five native human serum calibrators, two IQC materials and 22 human serum samples were distributed and quantified using the harmonized procedure.

Results

Seven serum apolipoproteins were quantified through 32 peptides. First, inter-peptide agreement was assessed in 51 comparisons. Within each lab, inter-peptide agreement for each of the proteins was high with a median correlation coefficient of 0.98. Importantly, the peptide-based quantitative results (72 comparisons) were also in agreement between the reference laboratories with median correlation coefficients of 0.98, median slopes of 1.05 and median biases of 2.81%. Between laboratory CVs of 3.9 – 8.2% were obtained for the seven apos.

Conclusions

A harmonized multiplexed candidate reference method for seven serum apolipoproteins has been developed. The LC-MS/MS results indicate test equivalence and acceptable inter-laboratory variation during this first harmonization attempt and direct towards the transferability of LC-MS based methods across continents. Our results show the potential of LC-MS for absolute, standardized (apolipo)protein quantitation.

doi: 10.1016/j.cca.2022.04.475

M257

Impact of water temperature on quality controls reconstitution for routine hemostasis testing

S. De Nitto [b], G.L. Salvagno [b], E.J. Favaloro [a], R. Gosselinc [c], G. Lippi [b]

[a] *Department of Haematology, Sydney Centres for Thrombosis and Haemostasis, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead Hospital, Westmead, New South Wales, Australia*
[b] *Section of Clinical Biochemistry, University of Verona, Verona, Italy*
[c] *Thrombosis and Hemostasis Center, University of California, Davis Health System, Sacramento, California, USA*

Background-aim

Background: Total quality assurance is a mainstay in laboratory diagnostics and develops through implementation of several activities for guaranteeing the quality of data throughout the testing process. Internal quality control assessment (i.e. IQA) is now universally recognized as a gold standard for establishing the reliability of laboratory data. The use of lyophilized internal quality controls (IQC) is widespread, since this material displays extended shelf-life and better stability compared to liquid controls. Though temperature of distilled water used for reconstituting lyophilized IQC materials may be a source of variability in test results, no clear indications exist on this matter concerning hemostasis testing.

Objectives: This study investigates whether the temperature of distilled water used for reconstituting lyophilized IQC materials for hemostasis testing may influence test results.

Methods

We performed tests testing for 10 consecutive days using two IQCs levels dissolved with distilled water at three temperatures (2-4° C, 22-24° C and 36-38° C). The tests performed were prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FBG), antithrombin (AT), protein C (PC), protein S (PS) and D-dimer (D-Dimer HS 500), using an ACL TOP 700 hemostasis analyzer.

Results

Overall, 50% (i.e., 7/14) IQCs measurements displayed statistically significant bias when with lyophilized material dissolved using distilled water at 3-5° C compared to 22-24° C. In two instances (Level I for both PT and D-dimer) such bias become also clinically significant. As regards lyophilized material dissolved using distilled water at 36-38° C, 21% (3/14) IQCs values displayed a statistically significant bias compared to using water at 22-24° C, in one instance (level 2 for PT) such bias become clinically significant.

Conclusions

These results show that the temperature of water used for dissolving lyophilized IQC may be critical for routine hemostasis testing, especially when using water at cold temperature. We hence advise to standardize water temperature, preferably between 22-24° C, before reconstituting lyophilized IQC used for validating routine hemostasis testing

doi: 10.1016/j.cca.2022.04.476

M258

Improving the diagnosis, treatment, and prevention of diseases through accurate and reliable laboratory measurements with CDC clinical standardization programs (CDC CSP)

O. Sugahara, U. Danilenko, K. Poynter, L. Collins, N. Khoshnam, C. Coffman, T. Buchannan, A. Ribera, B. Laughlin, K. Dahya, B. Smith, A. Arndt, F. Pokuah, D. Wirtz, C.Z. Ulmer, H. Zhou, H.W. Vesper

Centers for Disease Control and Prevention, United States

Background-aim

Laboratory measurements are critical for the correct diagnosis and treatment of patients with chronic diseases such as cardiovascular diseases, hypogonadism, bone-and kidney-diseases, and diabetes. Inaccurate measurements can lead to misclassification of patients and incorrect treatment. The CDC CSP helps to improve the accuracy and reliability of clinical assays by assessing their analytical performance against performance goals defined by clinical and medical organizations. The CDC CSP assist with assay calibration, and certifies and monitors analytical performance.

Methods

CDC CSP have programs in place for total testosterone (TT), estradiol (E2), vitamin D (VD), free thyroxine (FT4), total cholesterol (TC), total glycerides (TG), HDL-cholesterol, and LDL-cholesterol. The programs available for monitoring analytical performance during routine testing include TT, VD, TC, TG, HDL-C, apolipoprotein AI and B.

Results

Enrollment of assays and LDTs in CDC certification programs has resulted in improvements in calibration accuracy; i.e. the absolute mean bias of assays participating in the CDC Vitamin D Standardization Certification Program was well below the allowable bias of 5% each year. Assays standardized in CDC certification programs also demonstrated higher accuracy in routine patient testing; i.e. CDC VD and TT certified assays have a lower bias compared to non-certified assays. The CDC Lipid Standardization Program indicated that the majority of TC measurements performed in routine testing were consistently within the recommended bias limits of ±3% over past 10 years.

CDC CSP continue to improve the analytical performance of assays by addressing measurement bias caused by factors other than incorrect calibration such as interfering compounds. The programs are responding to new clinical and public health needs with the addition of new analytes such as PTH and glucose.

Conclusions

The CDC CSP work with stakeholders to educate the clinical and laboratory communities about the importance of using standardized assays in patient care, research, and public health.

doi: 10.1016/j.cca.2022.04.477

M259

Establishment of reference interval of iron profile and their relation with hematological parameters in Nepalese healthy adults

K. Acharya[a], K. Shrestha[b], B.K. Yadav[a]

[a] *Biochemistry, Department of Biochemistry, Tribhuvan University Teaching Hospital, Nepal*
[b] *Microbiology, Department of Microbiology, Tribhuvan University Teaching Hospital, Nepal*

Background-aim

BACKGROUND-AIM: For the proper management of the patient laboratories play an important role. Many clinical decisions rely on the test results provided by the clinical laboratory. In the context of the Nepalese population where the Clinical reference intervals are an ill-defined clinician and medical laboratories use the RIs set from the population very different from the Nepalese population. Thus, in this context, this study had been targeted to set the reference interval of CBC and iron profile among the healthy Nepalese population.

Methods

Methods: This study was done at Tribhuvan University Teaching Hospital (TUTH), Kathmandu. A total of 155 blood samples were taken especially form the people living around the TUTH for the analysis of different blood tests. Finally, data from only 130 study populations were selected as per our inclusion and exclusion criteria which were set according to the IFCC-CLSI guidelines for RIs calculation.

Results

Results: Hb and MCV in Nepalese women were found to be at lower levels and some were even under mild to moderate level of anemia as

defined by WHO. On the other hand, a significant difference of mean for iron, Ferritin, and the majority of CBC count (p-value <0.05) between male and female population is also shown by this study. Comparison based on age-group showed that there was no significant difference in the means obtained.

Conclusions

Conclusion: Using Sysmex automated blood analyzer we calculated the RIs from Complete blood cell count (CBC) from the Healthy Nepalese population and Iron profile using automated biochemistry (BT-1500) and immunochemistry analyzers (Vitrous 3600) according to the guidelines provided by CLSI and IFCC. Data of which is not available for the Nepalese population earlier. This reference standard can be useful for the Nepalese population for the clinical decisions of the patients which may be lifesaving by early diagnosis.

doi: 10.1016/j.cca.2022.04.478

M260

Towards the standardization of PCT assays?

A. Boeuf, H.H. Huynh, M. Derbez, C. Giangrande, B. Lalere, S. Vaslin-Reiman, V. Delatour

Laboratoire National de métrologie et d'Essais (LNE), Paris, France

Background-aim

The recent study of Rudd et al. shows that 48.9 million cases of sepsis were reported in 2017, resulting in 11 million deaths worldwide. These data, twice as many as the previous estimation, are confirming the urgent need to improve the prevention, the diagnosis and the management of sepsis. Procalcitonin (PCT) is one of the biomarkers used for the diagnosis of sepsis, allowing early and accurate diagnosis of bacterial infection, and is useful in guiding antibiotic treatment. Currently, PCT quantification is performed by immunoassays in biomedical laboratories, and there is no reference material and higher-order reference method.

Methods

Through the creation of an IFCC working group, LNE is evaluating the need and the feasibility of standardization of PCT assays. The discussion and close collaboration between clinicians, health authorities, in vitro diagnostic providers, External Quality Assurance (EQA) providers, and metrology institutes allows sharing the different views on the way of proceeding and underlining the issues to address during the process of standardization.

Results

Consultation of stakeholders highlighted the need for EQA materials of proven commutability to estimate the between-method agreement accurately. Thus, the collection of biological samples was initiated to produce candidate EQA materials consisting of pools of frozen human serum. In parallel, a candidate reference method for the SI-traceable quantification of PCT in human serum, based on an ID-LC-MS/MS approach, was developed. Results traceability to the SI units was achieved through in-depth characterization of the purity of primary calibrators consisting of two synthetic peptides and one recombinant protein. A first estimation of the limit of quantification (LOQ) was around 2 ng/mL.

Conclusions

Our developments allowed reaching a LOQ in the area of the clinically relevant cut-offs for diagnostic (0.25 ng/mL – 5 ng/mL). It will then be necessary to determine if the LOQ could be reduced, if the method meets the required performance specifications and robustness, if the variability of the results of the commercially available PCT assays should/could be improved through common calibration with commutable secondary calibrators, and if correlation between the candidate reference method and routine assays is sufficient.

doi: 10.1016/j.cca.2022.04.479

M261

Performance of Nova®-stat profile-prime blood gas analyzer compared with ABL80-FLEX® analyzer

M. Boudaya, S. Fendri, R. Marrakchi, F. Ayadi, N. Issaoui, F. Gargouri, L. Abid, K. Moalla, K. Jamoussi

CHU Hedi Chaker, laboratory of biochemistry, Sfax, Tunisia

Background-aim

The analytical performance of the Nova® STAT PROFILE Prime (Nova Biomedical) blood gas analyzer was evaluated and compared with that of the ABL80-FLEX® (Radiometer-Copenhagen) analyzer currently used in the laboratory for the determination of pH, partial carbon dioxide pressure (pCO2) and partial oxygen pressure (pO2).

Methods

A cross-sectional study was conducted with whole blood samples . Each sample was measured for pH, pCO2, pO2. The imprecision study was performed according to the French Society of Clinical Biology (SFBC) guidelines, using three levels of internal quality controls. The results of both devices were compared using the Bland and Altman test and the linear regression method (concordance test, R2).

Results

Twenty samples were included. The analytical imprecision performance was acceptable for all parameters tested, except for pO2 at a single level. The linear regression method showed a high correlation between results of the two analyzers. In Bland-Altman analysis, ε95% of the results were within the limits of agreement.

Conclusions

This comparison study of pH, pCO2 and pO2 measurements between the Nova® STAT Profile Prime (Nova Biomedical) and the ABL80-FLEX® (Radiometer-Copenhagen) ® analyzers showed good agreement.

doi: 10.1016/j.cca.2022.04.480

M262

Evaluation of a new blood gas analyzer system with microcaptor cards

M. Boudaya, S. Fendri, R. Marrakchi, F. Ayadi, N. Issaoui, F. Gargouri, K. Moalla, L. Abid, K. Jamoussi

CHU Hedi Chaker, laboratory of biochemistry, Sfax, Tunisia

Background-aim

Blood gas analyzers are important in assessing and monitoring critically ill patients. This study aimed to investigate the performance of a microcaptor card technology analyzer newly introduced in our laboratory.

Methods

A cross-sectional study was conducted with whole blood samples . Each sample was measured for pH, partial oxygen pressure (PaO2), partial pressure in carbon dioxide (Pa CO2) ,Bicarbonate ions (HCO3-), excess Bases (EB), arterial oxygen saturation (SaO2), total blood hemoglobin (Hb) and anion gap (TA) using Nova® STAT Profile Prime analyzer and ABL80 FLEX® analyzer. The results of both devices were compared using the Bland and Altman test and the linear regression method (concordance test, R2).

Results

Twenty samples were included. The linear regression method showed a strong correlation between the two analyzers except for anionic gap and hemoglobin. Means analytes concentrations were similar for the both devices except for pH and pO2 with a statistically significant difference (p = 0.008 and 0.019 respectively). In Bland-Altman analysis, ε95% of the results were within the limits of agreement.

Conclusions

Means analytes concentrations were similar for the both devices except for pH and pO2 with a statistically significant difference (p = 0.008 and 0.019 respectively).

doi: 10.1016/j.cca.2022.04.481

M263

Development of a stable isotope dilution mass spectrometry method for absolute quantification of fecal pancreatic elastase

D. Ritz [b], D. Guschin [a], M. Schneider [a], A. Schmidt [b]

[a] *BÜHLMANN Laboratories AG, Schönenbuch, Switzerland*
[b] *University of Basel, Basel, Switzerland*

Background-aim

Fecal pancreatic elastase is a proteolytic enzyme that plays an important role in the digestive process of humans. High stability of the enzyme during intestinal transit makes pancreatic elastase a very useful marker for the assessment of pancreatic function in patients suffering from conditions such as chronic pancreatitis or cystic fibrosis. Currently, there are neither reference materials nor reference methods for the determination of fecal pancreatic elastase concentrations available. A novel method for quantification of fecal pancreatic elastase based on isotope-dilution mass spectrometry was developed, evaluated and quantitatively compared to the BÜHLMANN fPELA® turbo assay.

Methods

Pancreatic elastase was quantified in extracts of human stool samples collected with the BÜHLMANN CALEX® Cap device. The sample pretreatment and digestion step were optimized to avoid unspecific digestion of elastase proteins. A set of 8 heavy isotope labeled human pancreatic elastase specific peptides was selected and carefully evaluated for application by stable isotope dilution mass spectrometry. Known amounts of reference peptides were spiked to pretreated stool samples. The peptide sample was analyzed on an Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer and the data were analyzed by the SpectroDive software. Only peptides matches with a SpectroDive q value δ 0.001 were considered to be significant. Set of fecal patient samples were analyzed by developed IDMS method and compared to the BÜHLMANN fPELA® turbo performed on Roche cobas c501.

Results

The developed assay can quantify the pancreatic elastase in native stool samples. Moreover, the developed assay can distinguish between clinically relevant isoforms of pancreatic elastase (CELA3A and CELA3B). A method comparison study shows good analytical agreement of the newly developed mass spectroscopy-based assay and the well-established particle enhanced turbidimetric immunoassay) BÜHLMANN fPELA® turbo.

Conclusions

The new stable isotope dilution mass spectrometry method assay for elastase quantification represents an independent method to determine pancreatic elastase levels in fecal samples. We believe that our developed method can improve global standardization of fecal pancreatic elastase quantification.

doi: 10.1016/j.cca.2022.04.482

M264

Towards an SI-traceable LC-MRM-MS based candidate reference measurement procedure for multiplex measurement of serum apolipoproteins (A), A-I, B, C-I, C-II, C-III and E

R. Ruhaak [b], F. Romijn [a], I. Begcevic-Brkovic [i,j], Z. Kuklenyik [d], J. Dittrich [i,j], U. Ceglarek [i,j], A. Hoofnagle [c], H. Althaus [m], E. Angles-Cano [f], S. Coassin [h], V. Delatour [k], L. Deprez [e], I. Dikaios [e], F. Kronenberg [h], G. Kostner [g], A. Lyle [d], U. Prinzing [l], C. Cobbaert [a], H. Vesper [d], o.N Behalf Of The Ifcc Wg Apo-Ms

[b] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands*
[a] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands*
[i] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Germany*
[j] *LIFE-Leipzig Research Center for Civilization Diseases, University of Leipzig, Germany*
[d] *Division of Laboratory Sciences, Centers for Disease Control and Prevention, Atlanta, GA, USA*
[c] *Department of Laboratory Medicine and Pathology, University of Washington, Seattle, WA 98195-7110, USA*

[m] Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany
[f] French Institute of Health and Medical Research (Inserm) - Université Paris Descartes, Paris, France
[h] Institute of Genetic Epidemiology, Department of Genetics and Pharmacology, Medical University of Innsbruck, Innsbruck, Austria
[k] Laboratoire National de Métrologie et d'Essais, Paris, France
[e] European Commission, Joint Research Centre (JRC), Geel, Belgium
[g] Gottfried Schatz Research Center (for Cell Signaling, Metabolism and Aging) Division of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria
[l] Roche Diagnostics GmbH, Penzberg, Germany

Background-aim

Medical results generated by CE-IVDs or in-house tests should be traceable to higher order Reference Measurement Procedures (RMPs) and Reference Materials (RMs). Currently, serum apolipoproteins (apos) are recognized as promising biomarkers for cardiovascular risk assessment and patient management. The former Reference Measurement Systems (RMS) for serum apo A-I, B and (a) are no longer available whereas no RMS exists for apos C-I, C-II, C-III and E. Consequently, there is an unmet need for an SI-traceable, robust, ideally multiplexed, and sustainable RMS for apos.

Methods

Mass spectrometry (MS) allows for quantification of proteins at the molecular level through their specific peptide measurands. An MS-based candidate RMP (cRMP) for apolipoproteins (a), A-I, B, C-I, C-II, C-III and E is developed using quantitative bottom-up proteomics. The method is provisionally calibrated with serum based calibrators that are value assigned to the current RMS where available. Peptide-based calibrators are being produced using a step-up approach, with prioritization for apo (a). The method is conditionally validated according to ISO-guideline 15193.

Results

The quantitation of serum apo(a) is by design independent of its size polymorphism and is linear over a range of 3.8 to 450 nmol/L, the LoQ for apo(a) being 3.8 nmol/L. Interpeptide agreement within individual proteins showed Pearson's Rs > 0.975, except for apoC-I (R = 0.953) and one comparison for C-III (R = 0.970). According to the EP-15 protocol average total imprecision of the cRMP was 9.8%, 4.5%, 3.7%, 7.4%, 5.1%, 5.9%, 5.9% and 4.5% for the quantifying peptides of apo (a), apoA-I, apoB, apoB100, apoC-I, apoC-II, apoC-III and apoE, respectively. Comparison of Roche ITA and MS for apo(a) quantitation resulted in a slope of 1.09 and bias of 8.3%.

