

Kidney diseases

W001

### **COMPARISON BETWEEN PARATHYROID HORMONE RESULTS OBTAINED FROM TWO DIFFERENT METHODS IN THE FOLLOWING OF HEMODIALYSIS PATIENTS**

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#### **BACKGROUND-AIM**

Introduction: Parathyroid hormone provides important information in chronic renal failure, especially in hemodialysis patients.

Objectives: It is known that parathyroid hormone (PTH) results obtained by Centaur and Liaison technologies are different due to the lack of international standardization. We intend to demonstrate that these differences are consistent through the measuring range of the assays and that both methods can be used to follow hemodialysis patients as they provide similar responses to physicians, as long as the dosing of PTH is based on the same method. Based on the results of this study and in the guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI), Euromedic Portugal aims to standardize the response of all the group's laboratories to requests by physicians for the determination of PTH in hemodialysis patients.

#### **METHODS**

PTH hormone was initially determined in 150 samples from hemodialysis patients for analysis of the correlation method. Subsequently, 20 patients were randomly selected, and two samples were taken at the intervals of 1 month, in order to evaluate PTH variation over time in both methods. Clinical features and laboratory tests were analyzed and related literatures were reviewed.

#### **RESULTS**

Good correlation was found between results of the two parathyroid hormone methods, but the intact parathyroid hormone levels from Centaur were higher than from the Liaison with the following formula: Centaur = 1,8121 Liaison + 22,36; R<sup>2</sup>=0,9737. In the analysis of sequential results from the twenty patients, it was observed that in 8 of these PTH values increased and in another 8 were lowered, which indicated similar behavior in both methods. The remaining four had minor differences, which did not exhibit clinical significance.

#### **CONCLUSION**

The comparison study confirmed that the Centaur PTH results are higher than the Liaison and these are consistent, as evidenced by the correlation coefficient obtained (R<sup>2</sup> value 0.9737). We conclude that the information provided by the laboratory to physicians in monitoring dialysis patients provides the same clinical value using either of the studied technologies. Consequently, Euromedic Portugal can standardize the service provided to all laboratories dosing PTH by using Centaur technology.

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### **ESTIMATING GLOMERULAR FILTRATION RATE BY DIFFERENT FORMULAS**

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#### **BACKGROUND-AIM**

Formulas for estimating GFR - MDRD and CKD-EPI with creatinine only, cystatin C only or with both biomarkers, are continuously modified as they include age, gender, race, concentration of the marker. GFR has been estimated in 617 individuals: 153 clinically healthy persons; 152 patients with type 2 diabetes mellitus without hypertension; 150 patients with essential hypertension; 162 patients with type 2 diabetes mellitus and concomitant hypertension. All people enrolled in the study have been tested for: albumin in urine, albumin % in total protein, albumin/creatinine ratio (ACR), protein/creatinine ratio (PCR); serum creatinine (Jaffe method) and cystatin C (PETIA). To estimate GFR the following equations are used: MDRD with creatinine only, CKD-EPI with creatinine only, CKD-EPI with cystatin only, CKD-EPI with creatinine and cystatin C.

#### **METHODS**

We find out differences between the different equations, but the mean value of GFR is reduced in all patients. According to the formula used the stages of CKD differentiate in different patients. The formula with cystatin C only is with the highest values in control group and in patients with diabetes mellitus and hypertension. The equations with creatinine show underestimation in low levels and overestimation in high levels of GFR, from 5 to 11%. Clinical reliability for the accurate measurement of GFR is also complemented by ROC curves in patients. The combined equation is with the highest diagnostic efficacy based on ROC curves. GFR correlation with albumin is strongest with the combined formula and weakest with MDRD.

#### **RESULTS**

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#### **CONCLUSION**

Upon data verification it was found that the simultaneous use of creatinine-cystatin C equation is more effective than applying equations using these biomarkers alone. It was found that formulas

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### **IS THERE ANY ASSOCIATION BETWEEN MEAN PLATELET VOLUME AND HIGH SENSITIVE TROPONIN T LEVELS IN CHRONIC RENAL FAILURE PATIENTS?**

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#### **BACKGROUND-AIM**

The mortality in chronic renal failure (CRF) patients is higher than that in normal population. Cardiovascular events are the major contributors in increased mortality rate in CRF patients since more than 50% of deaths are due to cardiovascular events. Cardiac troponins are highly specific and sensitive markers of myocardial damage. Previous studies showed that elevated levels of cardiac troponin T concentrations as measured with a highly sensitive assay are related to incidence of cardiovascular death in patients with acute coronary syndrome. In addition, high sensitive troponin T (hsTnT) elevation has also been related to an adverse clinical outcome and/or increase in all-cause mortality in patients without acute coronary syndrome in CRF.

Bleeding problems and thrombotic complications are common in CRF. Although the main responsible factor is platelet dysfunction, multifactorial and complex mechanisms play important roles. Mean platelet volume (MPV) is a marker of platelet activation and function. Increased platelet volume is associated with increased platelet activity. Various disorders such as inflammation, hypoxia, vascular injury, thrombosis and atherosclerosis were found to be associated with MPV.

In this study, we aimed to investigate the relation of hsTnT, which is a biomarker for adverse clinical outcomes, with MPV in CRF patients.

#### **METHODS**

A total of 76 CRF patients (31 female, 45 male, mean age:  $51.8 \pm 16.4$  years) and 41 healthy control subjects (22 female, 19 male, mean age:  $45.7 \pm 11.6$  years) were included in the study. Levels of hsTnT in serum (ECLIA method) and MPV in whole blood with EDTA (Siemens Advia 2120) were measured in all study subjects. Relation of hsTnT with MPV was also investigated using Spearman correlation test.

#### **RESULTS**

CRF patients had significantly higher levels of hsTnT ( $0.066 \pm 0.007$  vs.  $0.004 \pm 0.001$  ng/mL,  $p < 0.00001$ ) than control subjects. There was no significant difference between the MPV levels of CRF patients and control subjects ( $8.12 \pm 0.97$  vs.  $8.53 \pm 0.78$  fl, respectively,  $p > 0.05$ ). Serum hsTnT levels did not correlate significantly with MPV levels in CRF patients.

#### **CONCLUSION**

The finding of no significant relation of MPV with a well-known biomarker of myocyte damage and outcome, hsTnT supported the view that MPV is not a reliable indicator for cardiovascular events in CRF patients.

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### **CORRELATION OF THE FORMULAS TO ESTIMATE GLOMERULAR FILTRATION RATE USING CISTATIN C AND CREATININE VERSUS MEASURED CREATININE CLEARANCE.**

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#### **BACKGROUND-AIM**

The glomerular filtration rate is usually assessed by measured creatinine clearance which allows an accurate assessment of renal function. Currently the use of formulas developed for the calculation of estimated glomerular filtration rate has been stimulated and they became a practical tool for assessment of renal function without the need for urine collection. This study aims to evaluate four different formulas to estimate glomerular filtration creatinine clearance measurement.

#### **METHODS**

The samples of 50 outpatients with medical request for measured creatinine clearance and cystatin C were evaluated. The group consisted of 28 men and 22 women with a mean age of 52±14 years (range of 26-70 years). All urine samples were collected during 24 hours for measured clearance. The method used for measurement of serum creatinine was traceable to mass spectrometry with isotope dilution (IDMS). For comparison purposes, the following equations were used: CKD-EPI creatinine, CKD-EPI cystatin C, CKD-EPI cystatin C and creatinin and MDRD.

#### **RESULTS**

The mean values of glomerular filtration in mL/min/1.73 m<sup>2</sup>, were:

Measured creatinine clearance: 83.1±30.8

CKD-EPI creatinin: 65.7±24.4

CKD-EPI cystatin C: 77.8±29.9

CKD-EPI cystatin C and creatinin: 71.2 ± 26.2

MDRD: 60,7 ± 22,4

The analysis of the creatinine clearance measured (x) versus estimated (y) resulted in the following regression equations and correlation coefficients:

CKD-EPI creatinin :  $y = 0.66x + 10.2$  ( $R^2 = 0.713$ )

CKD-EPI cystatin C:  $y = 0.79x + 11.4$  ( $R^2 = 0.676$ )

CKD-EPI cistatin C and creatinin:  $y = 0.75x + 8.61$  ( $R^2 = 0.786$ )

MDRD:  $y = 0.59x + 11.3$  ( $R^2 = 0.667$ )

#### **CONCLUSION**

In this study the formulas to estimate glomerular filtration rate showed a tendency to underestimate the results in relation to the measured creatinine clearance. The formula using cystatin C and creatinine showed better correlation when compared to measured creatinine clearance.

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### **EVALUATION OF PLASMA BNP CONCENTRATIONS IN PATIENTS WITH DIABETIC CHRONIC KIDNEY DISEASE**

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#### **BACKGROUND-AIM**

Background: In patients with chronic kidney disease (CKD), as in other populations, elevations in cardiac biomarkers level predict increased risk of cardiovascular events. Plasma B-type natriuretic peptide (BNP) is produced and released from cardiac ventricles. BNP regulates excretion of water and sodium in the kidney and when renal function deteriorates, BNP level is increased. In this study we examined the value of BNP in assessing the risk of developing end-stage renal disease and prediction of congestive failure in diabetic patients with CKD.

#### **METHODS**

Methods: Our study consisted on 44 patients with CKD, type 2 diabetes and cardiomyopathy (group 1) and 45 patients with CKD and diabetes mellitus, without clinical evidence of congestive heart failure (group 2). In both groups we had 5 predialysis patients and 5 patients on dialysis. We were analyzed plasma BNP concentrations, serum creatinine and proteinuria for all patients.

#### **RESULTS**

Results: BNP concentrations were significantly elevated in the group 1, compared to the group 2 ( $p=0.0098$ ). The average BNP level of the 44 patients was 1529.0 pg/mL (from 143.2 pg/mL to 5000 pg/mL). Median plasma BNP level in group 2 was 450.0 pg/mL. Serum creatinine and proteinuria concentrations were not significantly different between groups, but BNP concentrations correlated positively with longer diabetes duration ( $p=0.001$ ) and higher proteinuria in both groups.