Conclusions

A robust, immuno-assay independent, MS-based cRMP is developed for simultaneous quantitation of serum apolipoproteins (a), A-I, B, C-I, C-II, C-III and E. The serum based calibration reveals on average exchangeable results. The envisioned peptide-base calibration will allow the establishment of an SI-traceable RMS for serum apolipoproteins, and availability of serum based commutable reference materials is prepared to aid manufacturers in their transition from the old to the new RMS.

doi: 10.1016/j.cca.2022.04.483

M265

Towards SI-traceability of lipoprotein (A) measurements: Comparison of a candidate LC-MRM-MS RMP method with commercially available immunoassays for evaluating commutability of candidate reference materials

I. Dikaios [e], L. Deprez [e], H. Althaus [n], E. Angles-Cano [f], I. Begcevic Brkovic [h], T. Boesche [m], U. Ceglarek [j,k], S. Coassin [h], V. Delatour [i], B. Dieplinger [a], J. Dittrich [h], A.N. Hoofnagle [c], G.M. Kostner [l], F. Kronenberg [g], Z. Kuklenyik [d], A.N. Lyle [d], U. Prinzing [l], H. Scharnagl [l], H.W. Vesper [d], C.M. Cobbaert [b], L.R. Ruhaak [b]

[a] Department of Laboratory Medicine, Konventhospital Barmherzige Brueder Linz and Ordensklinikum Linz Barmherzige Schwestern, Linz, Austria
[b] Dept of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, The Netherlands
[c] Dept of Laboratory Medicine and Pathology, University of Washington, WA, Seattle, USA
[d] Division of Laboratory Sciences, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA
[e] European Commission, Joint Research Centre, Belgium
[f] French Institute of Health and Medical Research (Inserm) Université de Paris, Paris, France
[g] Institute of Genetic Epidemiology, Dept of Genetics and Pharmacology, Medical University of Innsbruck, Austria
[h] Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig University, Germany, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Germany
[i] Laboratoire National de Metrologie et d'Essais, Paris, France
[j] LIFE-Leipzig Research Center for Civilization Diseases, University of Leipzig, Germany
[k] Division Clinical Mass Spectrometry of the German Society of Clinical Chemistry and Laboratory Medicine (DGKL), Germany
[l] Medical University Graz, Austria
[m] Roche Diagnostics GmbH, Penzberg, Germany
[n] Siemens Healthcare Diagnostics Products, Marburg, Germany

Background-aim

Elevated concentrations of lipoprotein(a) (Lp(a)) are directly related to an increased risk of cardiovascular diseases, thus making its determination a crucial factor for clinical diagnosis. However, the lack of global standardisation of current immunoassay-based measuring procedures (MPs) for Lp(a) leads to inconsistent care of patients. The former Lp(a) reference method and the associated WHO-IFCC reference material are no longer available. Recently, it was decided to evolve to a next generation reference measurement system (RMS) with SI-traceability. To accomplish this an IFCC working group on quantitating apolipoproteins by mass spectrometry (MS) was formed. The SI-traceable RMS will consist of a MS-based, peptide-calibrated candidate reference measurement procedure (cRMP) and secondary serum-based reference materials (RMs) certified for their apolipoprotein(a) molar concentration.

Methods

A correlation study was performed between the cRMP and immunoassay-based MPs using a panel of 39 clinical samples (CS) that cover the whole Lp(a) concentration range. In addition, the commutability of 14 different candidate RMs was investigated to select a suitable RM format for the future development of the serum-based RM.

Results

Comparison of immunoassay-based MPs with the cRMP for measurements of CS in nmol/L demonstrated a good linear correlation, but showed significant measurement bias and sample specific differences.

Conclusions

The results of the commutability study show that RMs based on human serum pools with endogenous Lp(a) are good candidates for future matrix-based certified RM, whereas human pools -spiked with recombinant apo(a) show different behaviour compared to CS, making them unsuitable as RMs in most of the currently available routine assays.

doi: 10.1016/j.cca.2022.04.484

Clinica Chimica Acta 530 (2022) S435–S442

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Therapeutic Drug Monitoring, Toxicology

W245

Pooled biospecimens for human environmental chemical biomonitoring: Potential utility in routine laboratory medicine for chemical risk assessment

J. Anetor Phd [a], A. Macdonald [b], D. Yu-Huang [a], D. Kinniburgh [a]

[a] *Alberta Centre For Toxicology, Cumming School of Medicine, University of Calgary & Laboratory for Toxicology, Chemical Pathology Dept., University of Ibadan, Nigeria*

[b] *Health and Environments Research Centre Laboratory, Dalhousie University, Halifax, Nova Scotia, Canada*

Background-aim

The chemical habitat in which the human population resides today calls for creative and resourceful approaches to the risk assessment of excessive exposure to environmental chemicals (ECs). Some sub- populations, such as pregnant women and their fetuses may be at elevated risk, justifying the need to assess their level of exposure to ECs. This can be very costly, necessitating the need for innovative, cost effective approaches for chemical risk assessment. The possible achievement of this in routine laboratory medicine practice is not widely recognized. We present here the pooling of remnant samples from routine clinical laboratory practice to be used in the measurement of exposure to ECs.

Methods

Assessment of environmental chemical exposure can be readily achieved by utilization of remnant biospecimens that would normally have been discarded. Pooling of biospecimens provides a direct index of cumulative exposure of a population to ECs at the time of specimen collection, as opposed to exposure estimates based on individual measurements. Biomonitoring, which refers specifically to chemical monitoring, may be defined as the systematic collection of human tissues and fluid such as blood, urine, hair or breast milk for the evaluation of chemical concentrations and or their metabolites and transformation products to facilitate exposure assessment. Pooling involves combining individual specimens into a single sample, using a set of grouping criteria that may include, age, sex and geography, and statistical analysis of the data allows for meaningful interpretation of the data not inferior to that of individual sample analysis.

Results

The use of pooled biospecimens is advantageous, in that it saves considerably on analytical costs, reduces time and resources needed for individual recruitment, and may permit quantification of specimens approaching limit of detection (LOD), as well as providing reliable population estimates of chemical exposure. Biomonitoring may also identify biomarkers of exposure and biomarkers of effect that could become routine clinical laboratory measurements in the future.

Conclusions

Pooled biospecimen approach may potentially be more beneficial to resource poor rapidly industrializing countries that employ significant amounts of chemicals without sound chemical management. This approach to human environmental biomonitoring promises to be a pragmatic and affordable strategy to biomonitoring as opposed to traditional methods of environmental chemical risk assessment and may be particularly attractive to emerging economies.

doi: 10.1016/j.cca.2022.04.486

W246

Systematic evaluation of different isotopes labelled internal standards for the determination of carbamazepine and carbamazepine-10,11-epoxide in human serum by liquid chromatography–tandem mass spectrometry

Y.H. Yu [b c], H.R. Lin [a]

[a] *Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan*

[b] *Department of Laboratory Medicine Medical, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*

[c] *Institute of Medical Biotechnology, Tzu Chi University, Hualien, Taiwan*

Background-aim

Carbamazepine (CBZ) is the first-choice antiepileptic drugs (AEDs). Epilepsy is the second most common central neuron system disease after stroke. CBZ is metabolized in the liver to the pharmacologically active metabolite carbamazepine-10,11-epoxide (CBZ-EP). Therapeutic drug monitoring (TDM) of AEDs helps to adjust dosage and to achieve an optimal therapeutic effect while avoiding side effect. Immunoassays applied in TDM provide rapid turnaround time but suffer with non-specific interferences. Different immunoassays with different cross-reactivity with metabolite CBZ-EP may reported discordant CBZ values and may cause confusion in interpreting CBZ serum levels. The accurate and precise analytical method for quantification of CBZ and CBZ-EP is necessary for clinical laboratory. LC-MS/MS assays have improved analytical specificity and have shown to be more accurate and precise. However, ion suppression effects are a major concern in quantitative LC-MS/MS

assays. The stable isotope-labeled internal standards (SIL-ISs) are used to correct for the variability arising from sample preparation and instrumental analysis. However, due to isotope effects, their behaviors are often not identical. Systematic evaluation of different isotopes labelled internal standards will improve the analytical performance in TDM field.

Methods

SIL-ISs are compounds where the atoms in a molecule are replaced by their stable isotopes, such as ^{2}H, ^{13}C, ^{15}N, and ^{18}O. The different isotope-labeled internal standards including CBZ-$^{2}H_{10}$, CBZ-$^{13}C_{6}$, CBZ-$^{2}H_{2}$,^{15}N, CBZ-EP-$^{2}H_{8}$, CBZ-EP-$^{13}C_{6}$ and phenacetin were investigated. Analytical parameters including sample preparation, chromatographic separation, ion suppression, precision and accuracy were systematically evaluated.

Results

Only minor differences in recovery of different sample preparations of CBZ, CBZ-EP and their related SIL-ISs were observed. The ^{2}H labeled ISs elute slightly earlier than their corresponding analytes. An improved ability to compensate ion suppression and better precision and accuracy were shown when ^{13}C or ^{15}N labeled ISs were used.

Conclusions

To the best of our knowledge, this is the first time to investigate and systematically evaluate of all commercially available SIL-ISs for determination of CBZ and its active metabolite CBZ-EP with LC-MS/MS. The use of ^{13}C or ^{15}N labeled ISs instead of ^{2}H labelled ISs is recommended for quantification of CBZ and CBZ-EP in human serum by LC-MS/MS.

doi: 10.1016/j.cca.2022.04.487

W247

A simple and robust mass spectrometric method for simultaneous determination of antidiabetic, antihypertensive, antidepressive, analgesic and antihyperlipidemic drug levels

A. Ünlü [a], S. Abusoglu [a], D. Eryavuz Onmaz [a], G. Abusoglu [b]

[a] *Department of Biochemistry, Selcuk University, Faculty of Medicine, Konya, Turkey*
[b] *Department of Medical Laboratory Techniques, Vocational School of Health, Konya, Turkey*

Background-aim

Most of the patients in hospital are commonly prescribed for chronic diseases. The aim of this study was to establish a simple mass spectrometric method which can determine commonly used drugs such as metformin (antidiabetic), atorvastatin (antihyperlipidemic), escitalopram (antidepressant), enalapril (antihypertensive), bisoprolol (beta blocker), carbamazepine (antiepileptic) and paracetemol (analgesic) simultaneously.

Methods

Imatinib, a tyrosine kinase inhibitör, was used as an internal standard. Internal standard was added into serum samples and the drugs standard mix then vortexed for 30 s. Protein precipitation was achieved by adding 500 ⌠ L of acetonitrile. The samples and standards were centrifuged, then supernatants were evaporated under nitrogen gas and the residue was dissolved with acetonitrile. Chromatographic separation was performed with Shimadzu Prominence HPLC system and Phenomenex Luna C18 column. The mobile phase A and B were HPLC grade water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid, respectively. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an electrospray ion source (ESI) operating in positive mode. Total run time was 5 min.

Results

The method was linear over for the concentration ranges 2-5 to 2000-10000 ng/ml depending to drug. This method is capable to detect the lowest level of therapeutic range of the targeted drugs. Retention times changed between 0.34-2.39 min. Intraassay and interassay CVs were ranged between 2 and 6.4 % for all drugs. There were no big interferences for the patients which are using two or more drugs.

Conclusions

We have developed a robust, rapid, simple and cost effective LC-MS/MS method to measure the most commonly used drugs simultaneously. This method can easily be used in small and moderate hospital laboratories for therapeutic drug monitoring.

doi: 10.1016/j.cca.2022.04.488

W248

Influence of occupational exposure to pesticides on serum biochemical markers reflecting hepatic citotoxicity among orchard workers

S. Cekovska [a], K. Tosheska-Trajkovska [a], V. Soleva [b], I. Kostovska [a], J. Brezovska-Kavrakova [a], S. Topuzovska [a], D. Labudovik [a], J. Bogdanska [a]

[a] *Department of Medical and Experimental Biochemistry, Medical Faculty, University Ss Cyril and Methodius, Skopje, The Former Yugolav Republic of Macedonia*
[b] *Private health institution Avicena laboratory, Skopje, Republic of Macedonia*

Background-aim

Background: Many studies have reported association between long-term exposure to pesticides and changes in a variety of biochemical parameters related to organ functions in human.

Aim: The aim of this study was to evaluate the effects of chronic pesticide exposure on several biochemical markers reflecting liver function among orchard workers. Serum concentration of total protein, total bilirubin and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and acethylcholinesterase (AChE) were examined.

Methods

Methods: In the study 50 peach orchard workers were included at the age from 19 to 60 years, chronically exposed to pesticides methomyl, mancozeb and chlorpyriphos-cipermetrin. All orchard workers kept to safety precautions. They had been handling pesticides from 1 to 35 years. The control group consisted of 40 healthy individuals without

occupational exposure to pesticides. All biochemical parameters were examined by using standard spectrophotometric assays.

Results

Results: There was significant difference for serum total protein and total bilirubin levels between control group and orchard workers ($p < 0.05$). In 21.3% of orchard workers was detected total bilirubin level higher than upper limit of reference interval. Very weak positive correlation was detected between total protein level and serum AChE activity ($r = 0.32$, $p = 0.003$). The significant decreased AChE activity was detected in 10.6% of peach orchard workers. There was no correlation between the serum AChE activity and years of occupational exposure to pesticides. In 12.8% of orchard workers increased ALT and GGT activities were detected. Significant difference between exposed and control group was detected only for ALP activity ($p < 0.001$). Very weak negative correlation was detected between AChE and ALP activities ($r = 0.34$, $p = 0.001$) in workers occupationaly exposed to pesticides.

Conclusions

Conclusions: Chronic exposure to pesticides affect serum level of examined biochemical markers reflecting hepatic citotoxicity. These biomarkers seem to be indicative of adverse effects of pesticides and should be used for monitoring of liver function in orchard workers and assess the individual risk of handling pesticides.

doi: 10.1016/j.cca.2022.04.489

W249

Evaluation of serum levels of heavy metals in saw millers and carpenters occupationally exposed to wood dust in Enugu state, south east Nigeria

O. Obianyido [b], H. Obianyido [a]

[a] *Faculty of Basic Medical Sciences, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu State, Nigeria*
[b] *Faculty of Medical Sciences, Department of Chemical Pathology, University of Nigeria, Ituku Ozalla, Enugu State, Nigeria*

Background-aim

Wood dust exposure is one of the commonest and the world's most aged occupational exposures causing several health challenge. The purpose of this study is to evaluate the serum levels of some heavy metals (arsenic, lead and cadmium) among carpenters and saw millers in Enugu state, south east Nigeria.

Methods

A cross sectional study was carried out using two hundred and fifty eight wood workers(saw millers and carpenters) in Enugu state south east Nigeria from October 2019 to December 2019. Venous blood samples were collected in plain bottles. These were centrifuged after clotting and clear serum used for the estimation of the concentrations of lead, arsenic and cadmium concentrations respectively. The concentrations of heavy metals (lead, arsenic and cadmium) in wood dust collected from different sites in the study area was also determined. All parameters were analyzed using a standard method. Subjects were grouped into two;

Group 1: saw millers
Group 2: carpenters
Group 3: control.

Results

The result showed a significantly higher concentration of Lead, Arsenic and Cadmium ($P < 0.05$) when compared to the control subjects, even though a higher concentration of lead (38.24 ± 0.61)ug/dl and arsenic (41.4 ± 0.64)ug/dl was observed in Group2. There was also an increased concentration of Lead, Cadmium and Arsenic in the wood dust collected from different sites on the study area.

Conclusions

Occupational exposure to wood dust brought about an increase in the serum concentrations of Lead, Cadmium and Arsenic of saw millers and carpenters. Higher levels of lead and arsenic seen in carpenters maybe because they are constituents of the chemicals used in the finishing and polishing of finished furniture. Increased concentration of these heavy metals maybe associated with some health challenges. Advice on the use of appropriate PPE (personal protective equipment and regular medical checkups is recommended.

doi: 10.1016/j.cca.2022.04.490

W250

Accuracy evaluation of automated electrochemiluminescence immunoassay for everolimus and sirolimus compared to liquid chromatography-tandem mass spectrometry

E.S. Shin [a], E.J. Lee [b], J. Hong [c], H. Kim [c], W. Lee [c], S. Chun [c], W. Min [c]

[a] *Department of Laboratory Medicine, Asan Medical Center, Seoul, Republic of Korea*
[b] *Department of Laboratory Medicine, Hallym University College of Medicine, Dongtan Sacred Heart Hospital, Hwaseong-Si, Republic of Korea*
[c] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea*

Background-aim

Therapeutic drug monitoring of everolimus and sirolimus is important for successful organ transplantation. Liquid chromatography-tandem mass spectrometry (LC-MS/MS), which is known as a gold standard method of everolimus and sirolimus assays until today, has many disadvantages such as difficulty to automate, requirement of complicated pretreatment process. In this study, we evaluated the analytical performance of newly developed chemiluminescence immunoassay for everolimus and sirolimus (Elecsys® everolimus and sirolimus, Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Methods

The Elecsys® everolimus and sirolimus assays were performed using the Cobas e602 module (Roche Diagnostics). According to Clinical and Laboratory Standards Institute guidelines, the analytical performance of precision, recovery, linearity, and carry-over were evaluated using quality control materials proved by the manufacturer. For correlation evaluation, the results of Elecsys® everolimus and sirolimus were compared with those of LC-MS/MS 120 samples from patients treated with everolimus or sirolimus.

Results

The within-run and total imprecision values were as follows: 2.3–4.5% and 4.5–6.4% for the everolimus assay; 3.3–4.8% and 4.7–8.1% for the sirolimus assay, respectively. The measured concentration was linear over the range of 0.718–27.585 ng/mL for everolimus analysis and 0.789–26.880 ng/mL for sirolimus analysis (all $R^2 > 0.99$). Recovery was 93.5–105.5% for the everolimus assay and 99.2–109.1% for the sirolimus assay (except lowest levels). Carry-over was -1.09% for the everolimus assay and -0.12% for the sirolimus assay. The results of the two chemiluminescence immunoassays showed acceptable correlations with those of LC-MS/MS ($R = 0.9585$ and $R = 0.9799$, respectively). The two immunoassays showed slightly proportional biases compared to LC-MS/MS.

Conclusions

The Elecsys® everolimus and Elecsys® sirolimus could rapidly and simply measure the concentrations of drugs using automated chemiluminescence immunoassay. And they showed acceptable analytical performance in precision, linearity, and correlation with LC-MS/MS. These methods can be adopted in the clinical laboratory for rapid therapeutic drug monitoring of patients who require treatment with immunosuppressants.

doi: 10.1016/j.cca.2022.04.491

W251

A study on selected serum inflammatory cytokines in occupationally lead and cadmium exposed workers of Jodhpur

T. Goyal, P. Mitra, S. Sharma, P. Singh, S. Gangam, P. Sharma

All India Institute of Medical Sciences Jodhpur, India

Background-aim

Heavy metals occur naturally in earth's crust but various anthropogenic activities result in their accumulation in environment, which may pose deleterious effects on human health. When accumulated inside human body, these heavy metals lead to various disturbances in normal physiological functioning. Among various heavy metals, lead and cadmium may produce lethal effect on human health affecting multiple systems like cardiovascular, respiratory, skeletal, immune, hematological, neurological, endocrine etc. Immune system is one of the most sensitive targets for these metals, however the exact mechanism involved is still unclear. Cytokines are the key modulators responsible for controlling immune response.In the present study, levels of immuno-regulatory cytokines were estimated in occupationally exposed workers.

Methods

The study enrolled 110 individuals from handicraft and welding factories of Jodhpur, who were occupationally exposed to lead and cadmium and 97 apparently healthy individuals without any history of occupational exposure. Blood levels of lead and cadmium were determined by graphite furnace atomic absorption spectroscopy. Serum levels of IL-2, IL-17, IL-10 and IL-4 were measured by commercially available ELISA kit.

Results

Blood lead and cadmium levels were found to be statistically different between the two groups ($p < 0.0001$). When compared to the control group, the serum levels of IL-2 ($p = 0.03$) and IL-4 ($p < 0.0001$) were significantly higher in the group of factory workers. The levels of IL-10 were significantly higher in non-exposed group ($p < 0.0001$). However, the levels of IL-17 did not show any statistically significant difference between the two groups ($p = 0.82$).

Conclusions

In conclusion, we found that exposure to heavy metals may result in significant alterations in immune-regulatory cytokine levels thereby resulting in altered immune response.

doi: 10.1016/j.cca.2022.04.492

W252

Levels of lead, cadmium, 8-OHdG and OGG1 expression in occupationally heavy metal exposed population

P. Singh, P. Mitra, T. Goyal, S. Sharma, P. Sharma

Department of Biochemistry, All India Institute of Medical Sciences Jodhpur, India

Background-aim

Heavy metals are the toxic substances that increase the susceptibility to various diseases. Lead (Pb) is a heavy metal, widely used in various industries and domestic settings due to its physical and chemical properties. Cadmium (Cd), a known carcinogen, has low rate of excretion from the body and is stored in soft tissues such as liver and kidney. The main sources of heavy metals are manual handling in industries like battery manufacturing, handicrafts, textile, paint, welding etc. Cadmium is absorbed significantly from cigarette, food, and water and air contamination. Though oxidative stress and DNA damage are mainly involved in heavy metal toxicity, there are many disparities among the results of studies on the genotoxicity of lead and cadmium. The aim of the study was to evaluate the Pb, Cd and 8-OHdG levels (oxidative DNA damage marker) and expression of DNA repair gene OGG1 in occupationally heavy metal exposed population.

Methods

The study comprised of 100 male workers, occupationally exposed to lead and cadmium from different factories and 100 healthy controls with no history of occupational exposure. Blood lead and cadmium levels were estimated by Graphite furnace atomic absorption spectrophotometry (ICE 3000, Thermo Fisher Scientific). Serum 8-OHdG level was estimated by ELISA based kit method and OGG1 expression was done by RT-PCR.

Results

The mean ± SEM of blood lead and cadmium was 5.66 ± 1.22 (μg/dL), 3.44 ± 0.13 (μg/L) in cases and 1.27 ± 0.11 (μg/dL), 1.01 ± 0.06 (μg/L) in controls. The 8-OHdG concentration was found significantly higher in heavy metal exposed group than control ($p < 0.006$). The expression of OGG1 was significantly decreased in heavy metal exposed group as compared to control group ($p < 0.001$).

Conclusions

Continuous exposure of heavy metals even at low concentrations is toxic and associated with increased oxidative DNA damage and impaired expression of DNA repair gene in occupationally heavy metal exposed workers.

doi: 10.1016/j.cca.2022.04.493

W253

Circulating levels of selected miRNAs in occupationally cadmium exposed workers of Rajasthan

P. Mitra, T. Goyal, S. Sharma, P. Sharma

Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India

Background-aim

Cadmium, a heavy metal, and a known carcinogen is widely distributed in the environment. Occupational cadmium exposure to workers may lead to serious health effects. Heavy metals exert their toxic effects mainly by oxidative stress and alteration in the immune system, although the mechanisms are unclear. Studies have demonstrated exposure to cadmium is associated with epigenetic alterations like DNA methylation, histone modification, and miRNA dysregulation. miRNAs are small non-coding RNA, which binds to 3'UTR region of target gene and inhibits translation. Recent studies have focussed on identifying novel molecular biomarkers that may aid in the understanding of the mechanisms of heavy metal toxicity. This study was planned to evaluate the changes in circulating miRNA levels (miR-155, miR-20b, and miR-221) in individuals occupationally exposed to cadmium and compare them with individuals without occupational exposure.

Methods

The study enrolled 110 individuals from handicraft and welding factories of Jodhpur with occupational exposure and 97 apparently healthy individuals working in offices. Blood levels of cadmium were determined by GFAAS. Circulating miRNA were isolated from serum by Qiagen isolation kit and subsequently converted to cDNA using Qiagen miRCURY LNA RT kit. miRNA expression was assessed using Qiagen miRNA PCR assay in RT-PCR.

Results

Blood cadmium levels (Mean ± SD) were significantly higher in exposed individuals (2.48 ± 1.20 μg/L) as compared to controls (1.9 ± 0.73 μg/L) ($p < 0.0001$). miR-155, mir-20b and mir-221 had a fold change of 1.62, 1.07 and 2.63 respectively. Functional analyses of miR-221 predicted 36 genes involved in multiple interacting pathways.

Conclusions

Findings of our study suggests that miR-221 is upregulated in individuals occupationally exposed to cadmium.

doi: 10.1016/j.cca.2022.04.494

W254

Risk factors associated with metabolic syndrome in HCV seronegative heroin dependants

Z. Pereska [c], D. Janicevic-Ivanovska [a], A. Spasovska Trajanovska [b]

[a] *Institute of Clinical Biochemistry, University Clinic Skopje, UGD Medical Fakulty Stip, The Former Yugolav Republic of Macedonia*
[b] *Psychiatric hospital, University Saints Cyril and Methodius, Skopje, The Former Yugolav Republic of Macedonia*
[c] *University Clinic of Toxicology, University Saints Cyril and Methodius , Skopje, The Former Yugolav Republic of Macedonia*

Background-aim

Heroin addiction is associated with a high prevalence of infective diseases such as chronic C virus hepatitis (HCV) and metabolic disturbances, which are not always causally separated in the literature. The aim of our study is to estimate the risk factors associated with development of Metabolic syndrome defined by IDF criteria in HCV naïve heroin dependants with normal BMI.

Methods

Glycemia and lipids were determined by standard methods.Insulinemia was determined by analyzer chemiluminescence immunoassay of CLIA methods Immulite 2000.

Results

The study was prospective, cross-sectional, including 220 heroin dependants and 60 controls. Blood samples from the heroin dependents were collected at the time of admission to the hospital, referring for inclusion in the Buprenorphine substitution program. The evaluated risk factors were gender (female/male), BMI (kg/m2), waist circumference (cm), age(years), HOMA-IR, HOMA-B%, triglycerides, HDL-C, glycemia, systolic and diastolic BP, duration of heroin dependence, Brinkman index. Insulin resistance was determined according to DR Matthews 's Homeostasis Model for Assessment of Insulin Resistance (HOMA-IR = (FPI/FPG) / 22.5) and beta cell function (HOMA-%B =(20xFPI)x(FPG-3,5), using the values of fasting glycemia (FPG), expressed in mmol/ l and insulinemia (FPI) in μU/ml. Heroin dependents with metabolic syndrome were presented with 9,3%. High triglycerides (OR = 3.13, P = 0.05), increased waist circumference (OR 2.39, P = 0.046) and HOMA-IR (OR = 0.82, P = 0.043) were significant predictors of developing metabolic syndrome in heroin dependents with normal BMI and HCV seronegativity.

Conclusions

Conclusion: Heroin dependence in HCV seronegative patients with normal BMI is associated with risk of developing metabolic syndrome, predicted with increased waist circumference, triglycerides and HOMA-IR.

doi: 10.1016/j.cca.2022.04.495

W255

A forensic toxicology investigation of 11 classes of abused drugs in human urine specimens

N. Chaouali, B. Moslah, O. Smaoui, K. Ben Salem, D. Amira, A. Hedhili

Laboratory of Toxicology, Research Unit of Environment and Toxicology LR12SP07, Center Mahmoud Yaccoub for Urgent Medical Assistance, Tunisia

Background-aim

According to the World Drug Report, 271 million people worldwide (5.5 % of the global population) aged 15–64 years are drug users. In Tunisia, North African country; drug addiction became an urgent public health problem that affects human health and social life. A medico legal toxicology investigation was conducted from January 2016 to December 2018, to monitor trends of illicit drugs consumption in Tunisia, during the post-revolutionary period.

Methods

An epidemiological cross-sectional study was carried out; data were collected from expertise files of 11 170 arrested individuals suspected to be drug abusers during the period lasting from January 2016 to December 2018. Fresh urine samples were collected and submitted by law enforcement agencies in the toxicology laboratory of Center Mahmoud Yaccoub for Urgent Medical Assistance, for forensic toxicological analysis. Drugs screening was carried out by immunoenzymatic assays. All positive samples were analyzed with gas chromatography coupled to mass selective detector (GC–MS) for confirmation.

Results

Out of 11 170 specimens analyzed, 5 409 (48.4%) were detected positives for at least one drug of abuse. Drug abusers were mainly male (sex ratio 37), the average age was 29 ± 7.91years. 65.4% were less than 30 years old and 91.3% were unmarried. Cannabis was the most widely consumed illicit drug (95%), consumption of cocaine and MDMA has increased (0.08% and 0.15% in 2014) versus (1.11% and 0.52% in 2018) respectively. Seventy-nine specimens were positive for more than one type of psychoactive substances, so-called polydrug use. This scourge has increased from 0.4% in 2014 to 1.5% in 2018.

Conclusions

These results could be helpful for authorities to combat traffic of narcotics and drug abuse. Given the new trend of consumption of new-generation synthetic psychoactive substances, investigations are currently underway in our laboratory to detect the presence of these compounds in biological samples using ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS).

doi: 10.1016/j.cca.2022.04.496

W256

Pitfalls of introducing the methotrexate method in a highly automated laboratory

S. Iskül, G. Zemtsovskaja

North Estonian Medical Centre, Estonia

Background-aim

Methotrexate (MTX) is used in chemotherapy of several neoplastic diseases.

MTX measurements for our hospital patients have been outsourced for several years. The results were frequently delayed. So we aimed to introduce a new method while comparing it to the previous method using patients samples and also assessing its precision (repeatability and reproducibility).

Methods

MTX was measured in 10 patient samples by Fujirebio Diagnostics Inc chemiluminescent microparticle immunoassay on Abbott ARCHITECT i1000SR analyzer in another laboratory and by ARK Diagnostics homogeneous enzyme immunoturbidimetric assay on Roche Cobas® 6000c501 analyzer in our laboratory.

The precision of our method was evaluated by measuring of ARK Diagnostics Internal Quality Control samples at 6 levels, twice a day for 3 days, for a total of 36 results.

Results

The correlation coefficient between the methods, the regression and the average bias were 0.9996, y = 1.0165x + 0.0274, and 2.16%, respectively.

The precision analysis was also successful with the coefficient of variation for repeatability being 0.98% to 5% and coefficient of variation for reproducibility being 1.39% to 5% at levels of 0.07 to 500 μmol/L.

However, we encountered two unexpected problems. One sample showed approximately 3 times lower result than in another laboratory. Unfortunately, we did not find a suitable explanation for this phenomenon. In addition, the MTX concentration in the first samples obtained after MTX administration usually exceeded the upper limit of the measuring range. Thus, requiring us to train the laboratory technicians of our highly automated laboratory to perform additional series of manual dilutions up to 10000 times.

Conclusions

The possibility of inappropriate results due to some endogenous interferences must be taken into account in MTX monitoring.

To be performed correctly, MTX measurements at high concentrations should be carried out in accordance with a specially developed protocol for manual dilutions.

doi: 10.1016/j.cca.2022.04.497

W257

Enzymatic hydrolysis of >10000 ng/ml of codeine, morphine, ethylmorphine and oxymorphone glucuronides in urine at RT for LC-MS-analysis

J. Oxelbark

Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Background-aim

Hydrolysis of drug conjugates before analysis facilitates analysis and saves time in data evaluation. Ethylmorphine and codeine glucuronides are however difficult to hydrolyse enzymatically, and typically demand higher temperatures and extended reaction times for acceptable recovery.

In drug testing it is also useful to establish the Codeine/Morphine ratio which is typically 10 for Codeine intake, but less if intake was supplemented with Morphine. Thus, a correct assessment of codeine levels far above cut-off is of interest.

The aim was to find a reasonably quick hydrolysis protocol for drug conjugates of codeine, morphine, ethylmorphine and oxymorphone at >10000 ng/ml each in urine.

Methods

A mixture of morphine-, codeine-, ethylmorphine-, and oxymorphone-glucuronide was spiked into 8 native blank urine samples, giving 20000 ng/ml of each drug conjugate, corresponding to approximately 13000 ng/ml free drug each. Methanol content in urine was 7 % after spiking. Then, 50 µl urine was mixed with 100 µl B-One glucuronidase (Kura Biotech Inc) in a 96-well plate, and left to stand for 30 minutes in RT. Internal standard was added after hydrolysis, which kept methanol concentration <10 % in the hydrolysis stage. Hydrolysis was quenched by adding 275 µl methanol and 100 µl reaction mixture was mixed with 400 µl water before analysis. Samples were analysed using LC-MS/MS. The protocol was repeated on three different days with different native urine samples.

Results

Recovery of free drugs in percent of expected amounts were calculated over the full set of 24 individual native urine samples, as shown below, with corresponding CV's.

Average Morphine Codeine Ethylmorphine Oxymorphone

Recovery 98% 93% 93% 97%

CV 1.9% 8.8% 8.6% 2.0%

The higher CV's observed for Codeine and Ethylmorphine indicate that individual urine composition has a larger impact on recoveries. However, out of the 24 samples, only one showed <80 % recovery of Ethylmorphine and Codeine

Conclusions

A quick and efficient method for hydrolysis of high levels of Morphine, Codeine, Ethylmorphine and Oxymorphone drug conjugates has been demonstrated. Using B-One glucuronidase (Kura Biotech Inc) hydrolysis for 30 minutes in room temperature of >10000 ng/ml of each drug resulted in good to excellent recoveries.

doi: 10.1016/j.cca.2022.04.498

W258

Evaluation of the renal effect of occupational exposure to wood dust among wood saw millers in Enugu municipality, south- east Nigeria

O. Obianyido [b], H. Obianyido [a], C. Okwuosa [b]

[a] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus, Nigeria*

[b] *Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology University of Nigeria, Enugu Campus, Nigeria*

Background-aim

Wood dust exposure is the commonest and prevailing occupational exposures causing several health challenges such as rhinitis, dermatitis, respiratory defects and cancer of the nasal and paranasal sinuses. Chronic exposure to wood dust is common among wood saw millers and workers in the furniture making industries.

The aim of this study is to evaluate the renal effect of occupational exposure to wood dust among wood saw millers in Enugu municipality, South –East Nigeria.

Methods

A case-control study was carried out between May 2021 and November 2021. One hundred and eighty four males between the age of twenty and seventy years were recruited for this study. One hundred and four were wood saw millers while eighty apparently healthy males within the same age bracket were used as control.

Phytochemical analysis and heavy metals concentration were carried out on the wood dust. Heavy metals concentration, serum urea and creatinine were also carried out on the subjects blood samples. Sociodemographic data were collected using structured questionnaires. All samples were analysed using standard methods.

Results

Our study showed an increased concentration of heavy metals (arsenic,cadmium and lead) in the wood dust collected from the vicinity of the wood saw millers.

Findings from this study also showed a significant increase ($p < 0.05$) in the serum concentration of arsenic(31.56 ± 0.88)µg/dl, cadmium (5.27 ± 0.38) µg/dl and lead (4.49 ± 0.60) µg/dl when compared to the control; arsenic(0.62 ± 0.10)µg/dl, cadmium (1.95 ± 0.71) µg/dl and lead ($2.03 \pm$) µg/dl.

Further more, there was a significant increase($p < 0.05$) in the serum concentration of urea(3.47 ± 0.07)mmol/l and creatinine (101.10 ± 0.24)mmol/l when compared with the control subjects urea(3.12 ± 0.03)mmol/l and creatinine(93.21 ± 0.28)mmol/l.

Conclusions

Occupational exposure to wood dust over a period amplifies the hap of heavy metal intoxication and may ultimately predispose wood sawmillers to renal damage. We recommend regular use of personal protective equipment and regular medical checkup among wood saw millers.

doi: 10.1016/j.cca.2022.04.499

W259

Short to mid-term monitoring of alcohol abstinence: Phosphatidylethanol is your biomarker of choice, even when still positive

K. Van Uytfanghe [b], L. Heughebaert [b], E. Abatih [a], C.P. Stove [b]

[a] *Fostering Innovative Research Based on Evidence (FIRE), Ghent University, Krijgslaan 281, 9000 Ghent, Belgium*

[b] *Laboratory of Toxicology, Department of Bioanalysis, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, 9000 Ghent, Belgium*

Background-aim

The role of phosphatidylethanol (PEth) as a direct biomarker for alcohol intake is more and more valued. PEth increases the sensitivity of uncovering alcohol consumption and monitoring abstinence. Upon chronic and excessive alcohol use PEth values are easily over 300 ng/mL. With a half-life of 7 – 8 days, it can take weeks for PEth values to drop below the decision limit for monitoring abstinence (20 ng/mL). The question arises whether abstinence can be confirmed based on two consecutive PEth positive results.

Methods

A large scale PEth monitoring study was set up in which over 500 participants agreed to stay sober for one month. During this period, they took 3 finger prick samples via self-sampling at home using volumetric absorptive micro sampling devices (VAMS, Mitra®, Neoteryx). PEth 16:0/18:1 was quantified using a validated liquid chromatography – tandem mass spectrometry method. A population-based algorithm capable of predicting abstinence with 95% probability was set up by fitting a linear mixed effect model to discern patterns in PEth elimination over time while accounting for intra- and interindividual variability in PEth scores. The latter was further incorporated in a 2-step decision tree, looking (i) whether or not PEth-values fall outside the prediction interval, and (ii) whether the slope between two PEth values is compatible with abstinence. Validation of this decision three was based on data of 74 people reporting to drink alcoholic beverages, alcohol free beverages or having eaten liquor-containing sweets.

Results

Allowing a cut-off of “4 units spread over 14 days”, the sensitivity and specificity of the decision tree was 89%. This is a realistic cut-off compared to other reports on detectability of a single dose intake of ethanol.

Conclusions

Monitoring the decrease in PEth, while the latter is still positive, can underpin claims of abstinence upon short term to mid-term monitoring.

doi: 10.1016/j.cca.2022.04.500

Clinica Chimica Acta 530 (2022) S443–S447

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Transfusion Medicine

W260

Urinalysis assessment of potential blood donors in a tertiary hospital, south-western Nigeria

A. Ajeigbe [d], T. Owojuyigbe [b], B. Olopade [c], R. Makinde [a], O. Ajose [a]

[a] *Department of Chemical Pathology, Obafemi Awolowo University/Teaching Hospitals, Ile-Ife, Nigeria*
[b] *Department of Haemtology and Immunology, Obafemi Awolowo University/Teaching Hospitals, Ile-Ife, Nigeria*
[c] *Department of Medical Microbiology and Parasitology, Obafemi Awolowo University/Teaching Hospitals, Ile-Ife, Nigeria*
[d] *Depertment of Chemical Pathology, Obafemi Awolowo University/Teaching Hospitals, Ile-Ife, Nigeria*

Background-Aim

Blood donors are routinely screened for their eligibility to donate blood safe for transfusion directed to protect the recipient while the donors are usually assumed healthy. There is no protocol in place for routine medical assessment of blood donors. Hence, this study. The aim of this study is to assess the urinalysis findings of potential blood donors at the blood bank of Ife unit of Obafemi Awolowo University Teaching Hospitals complex (OAUTHC) using a dipstick urine strip.

Methods

This was a cross-sectional study carried out between January and March 2019 at the Department of Hematology and Blood transfusion of OAUTHC. A total of 200 potential blood donors at the blood bank were recruited for this study. Ethical clearance was obtained from the Ethics and Research committee with a written informed consent obtained from each participant. Excluded from the study were those who declined consent. Data on sociodemography and past medical history were obtained using an interviewer questionnaire based method followed by collection of 10ml urine into a universal bottle for dipsticks urinalysis using a 10 panel urine strips. Data was analysed by SPSS version 22.

Results

The mean age of the study participants was 29.3 + 7 years in a range of 18 to 57years. There were more males (93%) than females (7%), majority (88.5%) of the participants had a minimum of secondary school education and 61.5% were self-employed. One hundred and forty-one (70.5%) of the subjects has no previous routine medical examination and abnormal urinalysis was found in 31.5%, 10.5%, 8.5%, 3%, 5.5%, and 1% for proteinuria, urobilinuria, hematuria, nitrituria leucocyturia, and ketonuria respectively.

Conclusions

This study found that the self-employed individuals with basic education form the readily available pool of potential blood donors with no routine medical examination and for which abnormal urinalysis is not uncommon. This may suggest that these individuals may not be healthy donors although found eligible to donate. Thus, potential blood donors will benefit from routine medical examination at the point of donation, as this will give a general overview of their health as well as promoting safe and healthy donation.

doi: 10.1016/j.cca.2022.04.502

W261

The increase of stromal cell-derived factor 1 (SDF-1) levels in packed red cells (PRC) as the indicator of storage lesion at Sanglah hospital-blood bank, Bali, Indonesia

A.A.W. Lestari, N.K. Mulyantari, I.P.Y. Prabawa

Department of Clinical Pathology, Faculty of Medicine, Universitas Udayana, Sanglah Hospital, Bali, Indonesia

Background-Aim

Packed Red Cells (PRC) is one of blood component obtained from the whole blood through the removal of blood plasma. The storage of PRC aims to maintain the viability and function of erythrocytes by reducing the cell metabolic activity. However, the biochemistry changes of PRC could be occurred through storage lesion by releasing Stromal Cell-Derived Factor 1 (SDF-1) that affect the erythrocytes metabolism and viability prior to transfusion. The purpose of this study is to evaluate the increase of SDF-1 levels in PRC as the indicator of storage lesion at Sanglah Hospital-Blood Bank, Bali, Indonesia.