#### **CONCLUSION**

Conclusions: Deterioration in kidney function, in both groups, increased BNP levels, and these values were the highest in patients on hemodialysis. Because of relationship between proteinuria and BNP, increased BNP may be a risk factor for the progression of renal disease. Measurement of BNP may improve the identification of patients with CKD who are closed to require renal replacement therapy, supporting a link between congestive failure and the development of end-stage of CKD.

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**CYSTATIN C AS A MARKER OF GLOMERULAR FILTRATION RATE IN TYPE 2 DIABETIC NEPHROPATHY**P. Gyawali<sup>1</sup>, B. Jha<sup>1</sup>, K.B. Raut<sup>1</sup><sup>1</sup>Institute of medicine, Tribhuvan University Teaching Hospital**BACKGROUND-AIM**

Diabetes is the most common cause of CKD worldwide. Growing body of evidence suggests, serum cystatin C as a superior marker than serum creatinine for assessment of renal function and in detecting early decline in renal function in diabetic nephropathy. This study examined the adequacy of the cystatin C as a marker of GFR for the assessment of nephropathy in the Nepalese patients with type 2 diabetes.

**METHODS**

101 patients diagnosed with type 2 diabetes, were categorized into different stages of nephropathy based on urine protein to creatinine ratio (PCR). Serum cystatin C level was measured using latex turbidimetry (Giese diagnostic), reference level 0.59-1.03mg/L. Serum creatinine was measured using modified Jaffe method with the reference level male (80-115 $\mu$ mol/L) and female (53-97 $\mu$ mol/L). Analytes were measured in Biotecnica 1500 chemistry auto-analyzer. GFR was estimated using MDRD equation and cystatin C based CKD-EPI (2012) equation. SPSS ver.20, t-test, one-way ANOVA, Pearson's correlation and ROC were used for data analysis and interpretation.

**RESULTS**

Cystatin C was elevated in 49 patients and serum creatinine was elevated in 38 patients out of 101 patients. Cystatin C level increased significantly with the progression of nephropathy ( $p < 0.01$ ). The mean serum cystatin C level in different stages of nephropathy were  $0.78 \pm 0.21$ mg/L (PCR  $< 15$ mg/mmol),  $0.95 \pm 0.33$ mg/L (PCR 15-50mg/mmol) and  $1.96 \pm 0.91$ mg/L (PCR  $> 50$ mg/mmol). Serum cystatin C level correlated significantly with urine PCR and serum creatinine ( $r = 0.516$ ,  $p < 0.01$ ) and ( $r = 0.90$ ,  $p < 0.001$ ) respectively. A significant ( $p < 0.001$ ) inverse correlation was observed between serum cystatin C and serum creatinine with eGFR (MDRD) ( $r = -0.89$ ,  $r = -0.81$ ) respectively. ROC analysis showed that the AUC was marginally better for serum cystatin C [(0.959) 95% CI: 0.925-0.993] than serum creatinine [(0.952)95% CI: 0.915-0.989] to detect eGFR  $< 60$ ml/min/1.73m<sup>2</sup> ( $p < 0.001$ ). To detect eGFR  $< 90$ ml/min/1.73m<sup>2</sup> AUC for cystatin C was 0.82 (95% CI: 0.734-0.906) and for serum creatinine was 0.88 (95% CI: 0.806-0.954) ( $p < 0.001$ ). The best cut off value of serum cystatin C to detect eGFR  $< 60$ ml/min/1.73m<sup>2</sup> and  $< 90$ ml/min/1.73m<sup>2</sup> was 0.993mg/L (sensitivity 92%, specificity 82%) and 0.775 mg/L (sensitivity 76%, specificity 84%) respectively.

**CONCLUSION**

Serum cystatin C is useful alternative or adjunct to creatinine as a marker of GFR for assessment of renal function in type 2 diabetic nephropathy.

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### **IGM ANTIBODIES AGAINST HLA : A CLINICAL STUDY IN TWO KIDNEY PATIENTS**

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#### **BACKGROUND-AIM**

Donor-specific antibodies (DSA) IgG against human leukocyte antigen (HLA) represent a significant barrier in kidney transplantation since they predispose to hyperacute rejection and reduce long-term graft survival. Recently were investigated also auto and allo-IgM antibodies, which don't seem to correlate with graft survival but probably affect rejection severity by switching to IgG isotype. Luminex® Single Antigen HLA class I and class II test (LSA I, II; One Lambda) can characterize and identify natural or not antibodies which might impact donor selection for transplantation.

#### **METHODS**

Luminex® LSA test was performed on two patients: 1) a 26 year-old woman, affected by systemic lupus erythematosus, waiting for a second kidney transplant, after a first transplant/explant due to a venous thrombosis; 2) a 27 year-old man affected by chronic interstitial nephropathy, waiting for a ABO incompatible living kidney donor transplant. Cross-matches between donor and patient were performed by CDC long-incubation assay and sera were analyzed to identify IgG, IgM and C1q-binding antibodies (C1qScreen, One Lambda) on Luminex® platform.

#### **RESULTS**

Woman post-transplant sera was positive at CDC test, but negative after diithiotreitol (DTT) treatment; indeed Luminex® test identified only IgM antibodies, including class I DSA (IgG negative, MFI < 1000) A26 specificity (MFI range 4000-12000). Initially cross-match assay on man was negative on T and B cells but positive after rituximab treatment and so after necessary transfusions. Luminex® results showed IgG antibodies levels not significant (MFI < 1000) in contrast to IgM antibodies level (MFI until 9000), including DSA A1 (MFI value 1500), before and after transfusion, C1q test negative.

#### **CONCLUSION**

Post-transplant woman sera showed auto and allo-IgM antibodies (MFI > 9000); some of these were DSA. It may be useful to consider IgM specificities, DSA or not, as forbidden antigens for next transplant, since IgM to IgG switch hasn't happened. Negative cross-match on T and B men cells and its positivity after rituximab treatment and transfusions suggest a real immunization. High levels of IgM antibodies, negative to C1q test, seem to be not correlated to transfusion, probably due to their "natural" origin.

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**COMPARISON BETWEEN A SECOND AND A THIRD GENERATION PTH ASSAY IN CHRONIC KIDNEY DISEASE PATIENTS.**M. Rodríguez Rodríguez<sup>1</sup>, D. García Seisdedos<sup>1</sup>, M. Rosillo Coronado<sup>1</sup>, M. García Collía<sup>1</sup><sup>1</sup>Ramón y Cajal Hospital**BACKGROUND-AIM**

PTH detection serve as noninvasive, diagnostic tool for the assessment of the renal osteodystrophy (ROD), highly prevalent among patients with chronic kidney disease (CKD). Biologically active PTH circulates as an 84 amino acid peptide. The bioactive PTH (1-84) (BI-PTH) peptide is metabolized to N-terminal, C-terminal and mid-regional fragments of varying lengths. PTH fragments and the PTH 1-84 intact accumulate in patients with CKD, presumably due to reduced excretion. Thus besides that, PTH fragments have a half-life that is 5-10 times longer than 1-84 PTH, it is important to know if intact PTH (I-PTH) assay, whose detection overestimates the true PTH concentration, could lead to diagnostic inaccuracies. The aim of this study was to compare the third generation BI-PTH assay with second generation I-PTH assay among different dialysis patients.

**METHODS**

Plasma samples were obtained from 89 patients with CKD stage 5. Plasma BI-PTH and I-PTH levels were measured using COBAS Elecsys PTH immunoassay (Roche Diagnostics, GmbH, Germany) on Cobas e411. It is a one-step sandwich electro-chemiluminescence immunoassay. The correlation between BI-PTH against I-PTH was performed using Passing and Bablok regression (PBR) analysis.

**RESULTS**

We have studied the relationship between estimated glomerular filtration rate (eGFR), using Modification of Diet in Renal Diseases formula (MDRD), and both assays BI-PTH and I-PTH. First, significant correlation between both assays was observed ( $r = 0.98$ ). Furthermore, the intact PTH is higher in dialyzed patients than in healthy individuals and it increases progressively with eGFR decrease. It is mostly due to impaired renal secretion. Second, the PBR analysis between BI-PTH against I-PTH was:  $BI-PTH = 0.54 (0.56-0.51) * I-PTH + 10 (16-5)$  (ng/mL), among the patients with CKD stage 5. The average increase of I-PTH relative to BI-PTH was of 42%, with a maximum of 60% and a minimum of 30%. But there was no significant shift between the different ranges of PTH chooses: from 0 to 150ng/mL, from 150 to 300ng/mL, and above than 300mg/mL. It was maintained the deviation between assays.

**CONCLUSION**

The third generation PTH (1-84) assay had comparable precision, performance and a strong correlation against second generation intact PTH assay. The difference between both method increases when baseline PTH increases. But there was a significant decrease, about an average of 46%, of the PTH levels. So, the target range of plasma levels of PTH ought to change if it is measured the PTH 1-84.



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### **COMPARISON OF TWO FORMS OF ESTIMATED DFG BLACK AFRICAN IN SUBJECTS: COCKROFT AND GAULT VS MDRD**

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#### **BACKGROUND-AIM**

The prevalence of kidney failure is increasing and also affects developing countries. In Africa particularly in Ivory Coast, in the absence of data on the MDRD formula, using the CG formula is still valid for estimating renal function, regardless of the epidemiological and clinical context. The present study aims to compare the CG and MDRD formulas in the diagnosis of chronic renal failure in black Africans subjects.