Methods

An observational cross-sectional study using randomized post-test only without control group design was conducted among 84 PRC at Sanglah Hospital-Blood Bank, Bali. The consecutive random sampling technique was used in this study to the PRC who met the inclusion criteria.

Samples were divided into 3 groups based on the storage time such as Group 1 (1-7 days), Group 2 (8-14 days), and Group 3 (15-21 days). The SDF-1 levels were evaluated for each group using Human SDF-1/CXCL12 Kit from Elabscience® by ELISA method. Data were analysed using SPSS version 25 for Windows software.

Results

Most of samples were O Rh + (51.2%), followed by A Rh + (33.3%), and B Rh + (15.5%) blood type. The median levels of SDF-1 in Group 1 group was 0.385 (0.050-1.020) ng/mL, followed by 0.455 (0.330-0.750) ng/mL in Group 2, and 0.490 (0.380-0.920) ng/mL in Group 3. There was a significant difference of SDF-1 levels in Group 1- Group 2 (P = 0.041) and Group 1-Group 3 (P = 0.000) groups, however, no significant difference found in Group 2-Group 3 (P = 0.133) using Mann-Whitney test. The Spearman's rho test was also found a moderate positive correlation between SDF-1 levels and storage time by days (r = 0.546; P = 0.000).

Conclusions

The increase of SDF-1 levels in PRC could be used as one of the indicators for storage lesion due to significantly correlated by the storage time at Sanglah Hospital-Blood Bank, Bali.

doi: 10.1016/j.cca.2022.04.503

W262

Proportion of different ABO and Rh phenotypes in voluntary blood donors at Tribhuvan university teaching hospital blood bank

A. Mishra, A.D. Pant

Blood Bank, Department of Pathology, Tribhuvan University Teaching Hospital, Nepal

Background-Aim

Background: A proper typing of ABO must be done of both the donors and recipients before transfusion of the blood. Rh system is another major blood group system after ABO system. Alloimmunization and complete restriction of the hemolysis cannot be achieved by just determining the ABO and Rh antigens due to the presence of other minor blood group antigens like Duffy, Kell, and MNSs on the RBCs. Being the major antigens, confirmation of only ABO and Rh-antigens can play a significant role in reducing the hemolysis and alloimmunization in the patients who are repeatedly transfused. Thus, their incorporation in the blood grouping panel is an urgent need in developing countries, keeping in view the heavy financial burden in determination of complete genotype of the individual.

Aim: To find the relative proportion of ABO and Rh antigens phenotypically in the screened voluntary blood donors. Also, the donors belonging to the 'O' blood group were further screened for the Bombay phenotype.

Methods

Materials and Method: Test-tube agglutination method was chosen. For that, 1 ml of blood was collected from the blood bag of donors containing CPDA-1 anticoagulant. Then, the collected blood was washed 3 to 5 times in a clean test tube with normal saline and finally a 2-5% of cell suspension was prepared. 7 clean test-tubes were taken and labeled as A, B, D, C, c, E and e where one drop of respective anti-sera was placed. Equal amount of 2-5% cell suspension was mixed with the respective anti-sera, centrifuged at 1000 rpm for one minute. Gently, the bottom of the tube was mixed and observed for the presence of agglutination. This, agglutination reaction confirmed the presence of the respective antigen on the RBC membrane.

Further, the confirmed A and AB-groups were analyzed for the presence of A1 antigen or A-variants and O-group donors were confirmed for the presence of Bombay phenotype using A1 lectin and H-lectin respectively by the same test-tube agglutination method as mentioned above.

Results

Results: ABO was tested in all the 320 blood donors and the frequencies were as follows: A (29.7%), B (25.9%), AB (8.4%) and O (31.6%) respectively. All the A antigens were tested for A1 subgroup where 96.9% were found to be A1 and rest 3.1% were other A-variants. O groups were tested for the Bombay Blood group but none of them were Bombay positive. The phenotypic frequencies of Rh antigens were D (96.4%), C (88.2%), c (46.6%), E (18%) and e (98.8%). Thus, e was the most common antigen and E was the least common of all Rh antigens. Phenotypically DCe/DCe was the commonest and DcE/DCE was the rarest phenotype observed.

Conclusions

Conclusion: Proper ABO typing can be life saving for the recipients and the Rh-phenotyping can be useful for preventing the alloimmunization and severe transfusion reactions in patients who are required to be repeatedly transfused.

doi: 10.1016/j.cca.2022.04.504

W263

Time-temperature indicators vs. temperature indicators for transfusion practice: Application in the real hospital setting

M. Park [d], M. Hur [b], H. Chung [b], H. Kim [b], K. Oh [e], D. Ko [c], Y. Chung [a]

[a] *Department of Laboratory Medicine, Hallym University College of Medicine, Seoul, South Korea*
[b] *Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, South Korea*
[c] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea*
[d] *Department of Laboratory Medicine, Yeungnam University College of Medicine, Daegu, South Korea*
[e] *Department of Nursing, Konkuk University Medical Center, Seoul, South Korea*

Background-Aim

Temperature indicators (TI) have been introduced to monitor surface temperature of red blood cell (RBC) units. We compared newly developed prototype time-temperature indicators (TTI), Freshzone TTI (Freshzone, Seoul, Korea), and two US-FDA approved TI, Safe-T-Vue 10 (STV10) (Temptime Corporation, Morris Plains, NJ, USA) and Timestrip Blood Temp 10 (BT10) (Timestrip UK Ltd, Cambridge, UK). They were compared in the hospital setting in terms of the 30-min rule.

Methods

Freshzone TTI, STV10, and BT10 were attached to 88 RBC units at issue and the time for their color change (CC) were monitored. Freshzone TTI shows CC indicating cumulative time above 10°C. STV10 and BT10 show CC indicating 10°C.

Results

In 88 units, the median time (interquartile range) for CC differed significantly between TTI and two TI (52.1 min [38.3 – 63.0] vs. 13.2 min [9.1 – 18.8]; 52.1 min [38.3 – 63.0] vs.10.5 min [8.4 – 13.8], both $P < 0.001$). Although 96.4% (n = 84) of Freshzone TI showed CC after 30 min after issue, 96.6% (n = 85) of STV10 and 98.9% (n = 87) of BT10 showed CC within 30 min after issue (both $P < 0.001$).

Conclusions

Prototype Freshzone TTI showed better performance in comparison with two TI regarding the 30-min rule. It would be possible for TTI to be implemented to transfusion practice.

doi: 10.1016/j.cca.2022.04.505

W264

A survey on RBC transfusion practice in Korea

M. Park [d], M. Hur [c], H. Chung [c], H. Kim [c], K. Oh [e], H. Kim [e], Y.H. Song [e], D. Ko [a], Y. Chung [b]

[a] *Department of Laboratory Medicin, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea*
[b] *Department of Laboratory Medicine, Hallym University College of Medicine, Seoul, South Korea*
[c] *Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, South Korea*
[d] *Department of Laboratory Medicine, Yeungnam University College of Medicine, Daegu, South Korea*
[e] *Department of Nursing, Konkuk University Medical Center, Seoul, South Korea*

Background-Aim

RBC transfusion practice is essential in patient's care. We conducted a survey on RBC wastage and awareness on the utility of temperature-sensitive indicators (TI) to reduce RBC wastage in Korea.

Methods

From June to August in 2019, 64 blood bank physicians (14 questions) in 64 secondary and tertiary hospitals and 673 nurses (13 questions) in 42 tertiary hospitals replied to the survey. We analyzed the results of the survey.

Results

In the survey to physicians, 97.0% (n = 62) of hospitals had transfusion guidelines. The RBC wastage in 2018 ranged from less than 5 units to more than 200 units. In the survey to nurses, 99.4% (n = 669) of nurses was aware of and complied with the 30-min rule; 13.5% (n = 91) of nurses had experience of RBC wastage due to violence of the 30-min rule. Both 67% (n = 43) physicians and 83.1% (n = 559) nurses answered that TI would help reduce RBC wastage.

Conclusions

This is the first survey on RBC transfusion practice in Korea. This study would be fundamental data for current RBC transfusion practice and understanding about RBC wastage and utility of TI.

doi: 10.1016/j.cca.2022.04.506

W265

Application of geometric mean criteria to anti-blood group antibody proficiency testing in Korean external quality assurance program

J.J. Yang [c], Y. Chung [a], H. Kim [b], D. Ko [c], S. Hwang [c], H. Oh [c]

[a] *Department of Laboratory Medicine, Kangdong Sacred Heart Hospital, Seoul, South Korea*
[b] *Department of Laboratory Medicine, Seoul National University Hospital, Seoul, South Korea*
[c] *Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, South Korea*

Background-Aim

Geometric mean criteria (GC) is another way to judge acceptability for proficiency testing (PT). Recently there is a report that applied GC for PT results provided by the College of American Pathologists. We tried to assess the feasibility of GC for anti-blood group antibody titration testing (ABT) in Korean PT program.

Methods

We reanalyzed the PT results for ABT in 2019. GC is calculated as the geometric mean (GM) ± 2 × geometric standard deviation (GSD). The number of acceptable results for GC was compared to that of mode criteria (MC, mode ± 1 titers). Only results with 10 or more peer groups were included.

Results

A total of 498 PT results (Anti-A: 249, Anti-B: 249) were analyzed. Titration methods included were as follows: column agglutination technique (CAT) with antihuman globulin (AHG), CAT with room temperature incubation (RT), tube technique with AHG, tube technique with RT, and tube technique with immediate spins. The number of acceptable results for GC was greater than the number for MC (Anti-A: 99.6% vs 94.8%, $P < 0.05$; Anti-B: 98.4% vs 94.0%, $P < 0.05$). GC almost always assigned more results as acceptable for every peer group.

Conclusions

Geometric mean criteria could serve as an alternative assessment criterion with a more robust statistical rationale. They are more precise for visualizing the central tendency.

doi: 10.1016/j.cca.2022.04.507

W266

Detection of hepatitis G virus (GBV-C) RNA in blood plasma in patients with lymphoproliferative diseases

S. Putkov [a], S. Akimova [a], S. Kazakov [b,c]

[a] *Burdenko main military clinical hospital of Ministry of defense, Moscow, Russia*
[b] *Russian medical Academy of continuing professional education of Ministry of health, Moscow, Russia*
[c] *Burdenko main military clinical hospital of Ministry of defense, Moscow, Russia*

Background-Aim

In Russia, the detection rate of the hepatitis G virus pathogen (GBV-C) ranges from 2% in Moscow to 8% in Yakutia. The use of the polymerase chain reaction (PCR) method in epidemiological studies has demonstrated a much wider spread of viral hepatitis G than previously thought. Worldwide, an average of 0.01-2% of donors are carriers of hepatitis G viruses. The question of whether to test donor blood for GBV-C is still open, despite the fact that this is the only way to prevent infection in patients receiving multiple transfusions of blood components as treatment. Given that the study of the presence of a direct marker of GBV-C RNA infection in the blood is performed by PCR, its mass introduction into routine screening remains unrealized.

The aim of the study is the Frequency of detection of GBV-C RNA in patients requiring multiple blood transfusions with lymphoproliferative diseases (LD).

Methods

A survey of 297 venous blood samples of patients of the hematology center for the detection of GBV-C RNA was conducted for the period from January 2016 to December 2019. The selection of viral RNA was carried out with a kit for extracting RNA/DNA in clinical material “Absorb” company AmpliSens (Russia). The reaction of reverse transcription and amplification of the obtained material with subsequent real-time detection was performed using the “Amplicens HGV-FL” test system (Russia). Blood was taken in vacutainers containing EDTA-K2, followed by centrifugation and plasma selection for the study. Positive samples with GBV-C RNA were additionally examined for the presence of DNA/RNA of oncogenic hepatitis C viruses (HCV), hepatitis B (HBV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human herpes type 6 (HHV6).

Results

Analysis of the results obtained showed that 31 patients (10.43%) were carriers of GBV-C RNA out of 297 examined samples of patients in the Hematology center departments. All patients have a history of LD. In 16 cases (5.38%), GBV-C was identified as monoinfection. There were 2 cases (0.67%) of co-infection with HCV, 2 cases (0.67%) with HCV and HBV and 11 cases (3.7%) associated with EBV virus.

Conclusions

Comparison of the study data allows us to show infection with the hepatitis G virus in a group of patients of the Hematology center, which may be due to multiple transfusions of donor blood components. The relationship between the detection of GBV-C and the presence of LD cannot be excluded. It is advisable to monitor patients of the hematology center for infection with the GBV-C virus. Consideration should be given to including the GBV-C RNA test in the blood donation study.

doi: 10.1016/j.cca.2022.04.508

W267

Cutaneous manifestation among transfusion patients

S.D. Joshi

Seti Zonal Hospital, DHN, Nepal

Background-Aim

The acute blood transfusion reactions are responsible for causing most serious adverse events. Awareness about various clinical features of acute and delayed transfusion reactions with an ability to assess the serious reactions on time can lead to a better prognosis.

To find out the common adverse effects on blood transfusion.

Methods

Patients having cutaneous and after blood transfusion case reports during 2018Jan-2019 March. Total number of patients were 21. Among them 10 males and 11 females.

Results

Among them four percent had mild reactions (agitation, sweating, pallor, cold feeling, sense of weakness, nausea, pain in transfusion site), and only 3(1 males and 2 female, had more severe disorders, including urticaria with angioedema, ecchymossis, vomiting, loss of consciousness, and convulsive syncope

Conclusions

Acute adverse reaction are although uncommon but we have to follow Evidence based management of it which helps to reduce mortality. Creating awareness about haemovigilance by conducting continuing medical education (CMEs), and training to healthcare professionals would lead to ian mprovement in reporting of transfusion reactions. Complacency and ignorance were the main factors which discouraged transfusion reaction reporting by doctors. Increasing awareness of haemovigilance among doctors and training on reporting transfusion reactions would likely improve spontaneous reporting and help to strengthen the blood transfusion system

Keywords: TRANSFUSION BLOOD; Skin; Reaction; Awareness; Developing country

doi: 10.1016/j.cca.2022.04.509

W268

Usefulness of cell-free hemoglobin in the prognosis of mortality in patients requiring massive transfusion protocol

D. Morell-Garcia[j], J. Rodriguez-Pilar[c], C. Ballester[i], M. Llabres-Comamala[f], A.M. Ferrer-Robles[a], C. Melero-Larrocha[a], M.M. Falco-Esteva[a], R.M. Ruiz De Gopegui[h], I. Hernandez-Bernal[l], C.M. Reina-Ferragut[l], J. Fernandez-Garcia[d], I. Martinez-Lopez[g], F. Enriquez-Palma[b], M. Blanquer-Jerez[m], M. Sansegundo[e], F.J. Garcia-Sola[f], M. Canaro[i], J.M. Sanchez-Raga[i], J. Relinque[i], A. Vela[i], M. De La Cruz[k], A. Perez[i], R. Amezaga-Menendez[c]

[a] *Anesthesia Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[b] *Cardiac Surgery Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[c] *Critical Care Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[d] *Digestive Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[e] *Emergency and 061 Service, Ib-Salut, Palma de Mallorca, Spain*
[f] *Emergency Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[g] *Genetics Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[h] *Gynecology and Obstetrics Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[i] *Hematology Department and Blood Bank Unit, Son Espases University Hospital, Palma de Mallorca, Spain*
[j] *Laboratory Medicine Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[k] *Massive Hemorrhage Commission, Son Espases University Hospital, Palma de Mallorca, Spain*
[l] *Pediatrics Department, Son Espases University Hospital, Palma de Mallorca, Spain*
[m] *Vascular Surgery Department, Son Espases University Hospital, Palma de Mallorca, Spain*

Background-Aim

The use of in-hospital massive transfusion protocols (MTP) aims to improve the survival of patients with life-threatening bleeding. Moreover, some biomarkers are independently associated with an increased risk of death due to hemoglobin-induced oxidative injury. The purpose of this study was to investigate the relationship between cell-free hemoglobin (Hb-cf) and other biomarkers to in-hospital mortality in patients with severe hemorrhage requiring MTP.

Methods

A prospective observational study was performed during 2019, patients requiring MTP were included, serum Hb-cf had been measured just prior to MTP's start (Architect ci16200,Abbott Diagnostics,US). A complete blood count, basic coagulation profile and serum glucose, lipids, liver and muscle enzymes, C-reactive protein (CRP), high sensitivity Troponin I (hs-TnI) and complete iron status profile were also measured. The Hb-cf levels higher than 150 mg/dL were excluded. A univariate analysis was performed using U Mann-Whitney test or Fisher's exact test, considering a statistical significance of 5%.

Results

A total of 15 patients were included (80% males, median age of 50 years old [IQR: 14-81]), no age differences found between subgroups. Four patients died during hospital admission, all men, which represents 26.7%. The causes of massive bleeding in the non-survivors group were abdominal stabbing, ruptured aortic aneurysm, upper gastrointestinal bleeding and polytrauma. The Hb-cf levels of non-survivors were 19.5 mg/dL (IQR: 5.6-33.5) and survivors 21.0 mg/dL (IQR: 9.5-49.0); $p = 0.484$. We found significant differences between non-survivors group in red blood cells (RBC) and platelets count, fibrinogen and serum transferrin levels, with lower levels than survivors group; and monocytes count, RBC mean corpuscular volume, activated partial thromboplastin time ratio, serum gamma-glutamyl-transferase, potassium, amylase, hs-TnI and iron, with higher levels than survivors ($p < 0.05$).

Conclusions

For MTP patients, alterations of hemogram, coagulation and serum iron status profile parameters before MTP starting could be useful as prognostic factors of in-hospital mortality. The use of Hb-cf levels may not be a relevant predictor, although larger studies are needed to verify these preliminary findings.

doi: 10.1016/j.cca.2022.04.510

Clinica Chimica Acta 530 (2022) S448–S452

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Transplantation Laboratory Medicine

W269

Adverse drug reactions of tacrolimus after liver transplant – Our Experience

M. Culafic [b], J. Djordjevic [d], S. Stankovic [d], M. Stulic [e], N. Pejic [a], S. Vezmar Kovacevic [a], B. Oluic [c], B. Miljkovic [a], D. Culafic [f]

[a] *Department of Pharmacokinetics and Clinical Pharmacy, Faculty of Pharmacy - University of Belgrade, Serbia*
[b] *Department of Pharmacokinetics and Clinical Pharmacy, Faculty of Pharmacy - University of Belgrade, Serbia 3 Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia*
[c] *School of Medicine – University of Belgrade, Serbia 5 Emergency Center, Clinical Center of Serbia, Belgrade, Serbia*
[d] *Centres of Biochemistry, Clinical Center of Serbia, Belgrade, Serbia*
[e] *Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia*
[f] *Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia School of Medicine – University of Belgrade, Serbia*

Background-aim

Outcomes and long-term survival rates after liver transplantation have remarkably improved over the last 50 years. However, longevity is accompanied by a variety of adverse drug reactions (ADRs), different in nature and frequency. Tacrolimus (TAC) is the most usually used immunosuppressant in liver transplant recipients. We aimed to describe the profile of ADRs of our patients on TAC.

Methods

We reviewed medical records of patients regularly monitored at Clinic for Gastroenterology and Hepatology following liver transplantation, from November 2015 to November 2019. Besides TAC concentrations, a standard biochemical analysis was performed to evaluate TAC safety. We noted all ADRs deemed to be TAC related.

Results

Medical records of 54 patients were examined, 66.7% male, 39.7 ±13.3 mean age. The most common diagnoses that led to transplant were: ethyl toxic cirrhosis 22.9% and autoimmune diseases 52.1% (primary sclerosing cholangitis 20.8%, autoimmune hepatitis 18.8%, and overlap syndrome 12.5%). We noted 103 ADRs Tac related. Following ADRs were observed as significant: Hypomagnesemia 90% (< 0.70 mmol/L), infections 22%, nephrotoxicity 18.7%, hepatotoxicity in 6%, and ineffectiveness in 23% of patients.

Conclusions

ADRs remain a challenge in immunosuppressive therapy. Physicians, pharmacists, and biochemists involved in the care of transplant patients need to be both vigilant and astute in timely detecting, solving, and reporting ADRs, consequently improving graft survival.

doi: 10.1016/j.cca.2022.04.395

W270

Impact of cryopreservation on hematopoietic progenitor stem cell dose and viability in autologous hematopoietic stem cell transplantation

U. Budhathoki, S. Neupane, B. Poudyal, S. Tuladhar, S. Shrestha, R. Pyatha

Clinical Hematology and Bone Marrow transplant Unit, Civil Service Hospital of Nepal, Kathmandu, Nepal

Background-aim

There has been a dramatic increase in the number of autologous peripheral blood stem cell transplants over the last decade for the treatment of patients with multiple myeloma and for relapsed or refractory Hodgkin or non-Hodgkin lymphoma. Cryopreservation of hematopoietic progenitor stem cells ($CD34^+$ cells) allows for more effective treatment of patients. However, Cryopreservation of $CD34^+$ cells might result in a significant variation in its number and viability. The aim of this study was to determine the effect of cryopreservation on $CD34^+$ cell dose, viability and its subsequent effect on engraftment time.

Methods

This study was conducted at the Civil Service Hospital of Nepal, only one hematopoietic stem cell transplant center in the nation. We performed a retrospective chart review of 21 consecutive patients with a diagnosis of multiple myeloma (MM), myeloid Chloroma (MC), Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), Acute Promyelocytic Leukaemia (APML) and T cell histiocyte rich large B cell Lymphoma(THRLBCL). Enumeration of $CD34^+$ cell dose and viability was analyzed by using FACSCalibur Flow Cytometer- Becton Dickinson and

Trypan blue exclusion test respectively. The Standard Operating Procedures (SOPs) of the Stem cell processing laboratory was strictly followed. The stem cells were cryopreserved at -90˚C by using a controlled rate freezing system- Forma Scientific Model 1050. Peripheral blood cell counts were determined daily in order to verify the time to platelet and neutrophil recovery by using Beckman coulter Unicel DxH 800.

Results

The median age was 47 years (Range 15-62); male to female ratio was 2.0, 66% had MM, 10% had MC, 9 % had NHL and HL, APML, THRLBCL cases were 5% each. Median $CD34^+$ cell dose was 4.20 x 10^6 cells/kg (Range 2-14.9) at the time of harvesting and 2.79 x 10^6 cells/kg (Range 1.38-10.1) at post-thaw. The median of the CD34 + cells viability was 99 % (Range 97-99), 80% (Range 70-97), and 70 % (Range 62-88) at the time of harvesting, before cryopreservation and post-thawing respectively (statistically significant between the three groups p δ0.001). Pre-cryopreservation $CD34^+$ viability showed significant positive correlation with the post-thaw $CD34^+$ cell viability ($p = 0.021$). $CD34^+$ cell dose at the time of harvesting was significantly correlated with the infusion $CD34^+$ cell dose (p δ0.001). It was noted that time to platelet engraftment (TTPE) was significantly correlated with the time to neutrophil engraftment (TTNE) with the p value δ0.001. When $CD34^+$ cell dose enumerated before reinfusion were grouped as <2.0, 2.0–5.0 and >5.0 x 10^6/kg, the median TTPE, and TTNE were 12, 15, 13 and 11, 13, 12 days, respectively (p values not significant between the three groups). The median TTPE and TTNE were not significantly associated with the reduced number of infusion $CD34^+$ cell dose ($p=0.99$ and $p=0.63$ respectively) and its viability before reinfusion ($p=0.31$ and $p=0.26$ respectively). No significant difference in median TTPE and TTNE between the multiple myeloma (13 and 12 days respectively) and other patients (15 and13 days respectively) was found in our study.

Conclusions

Standard Operating Procedures (SOPs) of stem cell processing lab should be amended and stringently followed in order to minimize the potential loss of viable $CD34^+$ cells during Cryopreservation. Our study showed no significant association of reduced $CD34^+$ cell dose and viability with engraftment time. This study holds further significance in being the first report of stem cell dose and viability from Nepal in autologous hematopoietic stem cell transplantation.

doi: 10.1016/j.cca.2022.04.396

W271

Creatinine and kidney function in icteric patients – Automated reflex protocol including cystatin C

P. Schenk [a], A. Inderson [b], C. Bouten [a], W. Rotteveel [a], J. Didden [a], S. Buurman [a], C. Cobbaert [a]

[a] *Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands*
[b] *Department of Gastroenterology, Leiden University Medical Center, Leiden, the Netherlands*

Background-aim

Previously, we did not report creatinine concentrations obtained by the Roche enzymatic assay in patients with strongly icteric sera (icteric index >342 μmol/L), based on significant interference by bilirubin yielding ~10-40% falsely lowered results. Since creatinine concentrations are required for Model for End-stage Liver Disease (MELD) score calculation, this impeded suitable clinical work-up of patients eligible for liver transplant, possibly harming such patients. Hence we aimed for an automated solution to this problem.

Methods

For all creatinine requests with icteric index >342 μmol/L, we generated an automatic reflex test determining creatinine using a dedicated dilution procedure. However, many liver (transplant) patients are cirrhotic and/or sarcopenic. Since creatinine concentration is undoubtedly influenced by muscle mass, the corresponding eGFR may not reflect renal function appropriately in this specific group. Therefore we validated the immunoturbidimetric Roche assay for serum cystatin C, which is produced by nearly all nucleated cells and thus independent of muscle mass.

Results

We successfully validated the Roche serum cystatin C assay. Results were in accordance with the UK NEQAS consensus, and there was no significant interference by bilirubin. Although it is likely that cystatin C and the corresponding eGFR allow a better estimate of kidney function for many liver transplant patients, creatinine remains critical for conventional MELD score calculation. After an unsuccessful attempt using a common 4-fold dilution, we adopted a 3-fold dilution and further improvements resulting in acceptable analytical performance of our dedicated measurement procedure (e.g., total CV $\leq$3.8% instead of $\leq$5.1%, and better equivalence to the routine assay), providing a creatinine result with $<10\%$ bilirubin interference.

Conclusions

We constructed an automated reflex protocol for patients with strongly icteric serum yielding both creatinine and cystatin C + eGFR (cys) results independent of bilirubin, potentially revealing more accurate renal function in this specific patient group.

doi: 10.1016/j.cca.2022.04.397

W272

Role of the ionized magnesium in the early post-liver transplantation outcome

S. Perkov [c], N. Filipčić Višković [a], T. Lupis [a], E. Matišić [c], A. Radeljak [c], I. Prkačin [b], M.M. Kardum Paro [c]

[a] *Department of Anesthesiology and Intensive Care, Merkur University Hospital, Zagreb, Croatia*
[b] *Department of Internal Medicine, Merkur University Hospital and School of Medicine, University of Zagreb, Zagreb, Croatia*
[c] *Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb, Croatia*

Background-aim

The frequent assessment of ionized magnesium (iMg) is essential for the correction of hypomagnesemia during liver transplantation (LT). The aim of this study was to determine if the perioperative magnesium con-

centrations and magnesium supplementation during LT are associated with the post-liver transplantation outcome.

Methods

48 patients undergoing LT at the Merkur Universitiy Hospital, Zagreb, Croatia from January 1st to June 30th 2019 were included. During LT 34 of them (71%) received magnesium supplementation therapy. Ionized magnesium were measured in arterial whole blood with direct potentiometry method accredited according to ISO 15189 norm and the concentrations of the iMg at the beginig and the end LT, and the lowest iMg concentrations during anhepatic phase were evaluated. Postoperative outcomes included length of stay (LOS) in the Intensive Care Unit Unit (ICU), mechanical ventilation (MV) duration, need for retransplantation and major adverse cardiovascular events (MACE) during first month after LT.

Results

In the magnesium supplementation group the median of the iMg concentrations were significantlly lower at the beginning of the LT (0,43 vs. 0,52 mmol/L, p = 0,0245) and significantly higher at the end of the LT (0,51 vs. 0,43 mmol/L, p = 0,0165). No significant differences of the lowest iMg concentrations were found. The median of the LOS in the ICU were significantlly higher in the magnesium supplementation group (6 vs. 3 day, p = 0,0353), while no significant differences in the MV duration, retransplantation and MACE during first month after LT were found.

Conclusions

Many different factors can affect outcomes after LT. Routine monitoring of iMg concentrations and magnesium supplementation contribute to avoid the unwanted side effects of hypomagnesemia during and after LT and may improve outcomes in LT recipients.

doi: 10.1016/j.cca.2022.04.398

W273

Induced pluripotent stem cells derived retinal ganglion cells for clinical use: Towards a retina in a culture dish

H.I. Ahmad Mulyadi Lai [c], C. Shih-Hwa [b], R.I. Ahmad Mulyadi Lai [a]

[a] *Clinical Biochemistry & Immunology, Geneflux Bioscience Sdn Bhd, Selangor, Malyasia*
[b] *Department of Medical Research, Taipei Veterans General Hospital, Taiwan*
[c] *Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan*

Background-aim

Optic neuropathies represent a group of ocular disease characterized by permanently damaged and loss of retinal ganglion cells (RGCs) disease conditions such as glaucoma, Leber's Hereditary Optic Neuropathy (LHON) and autosomal dominant optic atrophy (ADOA) that lead to vision impairment. One strategy for future retinal repair is to develop cell therapy that direct unlimited cellular source of stem cells into in vitro human RGC. Induced pluripotent stem cells (iPSCs) can be derived from patient somatic tissues and have the potential to differentiate into multiple cell lineages. In this study, we characterization and optimize 36-day protocol conditions under which RGCs can differentiate efficiently from iPSCs.

Methods

We applied 2 approach in making of retinal ganglion cells, including differentiating iPSC into retinal progenitor cells and differentiating retinal progenitor cells into retinal ganglion cells. iPSC is derived from peripheral blood mononuclear cells that have been reprogrammed back into an embryonic-like pluripotent state using viral mediated expression of the Yamanaka reprogramming factors. iPSC was maintained with StemFlex medium and validated using stem cell markers by immunochemistry and RT-PCRs. After 2nd passage, selected iPSC lines were allowed to form embryoid bodies for 9 days and developed towards the neural retina lineages. After day 35, RGC will be cultivate and characterized using neuronal cell markers by immunohistochemistry, western blot and qPCR.

Results

Embryoid bodies (EBs) were generated by day seven cultivated iPSC in suspension culture, which were then differentiated into neural rosettes and optic vesicles (OVs) by day 16. By day 25, RGCs with characteristic neural morphology were differentiated from OVs. Notch signalling pathway inhibitor N-(N-(3,5-difluorephenacetyl)-L-alanyl)-S¬-phenylglycine t¬-butyl ester (DAPT) were used to enrich neuron formation in day 25. At day 35, RGC harvested and characterized by expression of specific RGC nuclear markers BRN3B and Atoh7, as well as y-synuclein and abundant mitochondria distributed in the axons.

Conclusions

iPSCs can be patient-specific, making iPSC-derived RGCs a promising candidate for cell replacement. We reprogrammed iPSC under feeder-free, xeno-free and integration-free conditions to avoid the risk of graft rejection and genotoxicity. Our protocols also shown high-efficiency and simple methods to differentiate from suspension culture of optic vesicles to adherent culture RGCs. Establishment of protocols for iPSC derived RGC offer exciting potentials for modelling optic neuropathic disease and developing RGC replacement therapies.

doi: 10.1016/j.cca.2022.04.399

W274

The polymorphism rs1800471 of the TGFB1 gene is associated with cholestatic diseases in children awaiting living donor liver transplantation

O. Gichkun [b], R. Kurabekova [b], O. Tsirulnikova [a], I. Pashkova [b], G. Olefirenko [b], O. Shevchenko [b]

[a] *Sechenov University, Moscow, Russia*
[b] *Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russia*

Background-aim

Experimental and clinical evidence support the role of transforming growth factor beta-1 (TGF®1), a cytokine with complex immune and nonimmune effects, on the development of chronic liver disease. The level of TGF®1 in the blood and tissues of the patients may depend on many factors, one of which may be the genetic determinism of the cytokine production.

The aim to determine distribution of the TGFB1 genetic variants in pediatric patients (pts) with End Stage Liver Disease (ESLD) awaiting living donor liver transplantation.

Methods

The study included 118 children (54 boys) with ESLD different etiology; age from 3 to 144 (19 ± 30) months. The comparison group was healthy blood donors (64 men). Plasma concentrations of TGF®1 were measured by ELISA. Single nucleotide polymorphism (SNP) of TGFB1 gene (rs1800469, rs1800470 and rs1800471) was studied by TaqMan SNP genotyping assay.

Results

The pts. had next frequencies of the investigated alleles: rs1800469 - 21% AA homozygotes, 35% AG heterozygotes, and 44% GG homozygotes; rs1800470 – 77% AA, 15% AG, 7% GG; rs1800471 – 0% GG, 12% GC, 88% CC. The SNPs frequencies in the donors had next profile: rs1800469 - 15% AA, 34% AG and 51% GG; rs1800470 - 85% AA, 15% AG and 0% GG, and rs1800471 – 0% GG, 6% GC, 94% CC. There was deviation from Hardy-Weinberg equilibrium in distribution of SNPs rs1800469 and rs1800470 in pediatric pts. In healthy donors all the investigated SNPs were in Hardy-Weinberg equilibrium. For rs1800470 G allele frequency differed between the pts and the control group (odds ratio = 2.15, C.I. [0.994-4.667], p = 0.04). A comparative analysis of the frequency of genotypes and alleles of the studied SNPs in patients with cholestatic and non-cholestatic diseases was carried out. It was found that the occurrence of rs1800469 and rs1800470 did not differ statistically in both groups. In the pts. with non-cholestatic diseases the GC genotype rs1800471 was not found while in the pts. with cholestatic diseases there were 16%, p = 0.020. Carriage frequency of the G allele in these groups also differed significantly: 8% cholestatic diseases vs 0% non-cholestatic diseases, p = 0.020. The level of TGF®1 was higher in pts. With GG genotype rs1800469 than AG genotype.

Conclusions

The frequency of rs1800470 TGFB1 differs between children with ESLD and healthy individuals. Allele G rs1800471 TGFB1 is associated with cholestatic diseases in pediatric potential liver recipients. The revealed features of the distribution of the studied SNPs in children may be associated with the underlying disease, some of which are genetically determined.

doi: 10.1016/j.cca.2022.04.400

W275

Diagnostic value of duplex microrna-424 and CRP test as an indicator of post-transplant gram-negative bacteremia in heart and lung recipients

O. Shevchenko [d], O. Tsirulnikova [e], S. Sharapchenko [d], V. Poptsov [g], I. Pashkov [a], N. Gabrielyan [c], O. Gichkun [d], D. Velikiy [d], A. Shevchenko [b], S. Gautier [f]

[a] *Department of Surgery N°3, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[b] *Department of Cardiology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[c] *Department of endotoxicosis and purulent-septic complications, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[d] *Department of Regulatory mechanisms in Transplantology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[e] *Department of Transplantology and Artificial Organs, Sechenov First Moscow State Medical University, Moscow, Russian Federation*
[f] *Director of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[g] *Intensive care unit, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*

Background-aim

MicroRNAs (miR) are regulatory molecules that have potential value for post-transplant complications diagnostic. Nosocomial infections caused by gram-negative multidrug-resistant bacteria are associated with high morbidity and mortality, especially for recipients of solid organs who are in vital need of immunosuppressive therapy. The aim of the study is to determine the diagnostic value of miR-424 level for post-transplant infections caused by gram-negative multidrug-resistant bacteria.

Methods

The study enrolled 83 heart recipients, aged 16 to 70 (48 ± 13) years and 26 lung recipients, aged 10 to 74 (36 ± 16) years. The miR-424 plasma expression was detected by real-time PCR (Qiagen, USA). Infection was verified through microbiological identification in blood culture.

Results

The miR-424 plasma expression correlated with tacrolimus blood concentration (r = 0.38; p = 0.04) in recipients. The miR-424 level in recipients with and without acute graft rejection (n = 40 and n = 43, resp.) didn't differ (p = 0.47), but was significantly higher in heart and lung recipients with gram-negative bacteremia (n = 37; p = 0.02). The spectrum of pathogenes consisted of gram-negative bacteria Acinetobacter baumannii and Klebsiella pneumoniae (> 50 %). When the miR-424 level is above a threshold value (-5.72 fold change), the relative risk of bacteremia is RR = 3.84 [95% CI 1.94–7.61]; Se = 60.0 %; Sp = 89.2 %. The miR-424 levels didn't correlate with most routine complete blood count and blood chemistry indicators, but CRP (r = 0.75; p = 0.02) concentration, widely known as an effective marker of infection. CRP concentration measurement (above 7 mg/L) improves the diagnostic characteristics of miR-424 test: RR = 3.8 [95% CI 1.6–8.7]; Se = 69.2 %, Sp = 100.0 %.

Conclusions

The duplex miR-424 and CRP test has a diagnostic value for post-transplant gram-negative bacteremia in heart and lung recipients.

doi: 10.1016/j.cca.2022.04.401

W276

Diagnostic value of microrna-27, microrna-101 and st2 for heart transplant acute rejection

O. Shevchenko [c], S. Sharapchenko [c], O. Gichkun [c], D. Velikiy [c], N. Mozheiko [b], L. Makarova [c], N. Koloskova [a], A. Shevchenko [a]

[a] *Department of Cardiology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[b] *Department of Pathology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*
[c] *Department of Regulatory mechanisms in Transplantology, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation*

Background-aim

MicroRNAs (miR) are a new class of regulatory molecules that affect various cellular functions and have potential value for diagnostic of post-transplant complications. Acute rejection is one of them, associated with high mortality after heart transplantation (HTx). The aim is to determine the diagnostic value of miR-27, -101 levels for acute rejection and to evaluate its relationship with stimulating growth factor 2 (ST2) known as an effective proteomic biomarker of rejection.

Methods

The study enrolled 83 heart transplant recipients, aged 16 to 70 (48.6 ± 10.9) years. Expression levels of miR-27 and miR-101 were measured by PCR in plasma. Concentrations of ST2 were measured by ELISA. Acute graft rejection was verified through morphological analysis of endomyocardial biopsy specimens.

Results

MiR-27 and miR-101 levels in heart transplant recipients with acute graft rejection (n = 44) are significantly lower than in recipients without (n = 39) rejection (p = 0.01 and p = 0.02 resp.). When the miR-27 expression level is below -4.9 fold change the relative risk of acute rejection is RR = 1.6 [95% CI 1.11–2.26]; miR-101 – below -8.03 fold change RR = 1.8 [95% CI 1.3–2.4], but sensitivity (Se) and specificity (Sp) were insufficient for use in clinical practice: Se = 52.3 % and Sp = 77.8 % for miR-27; Se = 44.2 % and Sp = 92.0 % – for miR-101. When the ST2 concentration is above a threshold value (36.8 ng/mL), the risk of acute graft rejection is RR = 2.3 [95% CI 1.5–3.5]; Se = 54.2 %; Sp = 100.0 %. The diagnostic characteristics of miR-101 test have been improved in combination with ST2: RR = 3.8 [95% CI 1.6–8.7]; Se = 69.2 %, Sp = 100.0 %.