#### **METHODS**

The study involved 225 adult black Africans came to do routine checkups at a hospital in Abidjan. Serum creatinine was measured by colorimetric Jaffe method and the formula is the MDRD utilisée variable 4. In the absence of MDRD formula validated in black Africans, in our study we accept the hypothesis that the racial factor applied to American blacks is applicable to black Africans. Analysis of the collected data was mainly carried out with the EXCEL 2003 software and EPI info 6.04. the comparison is made by a concordance study from the kappa

#### **RESULTS**

The results showed that:

- In The men and women there is no difference between the DFG given by the two formulas ( $K = 0.62$   $Z = 9.65$ ,  $P < 0.001$ ); diagnostics agree significantly.
- There Is a discrepancy between the results given by both MDRD and CG formulas in the elderly over 65 years in the estimated GFR.
- For Obese subjects both formulas give different diagnoses ( $K = 0.128$ ,  $Z = 1.07$ ,  $P = 0.142$ )

#### **CONCLUSION**

Our results show that the choice of the DFG determination method should be based on context. So it becomes imperative to validate the MDRD method among black Africans in order to have more methods of determinations, and then choose the most suitable according to the clinical case for the diagnosis, classification and monitoring of CKD patients.

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### **CARDIAC MARKERS AND LEFT VENTRICULAR HYPERTROPHY IN ASYMPTOMATIC HEMODIALYSIS PATIENTS**

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#### **BACKGROUND-AIM**

**BACKGROUND:** Cardiac markers are often elevated in hemodialysis patients showing the presence of left ventricular dysfunction. **AIM** of the study is to establish the plasma levels of high-sensitivity cardiac troponin T (hs-TnT) and Amino-Terminal pro Brain-Type Natriuretic Peptide (NT-proBNP) and their relation to the presence of left ventricular hypertrophy (LVH) in patients on hemodialysis without signs of acute coronary syndrome or heart failure.

#### **METHODS**

**METHODS:** Were studied 48 patients undergoing hemodialysis - 26 men and 22 women. Were measured pre- and postdialysis levels of hs-cTnT and NT-proBNP at week intermediate procedure. Patients were divided in two groups according to the presence of echocardiographic evidence of LVH - group A - 40 patients (with LVH) and group B - 8 patients (without LVH). Blood concentrations of hs-cTnT and NT-proBNP was measured by commercially available assays – Roche Elecsys.

#### **RESULTS**

**RESULTS:** In the whole group of patients was found elevated predialysis levels of all two markers with significant increase ( $p < 0.05$ ) after dialysis with low-flux dialyzers. Predialysis values of NT-proBNP show moderate positive correlation with hs-cTnT ( $r = 0,47$ ). Such dependence is observed in postdialysis values of these markers. There is a strong positive correlation between the pre- and postdialysis levels: for hs-cTnT ( $r = 0,966$ ) and for NT-proBNP ( $r = 0,918$ ). It was found a significant difference in the mean values of hs-cTnT in gr. A and gr. B ( $0,07 \pm 0,01$  versus  $0,03 \pm 0,01$  ng /mL,  $p < 0,05$ ) and NT-proBNP ( $15\ 605,8 \pm 2\ 072,5$  versus  $2745,5 \pm 533,55$  pg /mL,  $p < 0.05$ ).

#### **CONCLUSION**

**CONCLUSIONS:** The results indicate the relationship of the studied cardiac markers with LVH in asymptomatic patients undergoing hemodialysis treatment.

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### **UROLITHIASIS: CHEMICAL COMPOSITIONS IN A SAMPLE OF 461 PATIENTS IN VOJVODINA, SERBIA**

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#### **BACKGROUND-AIM**

Urolithiasis represents the occurrence of stones in any part of the urinary system. The incidence of urolithiasis in some parts of the world is very different, so it can be said that the disease depends on the geographical area and age. In large number of cases illness is genetically predisposed, but some factors such as life-style and stress can be causes of illness as well. There are several theories about the origin of urolithiasis and microurolithiasis, but one thing is sure that when once occurs, it is highly likely that there is a recurrence. The aim of the research is to determine the incidence of certain types of stones in men and women. A total sample of 461 persons (229 men and 232 women, age 7 – 84 years) were analyzed.

#### **METHODS**

Chemical methods used for determining the composition of stone: oxalate was determined by reaction with resorcinol and sulfuric acid, phosphate with ammonium molybdate, urate xanthoproteic reaction and cystine in the reaction with sodium nitroprusside.

#### **RESULTS**

Both in men and women the most common stone of inorganic calciumoxalate was found, 46.3% in men and 23.3% among women. Calciumoxalate-phosphate is present in women in 8.6% and in men 4.4%. Pure phosphate stones were found in women in 1.3% only. And there the mixture of inorganic-organic stone called calciumoxalate-urate with 2.6% in males and 0.4% in females. Stones of organic origin are much rarer, urate is somewhat more common in men, with 3.5%, while in women in 1.3% of cases. Cystine type of stones in women occurs in 0.9% of cases, while in men the percentage was 0.4. The total number of analyzed oxalate stone formation is present in 34.7%, calciumoxalate-phosphate 6.5%, calciumoxalate-urate 1.5% and 0.7% phosphate. In relation to the total number, urate organic stones are found in 2.3% of cases, and cystine in 0.7%.

#### **CONCLUSION**

This study has shown according to the results that calciumoxalate takes first places in all stones, with much higher prevalence in males comparing with females.

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**RENAL PHOSPHATE HANDLING IN ANTIRETROVIRAL-NAÏVE HIV-INFECTED PATIENTS**T. Adedeji<sup>3</sup>, S. Adebisi<sup>1</sup>, N. Adedeji<sup>5</sup>, O. Jeje<sup>2</sup>, R. Owolabi<sup>4</sup><sup>1</sup>Department of Chemical Pathology, Benue State University, Makurdi, Nigeria<sup>2</sup>Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria<sup>3</sup>Department of Chemical Pathology, Obafemi Awolowo University, Ile-Ife, Nigeria<sup>4</sup>Department of Medicine, HIV Unit, University of Abuja, Nigeria<sup>5</sup>State Specialist Hospital, Ile-Ife, Nigeria**BACKGROUND-AIM**

Renal impairment is a common complication of human immunodeficiency virus (HIV) infection. We estimated the prevalence of moderate to life-threatening hypophosphataemia, and hyperphosphataemia associated with HIV infection before initiating antiretroviral therapy (ART).

**METHODS**

A cross-sectional analysis was performed on 212 consecutive patients within a hospital-based cohort. Controls were 50 HIV-negative subjects. Blood and urine were collected simultaneously for phosphate and creatinine assay to estimate fractional phosphate excretion (FEPi%). Estimated glomerular filtration rate (eGFR) was by Modification of Diet in Renal Disease equation.

**RESULTS**

Only 170 of the 212 selected patients submitted morning hours blood and urine; 99 (58.2%) were females. eGFR showed significant difference between patients' and controls' means ( $47.89 \pm 16.96 \text{ ml/min/1.73m}^2$  versus  $\geq 60 \text{ ml/min/1.73m}^2$ ,  $p=0.000$ ); implying a moderate chronic kidney disease in the patients. Of the 170 patients, 78 (45.9%) had normal plasma phosphate (0.6-1.4 mmol/L); 85 (50%) had hyperphosphataemia (range: 1.5-5.9 mmol/L). Grades 1 (0.5-0.6 mmol/L), 2 (0.4-0.5 mmol/L) and 3 (0.3-0.4 mmol/L) hypophosphataemia was observed in 3 (1.8%), 3 (1.8%), and 1 (0.5%) patient(s) respectively. None had grade 4 ( $< 0.3 \text{ mmol/L}$ ) hypophosphataemia. Overall, the patients had significantly higher mean plasma phosphate than the controls, 1.61 mmol/L versus 0.97 mmol/L ( $p < 0.001$ ); significantly lower mean urine phosphate than the controls, 1.78 mmol/L versus 17.09 mmol/L ( $p = 0.000$ ); but a non-significantly higher mean FEPi% than the controls, 2.27% versus 1.48% ( $p > 0.05$ ). Predictors of FEPi% were age (Odds ratio, OR 0.9, 95% confidence interval CI 0.7-1.3,  $p = 0.009$ ); weight (OR 2.0, 95% CI 1.49-2.8,  $p < 0.001$ ); and height (OR 1.76, 95% CI 1.04-5.5,  $p = 0.002$ ). Older age was associated with greater urine phosphate (OR 1.1, 95% CI 1.01-1.16,  $p = 0.019$ ). CD4+ predicted urine phosphate among males ( $p = 0.029$ ).

**CONCLUSION**

HIV infection induces renal insufficiency with reduced renal phosphate clearance that results in positive phosphate balance. Thus, hyperphosphataemia is highly prevalent, and there is mild to moderate hypophosphataemia but its life-threatening form (grade 4) is rare among ART-naïve HIV patients.

Kidney diseases

W013

### **β-TRACE PROTEIN AS MARKER FOR GFR IN RENAL TRANSPLANT RECIPIENTS**

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#### **BACKGROUND-AIM**

After renal transplantation monitoring and detection of slight-to-moderate changes in GFR is a prerequisite for an optimal patient management. Due to the limitations of serum creatinine and lack of validation of creatinine based GFR estimation equations in transplantation setting, β-Trace protein (BTP) has been proposed as an alternative marker for GFR.

Aim: The aim of this study was to evaluate the relationship between serum levels of beta-trace protein (BTP) and glomerular filtration rate (GFR) in renal transplant recipients

#### **METHODS**

We measured true GFR by <sup>99m</sup>Tc-diethylenetriaminepentaacetic acid (<sup>99m</sup>Tc-DTPA) and BTP and for comparison cystatin C and creatinine in 60 RTRs. We also conducted a study of the GFR estimates of the Cockcroft and Gault (C&G), and the abbreviated modification of diet in renal disease (aMDRD).

#### **RESULTS**

Serum levels of BTP progressively increased with the reduction of GFR. A good correlation was found between GFR and serum levels of BTP ( $r=0.938$ ), Creat ( $r=0.823$ ), Cys ( $r=0.907$ ). BTP has the highest sensitivity of 96% and specificity of 91% at a cutoff of 2.01 mg/L with area under the curve of 0.965. The BTP correctly classified 89% of patients compared to only 80% with cystatin-c, 75% with aMDRD equation, 69% with the Cockcroft–Gault equation

#### **CONCLUSION**

On the basis of the above results, we believe that BTP may be a useful and reliable analyte to estimate GFR in RTRs.