Conclusions

Decreasing miR-27 and miR-101 (above the -4.9 and -8.03 fold change resp.) and increasing ST2 (above the 36.8 ng/mL) threshold levels are associated with high risk of acute rejection after HTx. The best diagnostic characteristics has the duplex miR-101 and ST2 test.

doi: 10.1016/j.cca.2022.04.402

Clinica Chimica Acta 530 (2022) S453–S462

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Speaker Abstract

Diagnostic proteomic markers to detect kidney diseases

T. Ozben [a], E. Bellei [b], E. Monari [b], S. Bergamini [b], A. Ferrari [c], A. Tomasi [b]

[a] *Department of Biochemistry, Medical Faculty, Akdeniz University, Antalya, Turkey*
[b] *Department of CHIMOMO, Medical Faculty, University of Modena and Reggio Emilia, Modena, Italy*
[c] *Headache and Drug Abuse Study Center, University Hospital of Modena and Reggio Emilia, Modena, Italy*

Objective

Early detection of kidney disorders based on selective biomarkers could permit to diagnose patients at the initial stage of the disease, where the therapy is still possible to stop or prevent occurrence of advance disease. Urinary proteomics is primarily applied to the study of renal and urogenital tract disorders. Here are reported two distinct successful examples of this approach for the discovery of early urinary biomarkers of kidney-related dysfunctions: diabetic nephropathy (DN), a well-known complication of diabetes frequently leading to dialysis, and drug-induced nephrotoxicity, a possible condition caused by medication-overuse headache (MOH).

Methods

Urine samples were first concentrated and desalted. Subsequently, they were subjected to two-dimensional gel electrophoresis (2-DE) coupled to mass spectrometry (MS) for protein identification. Furthermore, some proteins were verified by Western Blot and ELISA test.

Results

In diabetes-related study, 11 differentially expressed proteins were detected (8 up-regulated and 3 down-regulated) in Type 2 Diabetic (T2D) and Type 2 Diabetic Nephropathy (T2DN) patients compared to the healthy control subjects. In MOH study, a total of 21 over-excreted proteins was revealed in urine of non-steroidal anti-inflammatory drugs (NSAIDs) and mixtures abusers versus controls. Particularly, 4 proteins were positively validated by immunoblotting and ELISA.

Conclusion

Urinary proteomics allows non-invasive assessment of renal diseases at an early stage by the identification of characteristic protein pattern.

doi: 10.1016/j.cca.2022.04.761

Standardization and harmonization of biological measurements: Successes, pitfalls and challenges

P. Gillery

University Hospital and Faculty of Medicine, UMR CNRS/URCA n°7369, Reims, France
Scientific Division of the International Federation of Clinical Chemistry and Laboratory Medicine, France

Standardization and/or harmonization of laboratory tests are key processes ensuring comparability of results and thus have a pivotal role in optimal patient care.

The Scientific Division (SD) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) develops reference measurement procedures (RMPs) and reference materials in order to improve assay standardization and result interpretation internationally. Whereas simple measurands were initially targeted, new candidates, mainly peptides, generate specific analytical challenges due to their complex structures, including existence of isoforms or occurrence of post-translational modifications.

However, solving analytical problems is not the end of the story, since later phases aiming at actually implementing standardized methods in clinical practice may prove full of pitfalls because of the number of involved stakeholders. Biological standardization may have unexpected consequences at the clinical level, and may face obstacles due to diverse specific regulations or cultural barriers throughout the world.

A typical example is the standardization of HbA1c, the gold standard for diabetes monitoring and diagnosis. All methods currently implemented worldwide are traceable to the IFCC-RMP, maintained by an international network of expert laboratories, and provide comparable values. However, the standardization process has led to recommend different units for HbA1c reporting, which has still not yet been implemented in all countries, because of sources of resistance mainly related to previously established guidelines and clinical recommendations.

The need for standardization must be shared by all stakeholders: scientific societies, clinical societies, national metrology institutes and reference laboratories, academia, manufacturers, individual clinical and laboratory professionals, patient associations, and eventually regulators. Therefore, standardization initiatives cannot be managed without considering the complexity of health organizations and regulations worldwide. IFCC-SD coordinates global and integrated approaches with all involved stakeholders, from the early phases of selection/prioritization of tests to the field implementation of methods. Specific topics are discussed in this session.

doi: 10.1016/j.cca.2022.04.762

doi:10.1016/j.cca.2022.04.110

Managing relationships and leading changes within the heterogeneous workplace

A.A. Khine Wamono

Division of Chemical Pathology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, National Health Laboratory Services, Tygerberg Academic Hospital, Cape Town, South Africa

Medical laboratories in both public and private sectors across the world have faced the changing environment of diverse needs, resources, political and economic landscapes and most importantly the workforce. Workforce diversity refers to the variety of differences among people in the organization related to age, gender, education, skills, employment status, designation, physical appearance, health, family and social status, nationality, ethnic background, culture, thinking style, religion, race and more. A complexity of managing diversity whilst having to ensure all employee maintain productivity in a highly stressful environment can be a daunting task for the lab managers. Diversity management aims to create and maintain a positive work environment by ensuring both similarities and differences of diverse individuals in the workforce are recognized and valued, so that they can all effectively contribute to the organizations' strategic objectives and goals. Cross cultural collaboration, inclusive work practices and teamwork are extremely crucial for organizational success and a mix of employees bring a variety of perspectives and ideas to the table, which can provide the organisation with unique insights. However, if diversity was not managed effectively, it could rather lead to personal conflicts, miscommunications, higher levels of employee turnover, and poor service delivery. Laboratory leaders and managers are accountable for managing the diversified workforce and are liable for the productivity and development of individual employee in the organizations. Employees also have to learn to realize their differences as assets, rather than liabilities in order to work in a productive way. Thus, managing a diverse work force is challenging to a great extent especially in times of change as strained relationships and conflicts are common. This presentation focuses on the benefits of a thriving diverse workplace as well as its potential risks and some strategies and examples on how to manage relationships and lead the change in such environment.

doi: 10.1016/j.cca.2022.04.763

The leader versus the manager and building leadership through emotional intelligence

P. Sharma

Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, India

Leadership and Management qualities although different, are both essential components for running an effective laboratory. Ideally, leaders have people who follow them while managers have people who work for them. Laboratories need both a strong leader and an effective manager to get their team on board to follow them towards their vision of success. Leadership is about getting people to understand and believe in a vision and to work together to achieve set goals while managing is more about administering and making sure that the day-to-day things are happening as they should. There are three ways of being effective towards successful leadership: leading self, leading others, and leading the organization. To develop leadership skills, one doesn't need to be mechanical and non-emotional, but rather focus on interpersonal and intrapersonal skills. Apart from technical skills, strategic thinking and knowledge, an effective leader also requires emotional intelligence. Emotional intelligence is defined as "the ability to accurately perceive and manage self and others' emotions and to understand the signals that emotions send about relationships." It has also been referred to a critical group of non-cognitive skills, capabilities and competencies which help individuals to control and manage their emotional response to events and pressures. There are five major emotional intelligence competencies that build the foundation of good leadership. They are Motivation, Self-awareness, Self-regulation, Social skills, and Empathy. Each of these competencies has its own components that a leader needs to imbibe for using emotional intelligence in their leadership strategies. For motivation, leaders need to lead by example, inspire, not get afraid of difficult stuff, remain focused and get driven. For self-awareness, leaders need to be confident, honest, direct, consistent. For social skills, Leaders should be good communicators, be approachable, and should listen to others. For empathy, leaders need to be compassionate and influential. Good leadership skills along with the proper implementation of emotional intelligence can evolve modern-day laboratorians into effective leaders of their field.

doi: 10.1016/j.cca.2022.04.764

Conflict resolution process and innovative solutions to complex challenges

M. Orth

Vinzenz von Paul Kliniken, Stuttgart, Germany and Heidelberg University, Medical Faculty of Mannheim, Germany

Routine group interaction is first disrupted by a conflict and the group is no longer united. Conflict escalation may give way to conflict resolution stage, after which the group can eventually return to routine group interaction. Conflict is more than a disagreement since one or both parties perceive a threat (real or not). Response to conflicts is based on our perceptions of the situation, not necessarily to the facts. Perceptions are affected by information available, life experiences, culture, values, and beliefs. Conflicts trigger strong emotions and are an opportunity for growth. However, there are healthy and unhealthy ways of managing and resolving conflict and resolving conflict requires skills.

Conflicts in medical laboratories are frequent and unique: diagnostic labs operate in a dynamic environment with operational and strategic needs changing, evolving, and expanding. Unlike other medical specialties, we face massive consolidations of facilities and merging of institutions to profit from economies of scale. We must be able to readily respond to huge increases in volume, assay menu changes, and staffing challenges. Labs must be focused on multitude of short-term goals while not losing sight of decisions for the future regarding staffing, automation, instruments and IT. However, clinical laboratories belong to healthcare, must obey specific legal and ethical limits of healthcare (such as services have to performed in person), and cannot expand their medical services without limits. Physician may not extend services by hiring employees unlike a commercial firm and e.g. there are specific laws for medical data protection and a special ethical code for physicians (Declaration of Geneva). Laboratory Medicine is the medical discipline with the second most patient contacts (after General Practitioners), the results of laboratory tests trigger many medical decisions, and the majority of laboratory tests performed are time-critical. However, personal encounters of laboratory personnel with patients are infrequent, lab testing is sometimes regarded as a commodity, not an individually-tailored medical service, and it is difficult to reach intensive personal involvement of associates with patients.

This session will enable the participants to:

- list specific causes of conflict in a medical laboratory
- describe different styles of conflict resolution
- select different skills to manage conflicts

- avoid detrimental mistakes during conflicts

doi: 10.1016/j.cca.2022.04.765

Real time patient QC - Middleware requirements and model validation

T. Badrick

Royal College of Pathologists of Australasia Quality Assurance Programs, Australia

Patient Based Real Time Quality Control (PBRTQC) techniques are becoming practical alternatives for many clinical chemistry assays because of commutability, greater sensitivity at detecting bias changes, application to pre-analytical error detection, and cost-effectiveness.

For PBRTQC to be effective and assay performance to be continuously monitored, laboratory data needs to be extracted and analyzed in real-time by the algorithm within the middleware of LIS. There should be an ability to reset the algorithm and exclusion of specific patients after a workup of the alarm.

Many analytical platforms will likely have onboard PBRTQC applications, but there will still be a need for laboratories to optimize and validate this middleware. There are several measures of performance for PBRTQC, including power function analysis, total error allowable (TEa) detection probability, average or median number of patient result affected before error detection (ANPed or MNPed), sensitivity/specificity of error detection, bias detection curves and the number of patient results required to detect error with a given probability. The performance verification of PBRTQC can be undertaken by in silico analysis. This can be performed without incurring a high cost beyond the human resources and computational power required.

To perform simulations, a set of representative patient data should be collected. It is often easier to use computer-based simulation where the actual patient population is used, adding varying levels of additional bias and block sizes to gauge the ability of the algorithm to detect the error while releasing as few erroneous patient results as possible.

doi: 10.1016/j.cca.2022.04.766

Down under EQA

T. Badrick

Royal College of Pathologists of Australasia Quality Assurance Programs, Australia

The Royal College of Pathologists of Australasia Quality Assurance Programs (QAP) is the leading supplier of EQA programs in Australasia as well as providing EQA to over 80 countries. The QAP provides programs in Chemical Pathology, Hematology, Microbiology, Anatomical Pathology, Cytopathology, Molecular Genetics, Serology, Immunology, Point of Care, and Biosecurity.

There is an emphasis on education and a large number of workshops are conducted by the QAP to provide support for participants in EQA interpretation and quality improvement. The organization is accredited to ISO 9001, ISO 17043, ISO 45001, and ISO 14001.

The QAP has been offering programs for over thirty years and has developed a range of unique programs in each discipline.

doi: 10.1016/j.cca.2022.04.767

TDM and Pharmacogenetics: Competitors or friends?

T. Van Gelder

Leiden University Medical Center, Leiden, Netherlands

Around 20 years ago, pharmacogenetics was predicted to revolutionize pharmacotherapy. Patients would no longer be treated with standard drugs in a standard dosage. Instead, the right drug in the right dose would be selected for each individual based on a genetic test. Individuals that were likely to experience adverse events would be identified before drug treatment, and personalized medicine would be within reach.

The time needed to genotype, and the cost associated with genetic tests, have been reduced to a level that is no longer prohibitive for widespread use. Not only detection of single-nucleotide polymorphisms, but also more extensive whole-genome sequencing is now widely available. The FDA has included pharmacogenetic information in the labels of more than 150 approved drugs. To assist physicians, the Royal Dutch Association for the Advancement of Pharmacy established a Pharmacogenetics Working Group that developed pharmacogenetics-based therapeutic (dose) recommendations following a systematic review of the literature. Electronic prescription systems in which the pharmacogenetic information of the patient is recorded now provide clinical decision support and encourage physicians to use the genetic information available.

Although pharmacogenetic studies have found correlations between pharmacokinetic parameters and gene polymorphisms for a large number of drugs the clinical application is still limited. For critical dose drugs physicians have a lot of experience with TDM, and for fine tuning drug dose TDM will remain necessary. Based on a pharmacogenetic test an optimal starting dose can be selected, with the aim of reaching target concentrations more rapidly. However, with efficient TDM it is possible to rapidly correct for the effect of genotypic deviations in pharmacokinetics, thus decreasing the utility of a genotyping approach. The influence of pharmacogenetics may be larger in populations where TDM is not performed.

In this presentation the basic principles of TDM and pharmacogenetics will be briefly explained, en examples will be shown of drugs for which the interplay between TDM and pharmacogenetics is highly relevant. Furthermore, you will find out whether TDM and pharmacogenetics are competitors or friends!

doi: 10.1016/j.cca.2022.04.768

Proteoform analysis to fulfill unmet clinical needs

C. Cobbaert, R. Ruhaak, Y. Van Der Burgt

Leiden University Medical Centre, Netherlands

In medical laboratories proteins in body fluids are routinely tested for diagnostic and prognostic purposes, as well as for therapy monitoring. It is however widely acknowledged that there is room for improvement with regard to sensitivity and specificity levels of current medical tests. Especially clinically effective and disease-specific tests that support diagnosis at an early and curable stage are lacking for a wide variety of diseases. Since the HUPO project, it is known that the complexity of the human body in health and disease largely arises from variations in protein expression between cells, tissues and body fluids, and from protein modifications. Consequently, identification and quantification of molecular alterations in proteins seems to be key for enabling Precision Medicine.

Aiming and searching for effective (protein) biomarkers should start by defining specific unmet clinical needs with the clinicians. To this end mass spectrometry (MS)-based proteomics has been applied as a discovery tool in retrospective studies of (large) clinical cohorts of body fluids. Mass spectrometry(MS) strategies have been further optimized for identification of primary amino acid sequences of proteins and their post-

translational modifications. Amongst other factors protein quantification requires in-depth knowledge about the nature of the measurand(s). This includes analysis of proteoforms by combining MS-strategies for quantitative peptide analysis (bottom-up) and intact protein analysis (top-down).

Notwithstanding the huge number of promising biomarkers from discovery studies, the number of new protein markers that made it from MS-based proteomics into the clinic is scarce. Key-reasons for this translation-lag are the use of invalid samples, lack of thoughtful study designs, silo-thinking of the stakeholders involved, inappropriate test evaluation and inadequate test standardization. Note that standardization should start with defining the measurands intended to be measured. Interestingly, proteoforms and post-translational modifications (PTMs) have hardly been taken into account because of technical challenges and the increased complexity of the resulting data. Proteoforms of existing protein biomarkers (such as PSA, antithrombin, apo(a),….) may provide an additional structural layer to quantitative levels of individual proteins with potential for Precision Diagnostics. The often ignored presence of proteoforms in protein tests and their implication for clinical chemistry proteomics and protein standardization will be discussed.

doi: 10.1016/j.cca.2022.04.769

Cap guideline: Initial detection and measurement of a monoclonal immunoglobulin protein

D. Keren

The University of Michigan, Ann Arbor, Michigan, USA

Advances in therapeutic options for multiple myeloma and related disorders has improved outcomes for patients and created a need for consistency in laboratory practices for the initial detection of monoclonal immunoglobulin proteins (M-proteins). A survey of 772 laboratories in 38 countries by the College of American Pathologists (CAP) found vast inconsistencies between testing practices that indicated an underutilization of methods needed to detect monoclonal free light chains or small quantities of M-proteins. In response to this problem, the CAP convened a panel of experts in laboratory detection of M-proteins to create a contemporary guideline for harmonizing this process. The panel had representatives from, and was performed in collaboration with the American Association of Clinical Chemists, American Society for Clinical Pathology, American Society of Hematology, and the International Myeloma Working Foundation. The 10 statements produced to assist laboratories in detecting M-proteins will be presented in detail with supporting literature.

doi: 10.1016/j.cca.2022.04.770

Importance of commutability for metrological traceability in standardization and harmonization

W.G. Miller

Virginia Commonwealth University, United States

Commutability is a property of a reference material such that the numeric relationship for results measured using different measurement procedures closely agrees with the relationship observed for results from a panel of patients' samples. Commutability is a required property for matrix-based secondary certified reference materials used in a metrological traceability chain. If a non-commutable secondary certified reference material is used as a calibrator, the non-commutability bias will be propagated through the metrological traceability chain and cause results for patients' samples to be different among different clinical laboratory measurement procedures. Commutability is not required for an IVD-manufacturer's measurement procedure specific working calibrator or end user calibrator. Commutability can be verified using several experimental designs. A critical experimental design element is the criterion for acceptable agreement between results for a reference material and a panel of patients' samples. The criterion is a fraction of the uncertainty required for a reference material's intended use in the metrological traceability chain related to the analytical performance specification for results from patients' samples. If a matrix-based certified reference material is not commutable for use with a specific measurement procedure, a correction for the non-commutability bias can be added to its metrological traceability chain so that results for patients' samples are correctly metrologically traceable to the value assigned to a non-commutable certified reference material.

doi: 10.1016/j.cca.2022.04.771

Commutability issues in standardization and harmonization of clinical laboratory results

W.G. Miller

Virginia Commonwealth University, United States

Commutability is a property of a reference material such that the numeric relationship for results measured using different measurement procedures closely agrees with the relationship observed for results from a panel of patients' samples. Commutability is a required property for matrix-based secondary certified reference materials used in a metrological traceability chain. If a non-commutable secondary certified reference material is used as a calibrator, the non-commutability bias will be propagated through the metrological traceability chain and cause results for patients' samples to be different among different clinical laboratory measurement procedures. Commutability is not required for an IVD-manufacturer's measurement procedure specific working calibrator or end user calibrator. Commutability can be verified using several experimental designs. A critical experimental design element is the criterion for acceptable agreement between results for a reference material and a panel of patients' samples. The criterion is a fraction of the uncertainty required for a reference material's intended use in the metrological traceability chain related to the analytical performance specification for results from patients' samples. If a matrix-based certified reference material is not commutable for use with a specific measurement procedure, a correction for the non-commutability bias can be added to its metrological traceability chain so that results for patients' samples are correctly metrologically traceable to the value assigned to a non-commutable certified reference material.

doi: 10.1016/j.cca.2022.04.772

Risk management and regulatory changes for POCT

J. Nichols

Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America

Point-of-care testing (POCT) is laboratory testing performed close to the site of patient care, outside of a formal laboratory. POCT is an increasingly popular means of performing laboratory testing because results are available faster than traditional testing. However, there is greater opportunity for error, because the test is often performed by clinical staff without experience or formal laboratory education. In the United States, CLIA non-waived devices must analyze 2 levels of quality control each day of patient testing, or the facility can develop a risk based Individual Quality Control Plan (IQCP) that will reduce the fre-

quency of external controls in lieu of internal control processes engineered by the manufacturer to reduce the probability of errors. ISO 14971 describes the application of risk management to medical devices, and regulatory and accreditation agencies in the United States now routinely inspect IQCPs as part of the clinical laboratory inspection process. This session will discuss the regulatory changes that introduce industrial risk management principles to the clinical laboratories and particularly POCT. Other recent regulatory changes will be discussed including new College of American Pathologist (CAP) checklist questions that apply to molecular POCT, as well as CLIA modernization efforts and changes under consideration to make nursing degrees equivalent to a biological sciences degree that would allow nurses to perform the same CLIA moderate and high complexity tests as medical technologists.

doi: 10.1016/j.cca.2022.04.773

LIS requirements for laboratory management

L. Je-Hoon

Department of Laboratory Medicine, Eunpyeong St. Mary's Hospital, The Catholic University of Korea, South Korea

The Laboratory Information System (LIS) is a software that records, manages, and stores the data needed by the clinical laboratory, which is an important element in operating the laboratory. LIS is traditionally required to transmit test orders to equipment, track orders and store results in searchable data form. It should also be possible to manage the flow of information between the laboratory, the workers and the patient, and be designed to optimize clinical care as well as laboratory operations. Modern LISs have evolved to include new features not previously seen, such as clinical decision support rules, system integration, and on-site test support.