Kidney diseases

W014

### **COMPARISON OF THE CKD-EPI EQUATION AND THE MDRD STUDY EQUATION FOR ESTIMATED GLOMERULAR FILTRATION RATE**

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#### **BACKGROUND-AIM**

Clinical laboratories are increasingly reporting estimated glomerular filtration rate by using estimating equations. The aim of the study was to assess of performance by comparing the two most used formulas for the estimation of glomerular filtration rate.

#### **METHODS**

A retrospective study was designed 242 participants ( 47.9 % women), with a median age of 55 years [Interquatile range (IQR)] 40-67. GFR was estimated by the use of following estimation equation: two most commonly used creatinine-based equations Chronic-Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD).

#### **RESULTS**

The median of the estimated glomerular filtration rate according to the CKD-EPI equation and the MDRD study equations were 73.84 mL/min/1.73 m<sup>2</sup> (IQR) 34.07-102.46] and 68.09 mL/min/1.73m<sup>2</sup> ( IQR 33.35-95.45), respectively. The median of the measured glomerular filtration rate was 75.98 mL/min/1.73 m<sup>2</sup> ( IQR 33.69-109.31 ). The significant factors were then included in a multiple regression analysis correlated to the measured GFR (mGFR). The equation of mGFR = ( -5,860) – 0,079 (gender) – 1.529 ( serum creatinine ) + 0.249 ( age) +1.164 (CKD-EPI)-0.122(MDRD).

#### **CONCLUSION**

Both equations estimate similar magnitudes of renal functions, although the CKD-EPI equation has less false positives.

Kidney diseases

W015

### **TO EVALUATE THE USEFULNESS OF RETICULOCYTE HEMOGLOBIN EQUIVALENT AND DF-HYPO XE INDEX ON THE SYSMEX XE 5000 IN HAEMODIALYSIS PATIENTS WITH IRON DEFICIENCY ANEMIA**

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#### **BACKGROUND-AIM**

Anemia is a common complication of chronic kidney disease (CKD), particularly in dialysis patients. European guidelines stress that iron status should be regularly assessed for the optimal management of renal anemia. These indicate reticulocyte hemoglobin content (CHr) and the percentage of hypochromic RBC (%Hypo) as markers for functional iron deficiency. CHr equate with reticulocyte hemoglobin equivalent (Ret-He) which was measured on the Sysmex XE-5000. DF-Hypo XE obtained from calculation of haemoglobin, haematocrit and Ret-He, as an index equate with %Hypo. The aim of this study was to evaluate the clinical usefulness of Ret-He and DF-Hypo XE index as predictors of iron deficiency anemia (IDA) in haemodialysis patients.

#### **METHODS**

In 375 blood samples from patients on chronic haemodialysis. Ret-He was compared with traditional parameters for iron deficiency (serum iron <40 µg/dl, Tsat <20%, serum ferritin <100 ng/ml, hemoglobin <11 g/dl) for identifying iron-deficient status. Biochemical parameters were measured on Hitachi 7180 and Ret-He were measured on the basis of automated fluorescent flow cytometry which in the reticulocyte channel, using a polymethine dye on a Sysmex XE-5000.

#### **RESULTS**

Overall dialysis patients, Ret-He and DF-Hypo XE index mean value was 35.2 pg and 5.2. Compared with IDA, mean value of 32.3 pg for Ret-He and 7.2 for DF-Hypo XE index. With the Ret-He cutoff value of < 34.0 pg and DF-Hypo XE index cutoff value of > 6.0. Receiver operating characteristic curve (ROC) analysis revealed values of the area under the curve [AUC] for Ret-He of 0.719 (p<0.05). IDA could be diagnosed with sensitivity of 71.4 %, and specificity of 63.6 %. However, [AUC] for DF-Hypo XE index of 0.636 (p>0.1), and sensitivity of 63.6 % and specificity of 58.0 % were not good.

#### **CONCLUSION**

Ret-He is a reliable marker of cellular hemoglobin content, and is easily measurable on the widely spread and popular blood cell counter and can be used to identify the presence of iron-deficient status in dialysis patients, but DF-Hypo XE parameters is not.

Kidney diseases

W016

**STABLE PLASMA CREATININE CONCENTRATION MAY INDICATE DETERIORATING RENAL FUNCTION IN SEPSIS**J. Chew-harris<sup>4</sup>, P. Chin<sup>2</sup>, J. Pickering<sup>5</sup>, C. Florkowski<sup>1</sup>, P. George<sup>1</sup>, Z. Endre<sup>3</sup><sup>1</sup>*Clinical Biochemistry Unit, Canterbury Health Laboratories, Christchurch*<sup>2</sup>*Clinical Pharmacology, Christchurch Hospital, Christchurch*<sup>3</sup>*Department of Nephrology, Prince of Wales Hospital, Sydney*<sup>4</sup>*Department of Pathology, University of Otago, Christchurch*<sup>5</sup>*Emergency Department, Christchurch Hospital, Christchurch***BACKGROUND-AIM**

Changes in renal function are reflected by plasma creatinine (pCr) concentrations only after some considerable delay. We hypothesised that patients with sepsis may have reduced renal function despite a stable creatinine.

**METHODS**

We used data from 119 hospitalised patients given gentamicin for sepsis. Glomerular filtration rate (GFR) was estimated by gentamicin clearance calculated from the plasma peak and trough gentamicin levels. Change in pCr was calculated from baseline to pCr when gentamicin concentrations peaked (within 30 minutes post infusion). Baseline pCr was defined using a hierarchical model according to (i) first available pCr requested by a general practitioner (GP) within 7 days to 12 months from gentamicin dose (74 patients), (ii) pCr just before hospital discharge prior to gentamicin therapy (38 patients) or (iii) lowest pCr available during hospital stay (7 patients). Patients were classified as having: increased creatinine (pCrincrease), defined as a  $\geq 20\%$  increase from baseline; decreased creatinine (pCrdecrease), defined as a  $\geq 10\%$  reduction from baseline; and the remainder as stable creatinine (pCrstable).

**RESULTS**

Twenty-eight (24%) patients were classified in the pCrdecrease group, 61 (51%) in the pCrstable group and 30 (25%) in the pCrincrease group. Mean  $\pm$  standard deviation baseline pCr were as follows;  $93 \pm 24$   $\mu\text{mol/L}$  for the pCrdecrease,  $91 \mu\text{mol/L} \pm 27$  for the pCrstable and  $81 \pm 23$   $\mu\text{mol/L}$  for the pCrincrease groups. Gentamicin clearance was higher in pCrdecrease group ( $90 \pm 47$  mL/min) compared with the pCrstable ( $69 \pm 37$  mL/min) and pCrincrease ( $56 \pm 29$  mL/min) groups ( $P = 0.02$ ). In the pCrstable group, 49% of patients had gentamicin clearance  $< 60$  mL/min, of which only 5 had a baseline pCr value of above  $125 \mu\text{mol/L}$ .

**CONCLUSION**

The results demonstrate that gentamicin clearance was reduced in patients with stable plasma creatinine. This may result from reduced GFR combined with decreased creatinine production. In patients with sepsis, a stable plasma creatinine may represent impaired renal function



Kidney diseases

W017

### **MIRNAS IN KIDNEY DISEASE**

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#### **BACKGROUND-AIM**

Microribonucleic acids (miRNAs), an abundant class of endogenous interfering RNAs, have become a source of research in biology and medicine.

MiRNAs are small single strand noncoding RNAs that inhibit gene expression through the post-transcriptional repression of their target mRNA and are key regulators of normal kidney development and function. Deregulation of miRNA expression is involved in various human diseases and evidence from clinical and experimental studies demonstrate their critical role in renal pathophysiology. These circulating miRNAs are present in a stable form in different biological fluids with technical advances permitting their accurate detection.

#### **METHODS**

A review of the literature was made in books and electronic databases without restriction on type of the article or publication year with the aim of collecting the current evidence involving miRNAs in kidney health and disease.

#### **RESULTS**

A variety of miRNAs were particularly abundant in kidney, including miR-215, miR-216, miR-146a and miR-886 while miR-192, miR-194, miR-21, miR-200a, miR-204 are present in the kidney as well as other organs. MiR-192 was much highly expressed in the cortex than in the medulla. We found in hypertensive kidney disease an increased expression of intrarenal miR-200a, miR-200b, miR-141, miR-429, miR-205 and miR-192 and in diabetic kidney disease the profile shifted to miR-192, miR29a/b/c, miR-377, miR-215 and the deletion of Dicer in podocytes. Decreased miR-200c and increased miR-141, miR205 and miR-192 were found in IgA Nephropathy and in systemic lupus erythematosus a higher expression of miR-146a and miR-155 was found in urine sediment. In Polycystic kidney disease miR-17 and miR-92 were upregulated. In acute renal allograft rejection a pattern of miR-142-5p, miR-155, miR-210 and miR-223 was found while a profile of miR-142-3p, miR-204, and miR-211 was observed in chronic rejection.

#### **CONCLUSION**

The unique expression pattern of these circulating miRNAs has been correlated with certain human diseases, and can help to distinguish diseased individuals from healthy controls and constitute due to their precocity promising biomarkers for diagnosis and prognostic for various kidney diseases

Kidney diseases

W018

### **EQUATIONS FOR ESTIMATION OF GLOMERULAR FILTRATION RATE: WHERE DO WE STAND?**

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#### **BACKGROUND-AIM**

Chronic Kidney Disease has become a serious threat worldwide and early impaired kidney function can have an asymptomatic course. Therefore accurate assessment of kidney function is essential. The glomerular filtration rate (GFR) is the best indicator of renal function and an estimation of GFR (eGFR) is now an integral part of routine patient care. Equations for eGFR represent an important aid in this task and considerable effort has been made in last years in order to improve their accuracy and predictive value.

#### **METHODS**

A review of the literature was made in books and electronic databases without restriction on type of the article or publication year with the aim of collecting the evidence involving the appropriate use of the different equations for eGFR.

#### **RESULTS**

In order to assess GFR the clinician should use a GFR estimating equation based on serum creatinine rather than rely on serum creatinine alone. Serum creatinine should be measured using a specific assay with calibration traceable to isotope dilution mass spectrometry (IDMS) reference methodology.

The Modification of Diet in Renal Disease (MDRD) Study equation, The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and their modifications were reformulated with standardized serum creatinine contrarily to the Cockcroft- Gault formula. CKD-EPI equation is more accurate than the MDRD Study equation, especially at higher GFR (greater than 60 mL/min/1.73m<sup>2</sup>) enabling reporting of numeric values throughout most of the range of GFR for age and creatinine, especially in younger individuals, women and whites.

In pediatric population the use of the Schwartz and the Updated "Bedside" Schwartz equation is recommended.

The 2012 creatinine-cystatin C equation is more accurate than equations using creatinine or cystatin C separately. Dosing of Cystatin C is recommended when a confirmatory test is needed and in particular clinical contexts as early kidney disease, kidney transplantation, acute kidney injury and cirrhosis.

#### **CONCLUSION**

The 2009 CKD-EPI creatinine is useful as initial test for decreased GFR and should replace the MDRD Study equation for routine reporting of serum creatinine based eGFR by clinical laboratories.

The 2012 CKD-EPI creatinine-cystatin C equation is useful as a confirmatory test in selected patients

Kidney diseases

W019

**ASSESSMENT OF THE VALIDITY OF PROTEIN - OSMOLALITY RATIO IN A RANDOM URINE SPECIMEN, IN ESTIMATION OF PROTEINURIA IN CHILDREN AND THE USE OF SCHWARTZ FORMULA IN DETERMINING GLOMERULAR FILTRATION RATE IN CHILDREN**

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**BACKGROUND-AIM**

Protein-creatinine and protein-osmolality ratios in a spot urine sample are used as alternative tools to estimate 24-hour urinary protein excretion. Furthermore, the Schwartz formula is used to estimate glomerular filtration rate (eGFR) which needs only a serum creatinine measurement. Effective spot urine analysis would be very useful in clinical practice as it would cut down unnecessary cost, time as well discomfort to patient and staff.

Therefore, this study was done to evaluate the reliability of these alternative tools in the assessment of renal disease.

**METHODS**

24-hour and spot urine samples were collected from 85 children with kidney disease and 56 healthy children aged 3-12 years. Urine protein-osmolality ratio and urine protein-creatinine ratio in spot urine samples were compared with 24-hour urinary protein excretion.

In the same population urinary creatinine clearance was determined and serum creatinine was measured to compare the measured creatinine clearance against eGFR.

**RESULTS**

The optimal values discriminating abnormal protein excretion from normal individuals was a protein-osmolality ratio of 0.38 mg/L: mOsmoles/kgH<sub>2</sub>O (sensitivity 85.7%, specificity 100%) and a protein-creatinine ratio of 28 mg/mmol (sensitivity 100%, specificity 94%).

The cutoff value for discriminating mild proteinuria from nephrotic range heavy proteinuria was a protein-osmolality ratio of 2.00 mg/L: mOsmoles/kgH<sub>2</sub>O (sensitivity 91.5%, specificity 100%) and a protein-creatinine ratio of 186 mg/mmol (sensitivity 93%, specificity 98.5%).

A statistically significant correlation ( $r = 0.476$ ,  $P < 0.0001$ ) was observed between measured creatinine clearance and eGFR in the whole population.

**CONCLUSION**

Both urine protein-creatinine and protein-osmolality ratios can be used to determine proteinuria as well to differentiate heavy proteinuria from milder forms. Urine protein-creatinine ratio was more sensitive than urine protein-osmolality ratio in detecting mild proteinuria from normal proteinuria.

eGFR, although weak, had statistically significant correlation and agreement with the measured creatinine clearance values. The constant value (k) in the Schwartz formula should be validated for accuracy in a given environment before routine use in clinical settings.

Kidney diseases

W020

### **CYTOKERATIN 18 AND ITS ROLE IN BLADDER CANCER**

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#### **BACKGROUND-AIM**

Bladder tumors rank second in frequency among tumors of the genitourinary system. The disease is more common among men as the sex ratio is 3: 1. About two thirds of those infected have been registered in the age over 65 years. We quantify serum cytokeratin 18 in bladder cancer patients and compare the results to a healthy control group.

#### **METHODS**

Serum cytokeratin 18 levels were measured, using monoclonal sandwich ELISA method in 30 patients diagnosed with bladder cancer. Sampling period was 2012 – 2014. Patients with bladder cancer and control group were divided into two groups – smoker and non-smokers.

#### **RESULTS**

Measured serum cytokeratin 18 levels in control group were  $2.78 \pm 0.8$  ng/mL. Cytokeratin 18 levels in patients with bladder cancer were significantly increased  $52.8 \pm 12.7$  ng/mL;  $P < 0.001$ . In the two groups we found a higher levels of cytokeratin 18 in smokers compared to non-smokers ( $r = 0.472$ ;  $P < 0.001$ ).

#### **CONCLUSION**

Our results are showing that serum quantification of cytokeratin 18 is reliable in diagnosis of bladder cancer. Smoking increases secretion of cytokeratin 18.

Kidney diseases

W021

**COMPARISON OF MEASURED GLOMERULAR FILTRATION RATE (INULIN CLEARANCE) AND CKD-EPI EQUATIONS**A. Jabor<sup>1</sup>, J. Franekova<sup>1</sup>, A. Parikova<sup>2</sup>, M. Stolova<sup>2</sup>, Z. Kubicek<sup>1</sup>, O. Viklicky<sup>2</sup><sup>1</sup>Dept. of Laboratory Methods, Institute for Clinical and Experimental Medicine, Prague<sup>2</sup>Nephrology Clinic, Institute for Clinical and Experimental Medicine, Prague**BACKGROUND-AIM**

Different approaches are used for the estimation of the glomerular filtration rate (GFR) as well as for the gold standard (inulin clearance) calculation. The aim of our work was to compare Jung model of inulin clearance calculation with recently recommended equations for the estimation of GFR.

**METHODS**

45 patients were evaluated (35 women, 10 men, both kidney donors or patients). Inulin clearance was measured after i.v. standard bolus 50 mg/kg of body weight. Blood samples were taken +10, +60, +120 and +240 minutes after load. Measured GFR (mGFR) was calculated according to the Jung model. Estimated GFR (eGFR) was calculated according to the KDIGO guidelines 2012 as follows: CKD-EPI 2009 (creatinine, CKD-Cr), CKD-EPI 2012 (cystatin C, CKD-Cyst), CKD-EPI 2012 (combined equation based on creatinine and cystatin C, CKD-Comb). For comparison, we calculated eGFR from MDRD equation (MDRD) and creatinine clearance (24 hours urine collection, CCr). All values are given in ml/s per 1,73 m<sup>2</sup>. Predictive performance was assessed according to Delanaye: absolute bias was calculated as the difference between eGFR and mGFR (negative value means that eGFR is lower than mGFR), relative bias as percentage of this difference of the mGFR. Accuracy was calculated as the proportion of the eGFR within +/- 30% of the mGFR.

**RESULTS**

Medians (interquartile range) for respective eGFRs were: CKD-Cr 1,26 (1,16 – 1,34), CKD-Cyst 1,18 (0,99 – 1,45), CKD-Comb 1,20 (1,08 – 1,38), MDRD 1,16 (1,07 – 1,21), CCr 1,53 (1,37 – 1,77) ml/s per 1,73 m<sup>2</sup>. Three best correlation coefficients between mGFR and eGFR were 0,627 (CKD-Cyst), 0,602 (CKD-Comb) and 0,504 (CKD-Cr). Maximal percentage +/- 30% were for CKD-Cr (80%), CKD-Comb (76%) and CKD-Cyst (73%). Minimal relative and absolute bias were for CKD-EPI (-2%), CKD-Cyst (-7,4%) and CKD-Comb (-7,5%).

**CONCLUSION**

Clearance of creatinine overestimates GFR, while MDRD equation underestimates GFR. The highest level of comparability between mGFR and eGFR were found for 2009 CKD-EPI (creatinine) and 2012 CKD-EPI (combined, creatinine and cystatin C).

Kidney diseases

W022

### **EVALUATION OF CYSTATIN C REAGENT ON THE HITACHI 7600 AUTOMATIC ANALYZER**

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#### **BACKGROUND-AIM**

Cystatin C is a low-molecular-weight protein sized 13kDa, which is constantly produced by nucleated cells and freely filtered through the glomerular and then fully reabsorbed and degraded at the proximal tubule. Compared to creatinine, cystatin C can track the changes in GFR with greater sensitivity and specificity, and it is not affected by muscle mass, diet, or gender. Using the Gentian cystatin C immunoassay (Gentian, Norway), a recently developed cystatin C reagent, this study conducted a performance evaluation of Gentian cystatin C on Hitachi 7600 Automatic Analyzer (Hitachi Ltd., Japan).

#### **METHODS**

Precision and linearity studies were conducted by comparing results between that of the Hitachi 7600 Automatic Analyzer using the Gentian reagent and that of the SPAPLUS® analyzer (Binding Site, Birmingham, UK) using Binding Site reagent, a human cystatin C kit. In doing so, a particle enhanced turbidimetric immunoassay method was used. In addition, traceability of the Gentian reagent and Binding Site reagent to a cystatin C standard reference material, ERM-DA471/IFCC was also analyzed.

#### **RESULTS**

The coefficient of variations (CVs) for within-run imprecision at low and high levels were 1.5% and 0.9% and the CVs for total imprecision at low and high levels were 3.4% and 2.6%, respectively. In the linearity test, the coefficient of determination (R<sup>2</sup>) was 0.9994 (range, 0.23 to 7.50 mg/L). Comparison with the results obtained by Binding Site reagent showed a correlation coefficient of 0.983. However, in the traceability test, the Gentian reagent was more accurate than the Binding Site reagent and the total accuracy was 96.7%.

#### **CONCLUSION**

The Hitachi 7600 Automatic Analyzer showed satisfactory results using the Gentian reagent. Evaluation results suggest that the Gentian cystatin C reagent can be useful in terms of monitoring and assessing post-transplantation renal function, renal function during chemical treatment, diabetic kidney disease prognosis, and renal function of cirrhosis or rheumatoid arthritis patients.