One of the methods designed to address the problems caused by the exponential growth in the amount and quality of information along with the development of new technologies is the Laboratory Information Management System (LIMS), which can be improved by integrating and managing many processes, and many in vitro diagnostic companies have also adopted this concept to use it for data management such as sample management, quality control, and workflow analysis. Additional functions with traditional LIS should be satisfied according to an increase in the field of test, development of test methods such as LDT (laboratory developed test), development of new markers, new data types such as genetic testing, and integrated reports.

When starting a new laboratory than the existing well-established one in terms of the operation of the laboratory, the total test process (TTP) should be understood and reviewed in conjunction with the laboratory certificate along with the workflow and related LIS functions of each analytical phase. Especially, it is needed to cooperate with clinicians in determining the items and ranges of critical value reporting (CVR), as well as in defining the turnaround time and considering a plan for its reduction. The role of the LIS in the use and management of appropriate quality control programs, utilization of automated verification systems, and administrative support such as document and inventory management will need to be understood and applicable. The development of middleware, in particular, has enabled us to facilitate these extended and advanced functions.

It is difficult to predict future LIS, but the function and form of LIS related to laboratory work will change in accordance with the Fourth Industrial Revolution, ultimately enabling accurate and error-free reporting that can be the mission of LIS.

doi: 10.1016/j.cca.2022.04.774

Laboratory monitoring of coagulation disorders by clot waveform analyais

M. Shima

Department of Pediatrics, Nara Medical College, Japan

Some current coagulation analyzers have a potential for monitoring of the changes in transmittance or absorbance during the APTT or PT assay. By processing the data, clot waveform analysis (CWA) can be performed for the assessment of the global clotting function. In addition to qualitative evaluation, clotting function can be quantitatively assessed by various parameters including coagulation velocity and coagulation acceleration. In 2013, we attempted to standardize the reagents, coagulation analyzers and parameters for CWA in the FVIII/FIX subcommittee of SSC (Shima M et al. J Thromb Haemost 2013; 11:1417-20). The CWA was useful for assessment of global clotting function of hemophilia patients. Furthermore, the CWA can be applied to the monitoring of the hemostatic treatment for hemophilia with inhibitor. We investigated the utility of the CWA for monitoring of bypassing therapies using modified trigger reagents with mixture of ellagic acid and small amount of TF (Elg/TF CWA). (Haku J et al. J Thromb Haemost 2014;12:355-62). We further attempted to apply CWA to the monitoring of FVIIIa mimicking bispecific antibody, emicizumab, which has been approved for the hemophilia A patients (Nogami K et al. J Thromb Haemost 2018;16:1078-88). Recently, we found that the coagulation velocity curve of hemophilia A is different shape from other clotting factor deficiencies. Therefore, we established new CWA with parameters of weighted average. We also applied the measurement of weighted average to template matching. We compared parameters of weighted average of each plasma sample with those of 158 templates obtained from coagulation factor deficient plasmas of various severity and lupus anticoagulant positive plasmas. This template matching was useful for quick diagnosis of hemophilia A with single APTT measurement.

doi: 10.1016/j.cca.2022.04.775

Korean actions taken for implementation of measurement traceability in laboratory medicine

S. Park, H. Lee, J. Jeong

Center for Bioanalysis, Korea Research Institute of Standards and Science, Daejeon, Rep. of Korea

Actions taken in Korea to implement measurement traceability in clinical diagnostic testing are introduced. KRISS (Korea Research Institute of Standards and Science), the NMI (national metrology institute) of Korea, has developed a number of reference measurement procedures and certified reference materials (CRMs) for major clinical diagnostic tests, which were validated through relevant international comparisons among NMIs. KRISS and the Korean community of laboratory medicine have worked together for implementation of measurement traceability. It began with KAEQAS (Korean Association of External Quality Assessment Service) who generously provided an opportunity for a special proficiency test (PT) to use KRISS CRMs first time as unknown samples, which let us grab an idea about potential deviations between NMI certified values and conventional consensus values of participating laboratories. Afterward, KRISS and KSLM (the Korean Society for Laboratory Medicine) collaborated in more organized manner to establish 'Accuracy Based Proficiency Testing' supported with measurement traceability provided by KRISS. A similar trial was made with KSNM (Korean Society of Nuclear Medicine) in which KRISS cortisol CRMs were used as unknown samples in a special PT for radioimmunoassay. The series of collaborations between the NMI and the major players of laboratory medicine dramatically raised the awareness of the potential impact of measurement traceability in practices of clinical diagnostic testing. Recently, the utilization of KRISS CRMs has been expanded for improvement of

data quality of nation-wide annual health check-up. The state of the art of the measurement quality of major testing facility has been assessed using KRISS CRMs, which is ought to be the first step to found valuable big data on public health monitoring.

doi: 10.1016/j.cca.2022.04.776

The laboratory and hypertension -adding value to the clinical guidelines

S. Bhargava

Sir Ganga Ram Hospital, New Delhi, India

Most diseases require the laboratory's results for diagnosis, prognosis and management. At the same time, knowledge of pathophysiology of a disease are constantly advancing.

To exemplify this, let us look at the role of laboratory in hypertension.

Understanding of hypertension has progressed considerably– from critical events (myocardial infarction/stroke/renal failure) to detailed pathophysiology. Accordingly, our work-up, as per the guidelines, has evolved to include markers of renal and cardiovascular damage (American College of Cardiologists and American Heart Association, 2017, and Ontario Guidelines Advisory Committee, 2008). However, involvement of the brain or vasculature is not assessed, nor is prevention of multiorgan damage enabled.

Towards this, a 4-stage laboratory workup is suggested:

a) at the time of a routine health checkup (risk biomarkers for those who are at risk, e.g. renin-angiotensin system markers, inflammatory markers, TSH, etc).
b) at the time of diagnosis (all baseline parameters and markers to guide therapy, e.g. blockers or angiotensin receptor blockers, urinary metanephrines, antioxidants, etc),
c) at the time of follow-up (markers of target organ damage like natriuretic peptides, homocysteine, C-reactive protein, etc), and
d) for research (assessing the utility of other associated biomarkers e.g. adrenomedulin, angiotensin II, aldosterone, ACE II, neuron specific enolase, etc). It is important that laboratories upgrade themselves as and when new molecules or new roles of existing molecules are identified.

Thus, appropriate utilisation of laboratory services could enhance prevention and better management of hypertension and associated target organ damage. This would reduce the morbidity of hypertension and decrease its economic burden.

doi: 10.1016/j.cca.2022.04.777

Clinical consultant in the field of genetics

D.D. Payne

CPDP Consulting, LLC, Denver, Colorado 80227-2230, USA

Molecular Diagnostics includes several subcategories such as: 1) Inherited d

Diseases, 2) Oncology, 3) Transplant Genetics, 4) Pharmacogenomics, 5) Prenatal

Diagnostics, and 6) Infectious disease. Rapid advances in all areas of Molecular Diagnostics can create a significant knowledge gap between the clinical chemist and the health care provider. The gaps can be characterized in four different phases of laboratory medicine specifically: 1) pre-examination, 2) examination, and 3) post-examination phase of testing. A fourth phase is characterized as therapeutic monitoring of the patient and represents situations when the same patient is retested using molecular methods. The fourth phase is most common in Personalized Medicine applications in Oncology, Transplant genetics, Pharmacogenomics and Infectious Disease testing. Because errors from healthcare providers can impact these phases of testing, the clinical chemist's participation in education and intervention can insure that the appropriate test is ordered and that the laboratory results are interpreted correctly. This clinical chemist-healthcare provider collaboration improves the management of the patient's disease or condition. A case-based approach will be used during this session to illustrate how the clinical chemist can serve as a clinical consultant in the area of genetics.

doi: 10.1016/j.cca.2022.04.778

Evidence- or eminence-based laboratory medicine

A.R. Horvath

New South Wales Health Pathology Department of Clinical Chemistry & Endocrinology, Prince of Wales Hospital, Sydney, Australia

We often like to highlight the importance of our profession by referring to the popular quote that pathology tests influence at least 70% of medical decisions. Even if this claim was true, do we know if those tests lead to the right medical decisions? Do we have enough high quality and robust evidence in laboratory medicine to confidently demonstrate that tests add value and deliver benefits to patients?

The key hurdle in evidence-based laboratory medicine is that patients rarely benefit directly from testing; the effects rely on the test results and the resulting management consequences – something laboratory professionals rarely have an insight into or follow up. Tests can also cause serious physical and psychological harms to patients if the wrong test is selected, or the test is used on the wrong patient, or for the wrong clinical question or at the wrong time – again clinical areas laboratory professionals have less control over. Inappropriately evaluated tests hitting the market too early can cause overdiagnosis, underdiagnosis or misdiagnosis.

On the one hand, we do not have enough high quality evidence to link testing with patient outcomes. On the other hand, we have too much 'evidence', often in the form of lengthy and sometimes poorly developed or even conflicting guidelines from various organisations, many of which do not even involve laboratory professionals when formulating recommendations. When health care professionals face such volume and quality of information, combined with time and financial pressures, no surprise that they revert to their own experience and judgment, and eminence-based medicine trumps evidence-based medicine.

Poor quality and lack of explicitness of recommendations on laboratory testing call for methodological and reporting standards for diagnostic trials, more regulation on the pre-market evaluation of new biomarkers, better quality systematic reviews and guidelines with transparent and graded recommendations to increase the validity, applicability, and the clinical and cost-effectiveness of laboratory services. These can only be achieved if laboratory professionals become more proactive partners of clinicians and patients and shift their focus from only perfecting analytical quality of testing to the entire diagnostic process.

doi: 10.1016/j.cca.2022.04.779

NGS for rare diseases

V. Shotelersuk

Center of Excellence for Medical Genomics, Faculty of Medicine, Chulalongkorn University, Thailand

Massive Parallel Sequencing or Next Generation Sequencing (NGS) can be applied to many medical areas including cancer, infectious diseases, pharmacogenomics, and non-communicable diseases. Since one of the most tangible benefits of clinical exome or genome sequencing currently is for care of rare diseases and my personal experience is in the area, I will focus on NGS application for rare diseases.

With more than 7,000 different disorders, rare diseases are collectively not rare. In addition, studying rare diseases could lead to better understanding of common diseases. My presentation will include the discoveries of new human disease genes in our lab (genes for an intellectual disability, a skeletal dysplasia, epilepsy, an autoimmune disease, a dilated cardiomyopathy, and a hypertrophic cardiomyopathy). Identification of the responsible genes is the first step toward deep understanding of the disease. Unveiling pathogenesis will sooner or later lead to specific and effective treatments for the disease.

In addition, I will share our real experience in the application of clinical exome and genome sequencing in clinical practice, including comparing yield of exome and genome for the diagnosis of rare diseases and using rapid exome sequencing to help diagnose and improve care of patients with acute and severe symptoms.

doi: 10.1016/j.cca.2022.04.780

Analytical performance requirements in therapeutic drug monitoring

M. Shipkova

TDM Competence Center, Synlab Holding Deutschland GmbH, Synlab MVZ Leinfelden-Echterdingen, Germany

Therapeutic Drug Monitoring (TDM) is a complex approach aimed at improving patient care by individually adjusting the dose of drugs. Typically, guidance of therapy by TDM requires consideration of a broad spectrum of patient characteristics: demographic, clinical, pharmacogenetic, pharmacokinetic and pharmacodynamic. Pharmacokinetic monitoring based on the determination of drug concentrations, their appropriate interpretation, and consequent use to optimize drug dosing for the individual patient is the most classical and broadly available tool to perform TDM. Although services in pharmacokinetic TDM are provided for many decades now, the respective analytical requirements are often not well defined. However, with increasing interest in personalized medicine the awareness about the importance of analytical issues steadily grows as do the efforts to achieve proper standards of practice worldwide.

This presentation will discuss specific determinants of the analytical performance required for methods to measure drug concentrations; sources of analytical variability in TDM including the impact of diverse laboratory practices; guidelines frequently used to set acceptance criteria for analytical methods in TDM; measures for improvement of analytical services and challenges to implement them. As examples of projects dedicated to fostering proper analytical performance in TDM published IATDMCT (International Association of Therapeutic Drug Monitoring and Clinical Toxicology) recommendations for the analyses of immunosuppressive drugs and for the application of dried blood spot (DBS) based measurement procedures in TDM will be shortly addressed.

doi: 10.1016/j.cca.2022.04.781

Accreditation of medical laboratories – An ongoing work

T. Zima

Institute of Medical Biochemistry and Laboratoiry Medicine, Charles University, Czechia

At the beginning of the 21st century is one of the priorities in laboratory medicine improvement of quality of whole lab processes for patient and staff safety including accreditation. Quality in the health care is the level of excelence of the health care provided in relation to the current level of knowledge and technical development.

Accreditation is an effective way to demonstrate competence of the laboratory, is a tool to recognize laboratories world-wide, is linked to periodical audits stimulating to keep and improve the quality, leads to high standard of services for clients (patients, health care providers, etc.). The first systems of QMS in labs are internal and external quality control and educational activities.

The strategic plans of IFCC and EFLM include improvement of quality on whole laboratory activities and focusing of accreditation of labs based on ISO standard (ISO 15189:2013 Medical laboratories – Requirements for quality and competence) encompasses all the assessment criteria specified in the policy of quality. Other national or international standards as Joint Commission International (JCI), SLIPTA –WHO, Clinical Pathology Accreditation (CPA) in UK, College of American Pathologists (CAP)) are also used in some countries for accreditation.

The process of accreditation was started 20 years ago and number of accredited labs increasing annually. Obligatory accreditation of labs exists in few countries (e.g. France) but in some areas as genetic testing are in many countries. Now, Czech Republic has 255 accredited labs in all areas of laboratory medicine but only few of them were accredited 10 years ago.

The quality management system provides integration of all processes required quality policy with needs and requirements of the users. The processes for selecting, evaluating and transfer of samples and results between the labs and referral laboratories are precisely described with responsibilities of both sites. Risk management is important part to identify the potential risk for patients safety and laboratory processes with aim to eliminate them. For personnel is important to precise job description with competencies and responsibilities with evaluation of effectiveness of continual professional development. Precise determination of biological reference intervals and critical values are important for correct communications connected to patient safety. The labs shall describe the quality quantitative indicators with regular reviewing at all laboratory processes. Also, ethical issues are normative and they are showing the impact of these topics for patients.

The benefits of accreditation are standardization of all processes, responsibility of each member of team. The accreditation of labs improves facilitation of accurate and rapid diagnostics, efficiency of treatment and reduction of errors in the all laboratory processes. Accreditation is not about who is the best, but who has a system of standard procedures with aim to improve the quality and patient safety. Quality system is about people, with people and for people.

Supported by RVO MZ CR VFN64165.

doi: 10.1016/j.cca.2022.04.782

Translational Lipidomics

M. Hersberger

Division of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Switzerland

Lipids are often classified into eight classes including fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterols, prenols, sachharolipids and polyketides. This nomenclature classifies the more than 24402 biologically active lipids archived in the structure database

of the LIPID MAPS Lipidomics Gateway. Lipids of several of these classes are essential in humans for energy production and storage, for the generation and integrity of membranes, for cell signaling, and for the regulation of inflammation and its resolution.

Targeted lipidomic approaches have been used for the last decades primarily to understand inborn errors of metabolism in the biosynthesis of sterols and in the metabolism of fatty acids, glycolipids (sphingolipids) and cholesterol. Such targeted lipidomic approaches are now routinely offered by specialized diagnostic laboratories to diagnose diseases in these pathways such as Smith-Lemli-Opitz syndrome, MCAD deficiency, Zellweger syndrome, Fabry and Niemann-Pick diseases. More recently, atypical deoxy-sphingoid bases have been identified, which are greatly increased in hereditary sensory and autonomic neuropathy type 1, another inborn error of metabolism, and intermediate degradation products of glycolipids emerged as novel biomarkers for a series of lysosomal storage diseases including Fabry and Gaucher.

In addition to the role of lipidomics in inborn errors of metabolism, this approach is now applied in the field of inflammation, its regulation and resolution. Lipidomics has propelled our understanding of the metabolome of the polyunsaturated fatty acids and cholesterol, which started to be identified more than fifty years ago. The development of more sensitive MS-technologies in recent years allowed the dramatic increase in the identification and measurement of enzymatically oxidized fatty acids often grouped as oxylipins. It is now clear that oxylipins have profound biological functions that orchestrate the onset and the resolution of inflammation. There are several classes of oxylipins which include the prostaglandins and leukotrienes, and the more recently discovered lipoxins, resolvins, protectins and maresins. Similarly, certain oxysterols were shown to regulate inflammation.

While targeted lipidomic approaches are usually used to measure the low abundant oxylipins and oxysterols in human tissue, untargeted lipidomic approaches covering a broad range of lipid classes evolved recently and have been used for biomarker discoveries and mechanistic studies in a variety of polygenic diseases. Using these untargeted lipidomic approaches, encouraging associations have been observed between several lipids and lipid mediators with diseases like MS, bacterial meningitis, bacterial pneumonia, acute respiratory distress syndrome, and Alzheimer's disease.

doi: 10.1016/j.cca.2022.04.783

Interpretation of HbA1c results, new targets and new uses. Is it time for change?