Kidney diseases

W023

### **MEMBRANE-BOUNDED HEMOGLOBIN AS PROBABLE PREDICTOR OF CHRONIC RENAL FAILURE DEVELOPMENT**

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#### **BACKGROUND-AIM**

Prediction of the rate of worsening of chronic renal failure is one of the urgent problems of clinical nephrology. The main purpose of the research was to study the membrane-bounded hemoglobin in erythrocytes from patients with various stages of chronic kidney disease (CKD) and degree of chronic renal failure.

#### **METHODS**

235 patients with various stages of CKD and degree of chronic renal failure (CRF) were divided into three groups. The first group included 59 patients with CKD of 1-2 stages (CRF 0). The second group consisted of 73 patients with 3 stages of CKD (CRF 1). The third group (n=103) included patients with 5th stage of CKD (CRF 3). Blood of 32 healthy donors has been used for control testing. In erythrocytes membrane-bounded hemoglobin concentration has been estimated following the protocol of Toktamysova & Birzhanova. Comparison of the results obtained has been performed using non-parametric Mann-Whitney U-test (for independent variables).

#### **RESULTS**

The results obtained have demonstrated increase in membrane-bounded hemoglobin in patients with CKD 1,2 (CRF0) in comparison with control ones (by 1.7 times,  $p<0.05$ ). In erythrocytes of patients with CKD 3 (CRF1) elevation of the membrane-bounded hemoglobin concentration was higher than in control group. We have noted that the membrane-bounded hemoglobin elevation depended on an initial clinical form of the disease. In erythrocytes from patients with CKD 3 (CRF1) with chronic glomerulonephritis as initial clinical form of the disease the membrane-bounded hemoglobin concentration was higher than in controls samples (by 2.3 times,  $p<0.05$ ). In erythrocytes of patients with CKD 3 (CRF1) with chronic pyelonephritis as initial clinical form of the disease the membrane-bounded hemoglobin concentration was higher than in controls samples (by 1.4 times,  $p<0.05$ ). At the same time in erythrocytes from patients with CKD 5 (CRF3) the membrane-bounded hemoglobin concentration was significant lower than in comparison with control subjects and with all previous groups of patients.

#### **CONCLUSION**

In our opinion, the membrane-bounded hemoglobin decrease was associated with the renal parenchyma damage and might be regarded as additional prognostic factor.

Kidney diseases

W024

### **UROLITHIASIS IN AN AFRICAN POPULATION; FINDINGS IN A NAIROBI HOSPITAL**

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#### **BACKGROUND-AIM**

Urolithiasis is a global problem whose incidence is reported to be increasing across the world. In the past urolithiasis was reported as being rare among the indigenous African population but more recent data in some African countries suggest otherwise. The number of cases seen at health facilities in Nairobi have increased over the years; 56 cases were reported at the national referral hospital over a fifteen year period (1980–1995) while a subsequent study at two private facilities reported 178 cases over a five year period (2004–2009). In these two studies chemical analysis of the stones was not performed.

We reviewed the chemical composition of the renal stones and clinical characteristics of patients seen at the Aga Khan University hospital (AKUH Nairobi).

#### **METHODS**

This was a retrospective study which utilized patients' clinical and laboratory records for year 2013. Sixty seven patients with confirmed urolithiasis formed the study. The analytical method for stones at AKUH is wet chemistry and can detect carbonate, cysteine, phosphate, magnesium, calcium, ammonium, uric acid and oxalate. Age, sex, symptoms, imaging and laboratory investigations performed, location of the stones and therapeutic procedures on patients were noted.

#### **RESULTS**

Ages ranged from 3 to 87 years with a median of 42; males were the majority (80%) and the commonest presenting symptoms were flank pain (91%), dysuria (19%), and nausea or vomiting (15%).

All stones contained calcium and oxalate, often in combination with one or more constituents that included bicarbonate, ammonium, phosphorous, magnesium, uric acid and cysteine.

Majority (48%) of the stones were located in the ureters, 24% at the pelviccalceal-ureteric junction, and 15% at the vesico-ureteric junction. The bladder, urethra and kidney parenchyma were the other sites affected. In a few of the patients multiple sites were involved.

Lithotripsy was the most performed therapeutic procedure (30%) closely followed by cystoscopy (26%), and only a single patient underwent open nephrostomy. One patient had spontaneous passage.

#### **CONCLUSION**

Urolithiasis is no longer a rare presentation in this part of the world and all age groups are affected with males more at risk than females. Calcium oxalate stones dominate in our patients



Kidney diseases

W025

**VITAMIN D AND LIPID STATUS IN PATIENTS WITH END STAGE RENAL DISEASE**N. Milinković<sup>2</sup>, A. Đorđević<sup>1</sup>, I. Dragašević<sup>1</sup>, N. Milenković<sup>3</sup>, M. Dajak<sup>1</sup>, S. Ignjatović<sup>2</sup><sup>1</sup>Center for Medical Biochemistry, Clinical Centre of Serbia, Belgrade, Serbia<sup>2</sup>Center for Medical Biochemistry, Clinical Centre of Serbia, Belgrade, Serbia, Institute of Medical Biochemistry, School of Pharmacy, University of Belgrade, Serbia<sup>3</sup>Primary Health Care Institution "Dr. Simo Milošević", Čukarica, Belgrade, Serbia**BACKGROUND-AIM**

Some observational studies indicate an association of vitamin D deficiency and unhealthy cholesterol levels. The aim of this study was to investigate effect of 25-hydroxy vitamin D (25D) levels (< 50 nmol/L and > 50 nmol/L) on lipid status in patients with end stage renal disease (ESRD), separately for predialysis and dialysis patients.

**METHODS**

Vitamin D levels and the lipoprotein profile was determined in predialysis patients (N = 40), chronic hemodialysis patients (HD) (N = 112), continuous ambulatory peritoneal dialysis patients (CAPD) (N = 120) and in control group (CG) (N = 50). The analysis included the measurement of 25D by HPLC, apolipoprotein (apo) A-I, apo B by nephelometry, and total cholesterol (TC), high density lipoprotein (HDL), cholesterol-rich low-density lipoprotein (LDL) and triglyceride (TG) by spectrophotometry.

**RESULTS**

We found that higher 25D levels (> 50 nmol/L) strongly correlate with HDL and apo A-I ( $r = 0.768$  and  $r = 0.642$  in HD and  $r = 0.798$  and  $r = 0.721$  in CAPD) ( $p < 0.05$ ). Also, we found significantly higher LDL/HDL and apo B/A-I ratio in dialysis patients than in predialysis patients (1.22 vs. 0.98 and 7.2 vs. 4.9) ( $p < 0.05$ ). CAPD patients had significantly higher concentrations of TC, LDL, TG and apo B than HD patients (6.7 vs. 5.2 mmol/L, 4.4 vs. 3.2 mmol/L, 2.4 vs. 1.7 mmol/L and 1.92 vs. 1.74 g/L,  $p < 0.05$ ).

**CONCLUSION**

Renal dyslipidemia is characterized to a greater extent by abnormal apolipoprotein rather than lipid profile. In addition, there is strong correlation between vitamin D repletion and healthier lipid profile in dialysis patients. Evaluation of lipid abnormalities, as well as vitamin D status are important in order to improve cardiovascular outcomes in ESRD patients.

Kidney diseases

W026

### **THE RATIONALE OF REFLECTANCE BORDER MODIFICATION FOR URISYS 2400 WBC ASSAY**

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#### **BACKGROUND-AIM**

Urine dipstick test is a valuable tool for screening and monitoring urinary tract and systemic diseases. Roche Diagnostics USA recommends that the reflectance cutoff for WBC be modified to overcome false positive problems in URISYS 2400 (Roche Diagnostics, Switzerland). We assessed the rationale of the adjustment recommended by the vendor.

#### **METHODS**

Fresh urine specimens taken for general health examination from 337 healthy persons were used. Each sample was analyzed by UF-1000i (Sysmex, Kobe, Japan), URiSCAN (YD Diagnostics, Korea) and 4 different strip lots for URISYS 2400. In case with any discrepancy in WBC between the methods, manual sediment analysis was conducted to dissolve the discrepancy. Reference values were determined by UF-1000i or manual microscopic analysis in case of discrepancy.

#### **RESULTS**

Of 337 specimens, 88.4% were negative for WBC by URiSCAN, while the proportion were much lower for URISYS 2400 (54.6% ~ 80.7%) in unmodified conditions. In one lot, about half of the asymptomatic subjects showed positive results. In comparison to the reference values, 0.6% of the specimens showed false positive reaction for URiSCAN. For URISYS 2400, false positive reactions were observed in 3.3% ~ 27.0%. The proportion of false negative was 8.6% for URiSCAN and 1.2% ~ 5.0% for URISYS 2400. After adjustment of reflectance borders, the proportion of negative results increased up to 79.5% ~ 88.4%. False positive reactions were observed in 0.3% ~ 5.9% and false negative occurred in 5.0 ~ 9.5% of specimens.

#### **CONCLUSION**

We can conclude that the reflectance border for WBC should be adjusted according to the vendor's recommendations.

Kidney diseases

W027

### **SIMPLE HPLC METHOD FOR ROUTINE SINGLE PLASMA IOPROMIDE DETERMINATION**

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#### **BACKGROUND-AIM**

Background: The single plasma sample method is widely used for later determining the glomerular filtration rate. The aim of the present study was to evaluate the potential simple HPLC method for routine determination of iopromide for later clearance determination. Iopromide was determined on plasma samples, withdrawn from patients, whose were administrated iopromide for radiological purpose.

#### **METHODS**

Method: Iopromide was determined in EDTA-plasma . 100µL was deprotenized with 400µl of perchloric acid. After centrifugation on 9000g, 50µL aliquouts was injected automaticly in isocratic HPLC system, consisted of: HPLC pump "Waters 1525", autosampler "Waters 2707", UV/VIS detector " Waters 2489". Determination has been done on 254 nm. All chromatographic data were evaluated by "EMPOWER-2, Waters". Isocratic HPLC separation has been done on " Lichrospher 60 RP-Merck", 125 X 4.6 mm, with mobile phase consisting of sodium dihydrogen phosphate solution, 60 mm, methanol 8%, tetrahydrofuran 2% v/v. Standard solutions ( 200 - 1000 mg/L), has been made from iopromide substance, delivered from "Sigma-Aldrich".

#### **RESULTS**

Results: Quantitative analysis has been performed on assesment of peak heights. The method was validated as linear in concentration range between 5.0 and 700 mg/L. within run precision for " low" sample concentration range, as 20 mg/L was 4.35%, and for "high" sample concentration as 500 mg/L was 3.9%. Between run precision, for same concentration were 5.65% and 5.0 %. Analytical and absolute recovery was 91% and 98%.

#### **CONCLUSION**

An accurate determination of iopromide concentration in plasma samples can be obtained, using this simple extraction and separation technique in biochemical laboratories which performed HPLC technique, for different analytical purposes. This modification can be easily performed as, fast reliable and cheap manner for iopromide determination and latter glomerular filtration ratio measurement.

Kidney diseases

W028

### **EVALUATION OF SERUM OSTEOPROTEGERIN AND FETUIN A LEVELS IN PATIENTS WITH CHRONIC KIDNEY DISEASE.**

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<sup>2</sup>Theodor Bilharz Research Institute

#### **BACKGROUND-AIM**

Chronic kidney disease (CKD) is one of the most problematic diseases worldwide with cardiovascular diseases (CVD) as the main cause of morbidity and mortality. Recent studies pointed out that accelerated vascular calcification (VC) is implicated as a main interplaying factor that orchestrate CVD in CKD. Physiological inhibitors of VC as fetuin A, an extraosseous calcification inhibitor and osteoprotegerin (OPG), a regulator of bone resorption play a major role in pathogenesis of VC. Our objective: was to evaluate the role of serum levels of OPG and fetuin A in CKD patients in a trial to unravel the pathogenetic mechanisms that might underly VC with chronic renal impairment.

#### **METHODS**

A total of 80 subjects were selected from Theodor Bilharz Research Institute : 60 CKD patients subdivided according to estimated glomerular filtration rate (eGFR)(MDRD formula) into:GpI(n=30) moderate to severe CKD(stages 3&4): eGFR:15-59 ml/min, GpII(n=30)end-stage renal disease (ESRD)(stage5):eGFR<15ml/min. In addition 20 age-and sex-matched healthy subjects were studied as a reference control group. 12ml fasting venous blood was withdrawn from all subjects, centrifuged, serum was used for estimation of urea, creatinine,uric acid, calcium, phosphorus, sodium, potassium. Further estimation of serum OPG and fetuin using ELISA. In addition ECG and echocardiography were performed to evaluate cardiovascular calcification.

#### **RESULTS**

A significant reduction serum fetuin A in both patients' groups (I&II) as compared to reference( $p<0.05, <0.05$ ). A significant negative correlation between serum fetuin and echocardiographic calcification score in patients' groups( $r:-0.61, p:0.004$ ). There was a crescendo significant rise of serum OPG in both patients' groups (I&II)as compared to the reference ( $p<0.01, <0.01$ ), being higher in ESRD GpII as compared to GpI( $p<0.05$ ). A significant positive correlation was found between serum OPG and calcification score in both patients' groups: $r=0.593, p:0.006$ .

#### **CONCLUSION**

It is apparent that serum fetuin and OPG might interplay in the pathogenesis of vascular calcification in CKD patients. Hypofetuinemia may be due to increased consumption in the uremic calcific milieu might have a role in enhancing CVD morbidity and mortality. Meanwhile elevated OPG might usher to a state of resistance to its action. So serum fetuin and OPG might be used as recent, non-invasive biomarkers mirroring VC in CKD patients.

Kidney diseases

W029

**THE CORRELATION BETWEEN THE LEVEL OF CREATININE, CREATININE CLEARANCE, CYSTATIN C AND RECIPROCAL VALUES OF CYSTATIN C OBTAINED IN PREGNANT WOMEN**R. Obrenovic<sup>1</sup>, B. Stojimirovic<sup>2</sup>, I. Vujosevic<sup>1</sup>, T. Tadic<sup>1</sup>, S. Stankovic<sup>1</sup><sup>1</sup>Center of Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia<sup>2</sup>Clinic of Nephrology, Clinical Center of Serbia, Belgrade, Serbia**BACKGROUND-AIM**

Normal pregnancy is associated with a number of metabolic and physiological changes. The increase of glomerular filtration rate (GFR) starts as early as the fourth gestation week. It is necessary to monitor renal function during pregnancy in order to avoid renal damage and preeclampsia. It has been recognized that serum cystatin C might well reflect glomerular filtration rate in various conditions, including pregnancy. The aim of this study was to determine the correlation between cystatin C, creatinin and creatinine clearance in pregnant women regardless of gestational age and try to answers whether cystatin C can use as a marker of GFR in pregnancy. A total of 109 pregnant women were included: group I-38 women (average age 29.63±4.3 years) in the first trimester, group II-32 women (average age 33.56±5.95 years) in the second trimester and group III-39 pregnant women (average age 30.1±6.95 years) in the third trimester.

**METHODS**

Cystatin C serum concentration was determined by the PENIA method using the SIEMENS (Marburg, Germany) tests, on BN II. Creatinine was determined with commercial kits (Hamburg, Germany) on Olympus 640 analyzer. Results were statistically analyzed using the ANOVA.

**RESULTS**

In group I serum creatinine inversely correlated with creatinine clearance ( $p < 0.026$ ) and directly correlated with cystatin C ( $p < 0.014$ ). Creatinine clearance inversely correlated with cystatin C ( $p < 0.0001$ ). Creatinine clearance directly correlated with the reciprocal value of cystatin C ( $p < 0.0001$ ). In a group II no correlation between serum creatinine and creatinine clearance ( $p = ns$ ). There are direct correlation between serum creatinine and cystatin C ( $p < 0.004$ ). No correlation between creatinine clearance and cystatin C ( $p = ns$ ) and no correlation between creatinine clearance and reciprocal value of cystatin C ( $p = ns$ ). In group III serum creatinine inversely correlated with creatinine clearance ( $p < 0.0001$ ) and direct correlated with cystatin C ( $p < 0.003$ ). No correlation between creatinine clearance and cystatin C and reciprocal value of cystatin C.

**CONCLUSION**

Serum cystatin C reflect GFR only in the first trimester pregnancy. Cystatin C is not a reliable marker of GFR in the second and the third trimester.

Kidney diseases

W030

**PERFORMANCE OF THE NEPHROCHECK® FOR VITROS® TEST\*\* ON THE VITROS® 3600 IMMUNODIAGNOSTIC SYSTEM**G. Ogbonna<sup>1</sup>, A.M. Sweeney<sup>1</sup>, S. Jackson<sup>1</sup>, M. Leeman<sup>1</sup>, T. Mangan<sup>1</sup>, J. Parsells<sup>1</sup><sup>1</sup>Ortho Clinical Diagnostics, Rochester, NY**BACKGROUND-AIM**

The NephroCheck for VITROS Test\*\* (VITROS) quantitatively measures Tissue Inhibitor of Metalloproteinase 2 (TIMP-2) and Insulin-like Growth Factor Binding Protein 7 (IGFBP-7) to generate an acute kidney injury (AKI) risk index (AKIRISK™ Score).

**METHODS**

We have evaluated the test performance on the VITROS® 3600 Immunodiagnostic Systems.

**RESULTS**

The test is linear across the range of 1.58 to 30.9 ng/mL for TIMP-2 and 20.6 to 647 ng/mL for IGFBP-7 yielding an AKIRISK™ Score range of 0.0325 to 20.0. Limits of Blank (LoB) were determined to be 0.52 ng/mL and 0.110 ng/mL for TIMP-2 and IGFBP-7, respectively. Limits of Detection (LoD) were determined to be 0.243 ng/mL for TIMP-2 and 1.994 ng/mL for IGFBP-7 resulting in LoB and LoD for the AKIRISK™ Score of  $2.8 \times 10^{-6}$  and 0.003 respectively. A 5-day precision study with samples at mean TIMP-2 concentrations of 1.26 ng/mL, 2.63 ng/mL, 9.67 ng/mL, and 10.6 ng/mL resulted in within-laboratory percent coefficient of variation (%CV) of 10.7%, 6.4%, 3.4%, and 3.7% respectively. Similar results were obtained for IGFBP-7 at concentrations of 35.1 ng/mL, 65.7 ng/mL, 138 ng/mL, and 202 ng/mL, resulting in within-laboratory %CV of 5.8%, 6.6%, 7.5%, and 8.0% respectively. The precision of the AKIRISK™ Score based on the two results were 11.5%, 7.9%, 9.0%, and 9.8% at AKIRISK™ Score of 0.04, 0.17, 1.34, and 2.14. The accuracy of the test was evaluated with 50 patient specimens against the Astute Medical NephroCheck® Test System (Astute) The following linear regression statistics were obtained: VITROS TIMP-2 =  $1.153 \times \text{Astute} - 1.24$ ; (r) = 0.960; VITROS IGFBP-7 =  $1.069 \times \text{Astute} - 1.717$ ; (r) = 0.984. The positive (PPA) and negative (NPA) percent agreement between the two assays were calculated based on the AKIRISK™ Score cutoff of 0.3 established on the Astute Medical NephroCheck® Test System, with AKIRISK™ Score greater than 0.3 being positive and AKIRISK™ Score less than 0.3 being negative.