S.L. Atkin

School of Postgraduate Studies and Research, Royal College of Surgeons in Ireland, Bahrain

The DCCT and UKPDS trials, for type 1 and type 2 diabetes respectively, highlighted the impact of intensive therapy to lower HbA1c, with a reduction in microvascular complications particularly for retinopathy and nephropathy at the conclusion of these studies. Subsequent long-term data in the EDIC study and the UKPDS cohort related the decrease in HbA1c to macrovascular disease and mortality. The suggestion that the lower the HbA1c the better the diabetes outcome led to the ADVANCE, VADT and ACCORD studies that confirmed the reduction in HbA1c was associated with a reduction in microvascular, but not macrovascular complications. However, 'the lower the better' HbA1c concept was questioned in the ACCORD study, with stringent HbA1c targets being associated with increased mortality in the intensive treatment group. This led to the HbA1c target pendulum swinging back to less stringent control post-ACCORD and the guidelines suggesting the need to individualize targets rather than issue prescriptive levels suitable for all patients. What has also emerged is that HbA1c variability may have an increasingly important role, with the studies indicating that greater HbA1c variability over time is associated with a greater risk of retinopathy, nephropathy and cardiovascular disease. However, all the data to date has been based on retrospective analysis and there is a need for prospective studies to confirm these findings. Furthermore, the role of other measures such as glycated albumin need to be addressed. Overall, the current data suggests that the laboratory calculation of HbA1c variability with diabetes risk prediction may have a future role in guiding clinical practice.

doi: 10.1016/j.cca.2022.04.784

Electronic apps and medical diagnostics data management

K. Adeli

The Hospital for Sick Children, University of Toronto, Canada

Laboratory medicine is a domain which offers a unique opportunity to analyze objective patient laboratory data and enable ready communication to both healthcare workers as well as patients. In recent years, an increasing number of web-based and mobile applications has been developed to improve access to laboratory test information and test result interpretation. They range from simple apps that provide reference lab value information to complex medical diagnostics data management. As examples, the "eLab" developed by Tru-Solutions Inc. is a comprehensive medical diagnostic center and lab management software that provides a user friendly interface and access control. It is linked iMedDx.com to allow flexible patient search and selection and includes an eLab Dashboard on mobile/tablet, allowing patients and labs/hospitals access to lab reports online. The Davis's Laboratory & Diagnostic Tests medical app provides another useful app with a wide-breadth of tests, as well as guidance on how to counsel and collect tests. The app is available on multiple platforms including the iPhone/iPad, Android and Blackberry. The "LabGear" is a medical lab reference app providing a pocket tool for medical laboratory test and is integrated with MedCalc with normal lab value reference information for over 200+ lab tests. There are several other medical apps that provide reference lab values including CALIPER, MedRef, Normal Lab Values, and Lab Tests. The CALIPER App has been developed in our laboratory for paediatricians, family physicians, and other healthcare workers worldwide. It is a user friendly and easy tool to assess a child's laboratory test results using the latest reference value database developed based on a study of thousands of healthy children and adolescents. The CALIPER apps allow pediatricians & family physicians to interpret laboratory test results for over 180 medical laboratory tests in children and adolescents using a comprehensive database of pediatric reference standards. WEB App: https://caliper.research.sickkids.ca/#/ In this presentation, I will review some of the key web and mobile resources in laboratory medicine and will discuss the critical importance of electronic apps in management of medical diagnostics data.

doi: 10.1016/j.cca.2022.04.785

Considerations of valuable technologies for total laboratory automation

Y. Lee

Department of Laboratory Medicine and Genetics, Soonchunhyang University Bucheon Hospital, Soonchunhyang University College of Medicine, Bucheon, Korea

Total laboratory automation started with automated specimen handling in the early 1970s. It has evolved into a more comprehensive hard-

ware with integration to software technologies that are applicable to many parts of the laboratory. With the healthcare environment rapidly changing, clinical laboratories are being challenged to achieve lesser overall costs, be more efficient in their process and improve quality.

The challenge to do better and at lower costs, coupled with the rapid evolution of automation technologies have triggered laboratories to approach clinical laboratory automation differently. To utilize these cutting-edge technologies, the clinical laboratory's strategy for total laboratory automation must use a multi-disciplinary approach, as these technologies enable laboratory to increase their productivity and meet their pre-analytical and post-analytical quality requirements.

Recent laboratory automation systems integrate various instruments and software to achieve process control across the board, from pre-analytical to post analytical process. By adopting these new approaches, many clinical laboratories can overcome the limitation of resources and maximize the efficiency of laboratory's overall operations.

Total laboratory automation is rapidly changing from volume-based solution to value-based solution.

doi: 10.1016/j.cca.2022.04.786

The arrival of a new era of clinical laboratories with fully automated LCMS systems

D. Kawakami

Shimadzu Corporation, Kyoto, Japan

A high-performance liquid chromatograph mass spectrometer (LC/MS/MS) systems are increasingly being used in recent years, due to their high sensitivity, high specificity, and ability to simultaneously measure drugs or other analytes in clinical laboratories. However, for clinical applications, the manual sample pretreatment operations required for using LC/MS/MS systems creates a processing bottleneck. Therefore, there was a need for a system that would enable anyone to safely and easily perform all steps, from sample pretreatment to LC/MS/MS analysis, automatically for large numbers of samples. The CLAM-2030, the world's first fully automated sample preparation module for LC/MS/MS released by Shimadzu Corporation in 2015 is such a system. Once blood collection tubes are placed in the system, it can automatically pretreat and, in combination with the LC/MS/MS system, fully automatically analyze the blood samples. This presentation will introduce innovative workflow of this system and its applications.

doi: 10.1016/j.cca.2022.04.787

Complementary markers of glycaemic control: Uses and limitations in clinical practice

E.S. Kilpatrick

Sidra Medicine, Doha, Qatar

HbA1c can be used diagnostically for type 2 diabetes and is the most commonly used marker in diabetes practice to monitor longer term glycemic control to guide disease management. However, HbA1c can be are unreliable in individual patients with or without diabetes including those with conditions such as anaemia or chronic kidney disease. Alternative or complementary glycaemic markers such as glycated albumin, fructosamine and 1,5 anhydroglucitol have been suggested in this group of patients. However, their widespread adoption has been partly hampered by a lack of evidence directly linking these markers to the risk of developing diabetes complications. New studies have helped address this shortfall and so there is now accumulating evidence that these tests are genuine alternatives to HbA1c. This lecture will touch on the advantages and disadvantages of these alternative glycemic markers within clinic practice.

doi: 10.1016/j.cca.2022.04.788

Guidelines, quality control and standardization of NGS testing

C. Endrullat

MSD SHARP & DOHME GMBH - Field Project Manager CDx, Germany

Next-Generation Sequencing (NGS) has been developed as a key player in sequence analysis within the last 15 years and exerts nowadays a decisive influence on academia, industry and the clinical environment. However, due to the fast development, NGS is still characterized by a lack of standard operating procedures, quality management/quality assurance specifications, validation strategies, proficiency testing systems and even less approved standards along with high cost and uncertainty of data quality. These aspects represent major obstacles for essential implementation of NGS in important areas such as clinical diagnostics, where reliable results, traceable and reproducible data as well as fast processing is crucial. On the one hand, appropriate standardization approaches were already performed by different initiatives and projects in the format of accreditation checklists, technical notes and guidelines for validation of NGS workflows. On the other hand, those efforts remain fragmented, linked to missing harmonization and less consent across all involved stakeholders. The presentation will provide an overview about existing standardization efforts, enlighten the problems associated with developing standards in NGS and give examples about possible standards and de facto standards, ultimately leading to reliable, traceable and reproducible results within a standardized environment.

doi: 10.1016/j.cca.2022.04.789

Expanding application of next generation sequencing in clinical laboratory

J. Smetana [b], M. Wayhelova [b], V. Vallova [b], E. Zrnova [a], I. Synkova [a], M. Vaňásková [a], J. Vinohradská [a], E. Ošťádalová [a], I. Valášková [a], P. Kuglik [b]

[a] *Department of Medical Genetics, Faculty Hospital Brno, Czech Republic*
[b] *Masaryk University Brno, Faculty of Science, Department of Experimental Biology, Czech Republic*

Brno, second largest city of Czech Republic and metropole of South Moravia region is well known in scientific community due to genesis of modern genetics based on discovery of fundamental laws of heredity by Johan Gregor Mendel.

In accordance on his legacy, Department of Medical Genetic of University Hospital Brno in cooperation with Masaryk University is able to provide the highest standards of scientific excellence and medical care in field of clinical genetics and genomics.

Technology of massive parallel sequencing (MPS) represents a significant breakthrough in the field of human genetics. This technology has largely contributed to the identification of new disease-causing genes and is nowadays well-established in clinical laboratories. Broad spectrum of diagnoses ranging from prenatal testing to evaluation of genetic oncological markers makes this technology essential diagnostic tool used on daily basis in our laboratory.

The presentation will show our portfolio of genetic diagnoses, algorithm of genetics analyses, results and impact of NGS based DNA analyses for patients in University Hospital Brno. During 2019, over 500 samples was investigated on Illumina MySeq platform. Amplicon-based

sequencing technology is used in diagnoses such as LQT syndrome, sudden cardiac death (SCS), Willebrand disease or for detection of high-risk mutations in patients with medulloblastoma, while in diagnostics and research of molecular bases of intellectual development (DID) we applied trio-based approach together with exome sequencing based on Agilent ClearSeq panel or recently Twist Human Core Exome Kit.

The novel genomic techniques based on allow generally faster and more effective patient´s care and brought countless number of possible utilization in clinical diagnostics.

Supported by the project of Faculty of Science of Masaryk University MUNI/A/1127/2019 and IP UH Brno 2019 PIG 5/19.

doi: 10.1016/j.cca.2022.04.790

Artificial intelligence and pathology data

T. Durant

Yale School of Medicine, United States

The field of artificial intelligence (AI) has undergone a noticeable and positive evolution of its technical capabilities throughout the past decade. As a result, a multitude of industries have become increasingly focused on identifying opportunities where the integration of AI-based technology may benefit current or future software applications. In the realm of clinical medicine, pathology and laboratory medicine are perhaps on the leading edge of specialties seeing this widespread adoption, both in academic and commercial echelons. Indeed, the modern clinical laboratory in the United States (US) now contains FDA-cleared IVD devices for automated complete blood cell counts and rapid antimicrobial susceptibility testing, both of which incorporate computer vision and machine learning as part of their assay. We as a field are now faced with the responsibility of serving as a learned intermediary for the implementation, stewardship, validation, and monitoring of these novel systems. While we remain in the early stages of adoption, academic and commercial use cases provide valuable lessons to consider when developing a general strategy to evaluate the integrity and utility of results produced by AI-based assays. Provided as a pragmatic approach, this session will explore contemporary examples of where AI has been applied to clinical laboratory practice. At its conclusion, attendees should be able to define fundamental machine learning concepts and begin to explicate a process for the implementation and monitoring of AI-based assays, as they become increasingly available to the clinical laboratory.

doi: 10.1016/j.cca.2022.04.791

Risk management in clinical laboratories

S. Kim

Department of Laboratory Medicine, Inje University, Ilsan Paik Hospital, Goyang 10380, Republic of Korea

Risk is the effect of uncertainty on the goal, which can be both an opportunity and a threat. Risk management is the analysis of risks and the creation of protective measures to keep risks at acceptable levels, and the purpose is to create new values or to protect existing values. Risk management should be part of organizational leadership and integrated into all activities of the organization through a structured and comprehensive approach. Risk management has long been used in the financial, manufacturing, transportation and service sectors, but it was introduced late in the medical sector. In particular, clinical laboratories began to introduce the concept after 2000.

The risk management process consists of establishing the context through communication and consultation, and the assessment and treatment of the risk. The laboratory should systematically apply risk management to the policies, procedures, and practices of the laboratory so that it can observe/review and record/report the risk management process. There are dozens of risk management tools, but many use risk registers, Failure Mode and Effects Analysis (FMEA), Failure reporting, analysis, and the corrective action system (FRACAS), fault tree analysis (FTA), fishbone diagram, and fault tree analysis. It doesn't matter which of these are used.

The risk management areas of clinical laboratories are very broad. It is important to establish a quality management system based on "risk-based thinking" when performing all administrative activities and patients' testing in the laboratory. However, since it is difficult to manage all areas at once to the detail, it can be a good strategy to start from the most needed area to the required level under each laboratory situation.

< The risk management areas of clinical laboratories >

- Effective laboratory management: business planning, financial, regulatory, operational, quality management including 12 quality management essentials
- Reducing patients' testing error: pre-pre-, pre-, analytical, post-, post-post- phase

The medical institution inspection in Korea requires the establishment of a risk management framework and improvement activities through risk management in each area (patient safety, infection control, facility environment, disaster preparedness, security, finance, employee safety, etc.). It is recommended to be a part of hospital risk management since laboratory medicine is a very important area for patient diagnosis and treatment, and collaboration with other departments is essential for pre- and post-analytical management. Improper requests, specimen nonconformity, swapped samples, delayed results caused by testing system problems, and errors in results will be good topics of laboratory risk management as a part of hospital risk management for patient safety.

At this symposium, I would like to share some examples of my experience in applying risk management in the laboratory. The following literature can be used when making risk management strategies in clinical laboratories.

The basic concept of risk management: ISO 31000: 2018 Risk management – guidelines

- risk-based thinking: ISO 45001:2018 Strategic Approach to Risk-based Thinking

Tools for risk management: ISO 31010:2019 Risk management — Risk assessment techniques

- Risk management tools to reduce patient examination errors: CLSI EP18-A2:2009 Risk Management Techniques To Identify And Control Laboratory Error Sources; Approved Guideline - Second Edition

Risk-based quality control plan

- (non-statistical): CLSI EP23-A: 2011 Laboratory Quality Control Based on Risk Management, 1st Edition (Individualized Quality Control Plan, IQCP)
- (statistical): CLSI C24-ED4:2016 Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions, 4th Edition

Laboratory quality management system (including 12 quality system essential, QSE) and patients' safety

- ISO 9001:2015 Quality management systems — Requirements
- WHO Laboratory Quality Management System Handbook 2011

doi: 10.1016/j.cca.2022.04.792

Clinica Chimica Acta 530 (2022) S463–S464

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Monday 27 June - 14.30–15.30. Tuesday 28 June - 16.00–17.00

Educational Abstract

EduW 5 Clinical utility of procalcitonin as a diagnostic and prognostic biomarker

S. Tan

Department of Laboratory Medicine, National University Hospital, Singapore

Procalcitonin (PCT) is a 116 amino acid peptide belonging to the calcitonin family of super peptides. PCT is encoded by the CALC-1 Gene located on chromosome 11. Physiologically, in the absence of infection, the transcription of the CALC-1 gene will be suppressed. In infective states, non-neuroendocrine tissues upregulate CALC-1 gene which in turn produces PCT. This biomarker is valuable in the diagnosis and management of sepsis because it increases within 3-6 hours of initial triggers, has a moderate half-life of 25-30 hours, and has demonstrated to rise significantly after endotoxin exposure but its increase is minimal in viral infections. As such, it has greater specificity than other pro-inflammatory markers in early identification of bacterial infections and sepsis. In this lecture, the speaker will explore the utility of procalcitonin which had served as a diagnostic modality in lower respiratory tract infections as well as its use in guiding clinical antibiotic stewardship. Further, we will review the literature and discuss its prognostic value in morbidity and mortality in septic patients, comparing its efficacy against other commonly utilized laboratory pro-inflammatory markers. Lastly, we will explore and compare the performance of contemporary assays quantifying PCT and their methodo.

doi: 10.1016/j.cca.2022.04.756

EduW 5 Epidemiology and diagnosis of nasopharyngeal carcinoma

E.W Pang

Director of Central Laboratory in Quality Healthcare Medical Services Limited, Hong Kong

Nasopharyngeal carcinoma (NPC) is the predominant tumor type arising in the nasopharynx, the narrow tubular passage behind the nasal cavity. Worldwide, there are approximately 80,000 incident cases and 50,000 deaths each year, but there are significant differences in ethnicity and geographic distribution. Especially the Cantonese living in the central region of Guangdong Province in Southern China.

Serology is not commonly used in other head and neck cancers. However, if NPC is suspected or known, a blood test is needed to see if any antibodies against to Epstein-Barr virus (EBV) are present. Because of the preclinical phase of NPC is often associated with EBV reactivation, assessing the serological status of EBV is an important tool for predicting the subsequent development of symptoms.

doi: 10.1016/j.cca.2022.04.757

EduW17 Hepatic fibrosis markers - hyaluronic acid, PIIIP NP, C IV, laminin, cholyglycine

T. Waleed

Member of Executive Board of SSCC, Consultant and Head of Clinical Chemistry Section at King Abdulaziz Medical City, National Guard, Riyadh, Saudi Arabia

Noninvasive measurement of liver fibrosis is very crucial to diagnose and monitor liver fibrosis. Currently, the liver biopsy is the standard of care which is an invasive technique is liver biopsy which required intensive and long turn-around-time (TAT) and subject to many errors. New multiple biochemical serum markers have been evaluated and appeared in the diagnostic market. Some of these markers include the N-terminal propeptide of collagen III, hyaluronic acid (HA), laminin, and cholyglycine. The use of these markers has increased the sensitivity and specificity in diagnosing cirrhosis and fibrosis. In addition, automated systems to measure these markers have been developed and introduced in the international market which will help to improve TAT and reduce operation errors. The utility and benefits of these markers will be discussed in this workshop. In addition, the clinical validation of hyaluronic acid (HA) is one of these markers will be presented.

HA is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units which is widely distributed in connective tissue and is produced mainly in mesenchymal cells. HA has several functions, including lubrication in joints, prevention from bacterial invasion and internal body hydration. HA has been used to assess liver cirrhosis. It is also consistent with the stage of fibrosis and thus, may be useful for non-invasive hepatic fibrosis assessment. Various methods and assays have been developed to measure HA, however, they need the clinical validation to prove optimum clinical utility. Therefore, the aim of this study was to clinically validate two available methods from two different manufacturers for HA.

Blood samples were collected from patients with liver diseases and control healthy subjects and samples were used to evaluate these methods. The targeted method was competitive chemiluminescent immunoassay (CLIA) MAGLUMI (Snibe, China) and was compared to other commercial immunetroubdmetric method.

The agreement between the two assays was based on each assay cut-off. The bias, the clinical utility including, area under curve (AUC), the clinical sensitivity and clinical specificity in evaluating patients with cir-

rhosis and HCC were calculated and compared. In this presentation, we will address all these issues and the results and conclusion will be discussed at the end of the presentation.

doi: 10.1016/j.cca.2022.04.758

EduW17 Liquid biopsy for gastric diseases

M. Plebani

Full Professor of Clinical Biochemistry and Clinical Molecular Biology, Past-Dean of the Medical School of the University of Padova, Director of Department of Laboratory Medicine University Hospital of Padova, Editor in Chief of the Journal CCLM, Italy

Upper gastrointestinal symptoms are common in the community of developed countries and, in particular, dyspeptic symptoms are among the most common gastric complaints, experienced by 25-40% of the people during their lifetime. Endoscopy (Esofagogastroduodenalscopy, EGD) is the accepted reference standard test for diagnosing gastric and duodenal ulcers, esophagitis, and esophageal and gastric malignancy, with very high sensitivity and specificity (>95%). However, because 40% of the whole population have upper gastrointestinal symptoms, it is not possible to investigate everyone with dyspepsia and non-invasive strategies should be implemented to reduce endoscopy workload. Since 1980 a serological test based on simultaneous assays of two aspartic proteinases called Pepsinogen I and II (PGI and PG II) has been proposed as an effective test-and-treat strategy to identify the severity of gastric mucosa inflammation. In addition, the evidence that Helicobacter pylori (Hp) infection represents the most common cause of gastritis and that the assay of serum gastrin-17 (G-17) should be used as an indicator of the morphological status of the antral mucosa, paved the way to the adoption of a panel of multiple stomach-specific serum biomarkers as a diagnostic tool for the first-line examination of dyspeptic patients, as well as for the screening of patients at risk of gastric cancer. This panel of tests (PG I, PG II, G-17, and Hp antibodies), commonly termed as GastroPanel, represents a valuable “serological/liquid biopsy” with very high specificity and sensitivity. In a recently performed meta-analysis of 27 published studies, GastroPanel was shown to allow the diagnosis of atrophic gastritis in corpus and fundum (AGC) and atrophic gastritis in antrum (AGA) with 70.2% pooled sensitivity and 93.9/ pooled specificity, respectively. In another meta-analysis, the pooled sensitivity resulted to be 74.7% and the pooled specificity 95.6%. Traditionally, GastroPanel was performed using manual or semi-automated ELISA assays developed by Biohit Oyi (Helsinki, Finland), but there is the need for more automated and time-saving methods to allow easier adoption of the panel in clinical practice. The aim of the present work was to evaluate the analytical and clinical performances of PG I, PG II, G-17 and Hp antibodies chemiluminescent assays with reagents provided by SNIBE Diagnostic (Shenzhen, China) and totally automated on Maglumi 1000. A very satisfactory analytical imprecision with the coefficient of variations (CVa) between 2.87% and 7.29% fo all tests was found. Excellent analytical linearity was observed for PG I and PG II (up to 250 and 50 g/L), for G-17 up to 70 pmol/L and for IgG –Hp up to 150 EIU. The analytical correlation between ELISA and CLIA assays resulted to be satisfactory with regressions ranging from 0.89 to 0.98. Finally, a full clinical agreement between the data obtained with CLIA and ELISA was observed.

doi: 10.1016/j.cca.2022.04.759