**CONCLUSION**

Compared to Astute, the VITROS AKIRISK™ Score had a 93.8% PPA and a 100% NPA. (\*\* under development)

Kidney diseases

W031

**ESTIMATION OF CARDIOVASCULAR RISK AND CHRONIC MYOCARDIAL DAMAGE IN KIDNEY DISEASE**A.V. Oláh<sup>1</sup>, T. Szalkó<sup>3</sup>, E. Szánthó<sup>1</sup>, J. Mátyus<sup>2</sup><sup>1</sup>Department of Laboratory Medicine, University of Debrecen<sup>2</sup>Department of Medicine, University of Debrecen<sup>3</sup>Faculty of Medicine, University of Debrecen**BACKGROUND-AIM**

Cardiovascular risk is increased in all stages of chronic kidney disease (CKD). We analysed the relation between cardiovascular risk (CV) estimated from the non-renal clinical parameters and kidney score based on GFR-proteinuria. Then we evaluated the diagnostic value of high sensitive troponin T (hsTnT) in CKD.

**METHODS**

Clinical data in Group-A (15 men, 5 women, age: 65±15 years) were followed for four years. Cardiovascular risk was estimated based on the Framingham study: age, BMI, blood pressure, lipids, patient history (diabetes, HbA1c, myocardial infarction, stroke). Kidney score was established from GFR-EPI and urinary protein/creatinine or albumin/creatinine, then patients were sorted into groups (1:mild, 2:moderate, 3:severe, 4:very severe CV-risk). In Group –B hsTnT was determined in 21 patients (19 men, 2 women, age:67±13 years). Laboratory tests were made on Roche Cobas-8000 system.

**RESULTS**

In group-A the mean of GFR decreased from 66 to 47 ml/min in four years. In most cases the decrease of GFR was followed by progressing proteinuria, and proteinuria correlated to clinical score (R: 0.775). The CV risk clinical score was proportional to kidney score: mean of clinical score was 21.8 at kidney score <3.5 and 29.2 at kidney score >3.5 (p:0.09). The atherogenic non-HDL showed stronger correlation during four years of lipid lowering therapy. Average of non-HDL decreased from 3.62 to 2.92 mmol/L (p:0.003) without proteinuria, and remained 3.5 mmol/L with proteinuria. In Group-B hsTnT increased with clinical scores (R=0.655). The mean of hsTnT was 14.8 ng/L at mild-moderate CV risk (kidney score 1-2), whereas hsTnT was 32.8 ng/L at severe and very severe CV risk (kidney score 3-4).

**CONCLUSION**

Correlation of Framingham CV risk and GFR-proteinuria shows the diagnostic value of kidney score. The increase of hsTn and non-HDL with impairment of CKD suggest their prognostic value in CV risk estimation.

Kidney diseases

W032

### **EFFECTS OF DIET AND GEMFIBROZIL ON POST-TRANSPLANT HYPERLIPIDEMIA IN KIDNEY TRANSPLANT PATIENTS**

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#### **BACKGROUND-AIM**

Post-transplant hyperlipidemia increases cardiovascular morbidity and mortality rate in kidney transplant patients. It also leads to graft loss due to atherosclerosis and glomerular damage. It is essential to control hyperlipidemia in kidney transplant patients to prevent these events.

#### **METHODS**

In our study, we determined lipid profiles in 59 kidney transplant patients. 20 of the patients had hyperlipidemia; 9 patients had type IV, and 11 patients had type II hyperlipoproteinemia. 14 patients were treated with American Phase 3 diet for one month and 6 of the patients received their regular diet as a control group.

#### **RESULTS**

Lipid profile was normalized in 9 patients on diet. The lipid profile of 5 patients on diet did not change. These 5 diet resistant patients were given gemfibrozil (600 mg twice a day) for two months. At the end of therapy period, their cholesterol and triglyceride levels decreased significantly. No change was observed in LDL-cholesterol and HDL-cholesterol levels.

#### **CONCLUSION**

We conclude that American phase 3 diet and/or gemfibrozil are effective in controlling post-transplant hyperlipidemia in kidney transplant patients.



Kidney diseases

W033

### **ISOLATED HEMATURIA - AN INDICATION FOR RENAL BIOPSY, BASED ON MORPHOLOGICAL EXAMINATION OF URINARY ERYTHROCYTES BY THREE METHODS**

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<sup>1</sup>University Hospital St. Marina Varna

#### **BACKGROUND-AIM**

Microscopic examination of the urinary sediment could itself suggest the origin of the hematuria. The aim of this research is to prove the indication for renal biopsy. A characteristic marker for glomerular bleeding is urinary acanthocyturia. We studied the urinary red blood cell (RBC) morphology in case of persistent microhematuria. Retrospective, it observed a girl of 5 years/2013 to present/ with episodes of transient macrohematuria during respiratory infections and persistent microhematuria; in absence of proteinuria, hypertension, edema, calciuria, family history, immunological and radiological changes.

#### **METHODS**

In the present review we observed day to day 10 fresh urine samples from 5 years old child with microhematuria. Minimum 100 RBCs were examined in each sample for dysmorphism by 3 used methods: 1) Light microscopy, 2) Phase contrast microscopy of the unstained urinary sediment and 3) FUS 100-Urine sediment Analyser in order to predict the site of hematuria. It is established that the phase contrast microscopy is the most sensitive method detecting dysmorphic RBCs in the urine. FUS-100 is characterized by utilizing flow digital imaging analysis technology. It leads to the possibility of the analyser to provide erythrocyte morphologic information.

#### **RESULTS**

All of the observed urine samples were with specific gravity (SG) varying from 1.010 to 1.020 and blood 3+, lacking of protein. More than 50% of red cells in each sample showed features of dysmorphism. We described the presence of variety of erythrocyte shapes: discocytes, echinocytes, schizocytes and many acanthocytes. As a result of the present review it is concluded, there is no a statistical significance between three methods.

#### **CONCLUSION**

Based on the sensitivity and specificity of the used methods, we concluded the presence of dysmorphic RBCs is due to glomerular origin in contrast to other presented pathologies. If the microhematuria persists between 6 to 12 months as in the introduced case, kidney biopsy should be considered.

Kidney diseases

W034

### **CREATININE AND CYSTATIN C IN PATIENTS WITH PLASMA CELL DYSCRASIAS**

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#### **BACKGROUND-AIM**

The plasma cell dyscrasias are often complicated by various forms of kidney diseases .

The damage depends on the type of plasma cell dyscrasias and monoclonal protein produced .

The immunoglobulin free light chains (FLC ) kappa (FLK) and lambda (FLL) are the mediator of nephropathy. With this work we tested the ability of the two markers to identify kidney injury in critical pharmacologically treated patients.

#### **METHODS**

Thirtyfour patients were evaluated: 15 females and 19 males of average age 67 years ( 47-81 ) , from O.U Haematology and Transplant Center of Piacenza with different plasma cell dyscrasias. The Cystatin C(Cys C) and FLC were determined by nephelometry on the instrument Immage 800 Beckman-Coulter, respectively, using the kit Dako Cys C and Freelite ( Binding Site Ltd. Birmingham , UK). Creatinine(creat) was determined by Jaffe method on Olympus 5800 (Beckman-Coulter).

#### **RESULTS**

Sixteen patients had normal values of serum creatinine (mean 0.78 mg/dL) and Cys C ( mean 1.14 mg/L) whit preserved renal function.

Five patients had serum creatinine (mean 1.3 mg/dL) and Cys C ( mean 1.57 mg/L) moderately increased with renal injury (RI) stage 2-3.

Five patients had serum creatinine ( mean 2.48 mg/dL) and Cys C (mean 3.55 mg/L) increased with RI stage 3-4.

Four patients had normal values of creatinine ( mean 1.0 mg/dL) and moderately increased values of Cys C ( 1. 57 mg/L) with preserved renal function.

A patient with AL amyloidosis lambda and RI stage 2 showed the following values: creat 0.82 mg/dL, Cys C 2.03 mg/L, FKc 6.47 mg/L, FLC 53.6 mg/L.

A patient with multiple myeloma(MM) IgG-K in lenalidomide therapy and preserved renal function showed the following values: creat 0.68 mg/dl, Cys c 1.53 mg/L, FKc 41.9 mg/L, FLC 2.25 mg/L.

Apatient with MM IgG-K in carfilzomib therapy and RI stage 3 showed the following values: creat 1.11 mg/dL, Cys C 2.01 mg/L, FKc 6.44 mg/L, FLC 11.5 mg/L.

A patient with MM IgG-L IRA rersolved showed the following values : creat 1.01 mg/dL, Cys C 2.56, FKc 3.15, FLC 225.

#### **CONCLUSION**

In conclusion the serum Cys C has a more sensitive marker than creatinine monitoring kidney injury in patients with plasma cell dycrasias stage 2-3.

Kidney diseases

W035

## EVALUATION OF LABORATORY VARIABLES ASSOCIATED WITH ANEMIA IN PATIENTS WITH KIDNEY DISEASE

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### BACKGROUND-AIM

In patients with chronic kidney disease, anaemia mainly develops from decreased renal synthesis of erythropoietin. The anaemia becomes more severe as the glomerular filtration rate (GFR) progressively decreases. In our study we like to identify the occurrence of anemia in patients with kidney disease (KD).

### METHODS

Our study included 88 female patients with KD, hospitalized at Department of Kidney disease at the University Clinics Center of Sarajevo. We investigated levels of iron, hemoglobin, ferritin and creatinin. The patients were divided in four groups according their level of creatinin I group creatinin level (88.5-221  $\mu\text{mol/L}$ ); II group creatinin level (221-505  $\mu\text{mol/L}$ ); III group creatinin level (506-981  $\mu\text{mol/L}$ ) and IV group creatinin level more then 981  $\mu\text{mol/L}$ . We measured ferritin using CMIA Architect I 2000 SR (ABBOTT), creatinin using Jaffa method and iron we measured using Dimension Xpand (Dade Behring). Statistical analysis was performed with Mann Whitney test, Student's t-test and using SPSS 20.0, assuming significance level of 5%.

### RESULTS

Hemoglobin showed a mean (standard deviation) value of 9.20 (1.8) g/dL, with the occurrence of anemia in 45.3% of cases. Anemia was associated with low iron concentration, high values of ferritin and creatinin. The I group have value of iron 14.1  $\mu\text{mol/L}$  (with iron treatment); 7.2  $\mu\text{mol/L}$  (without iron treatment); and ferritin 235.18  $\mu\text{g/L}$ ; II group have iron 10  $\mu\text{mol/L}$  (with iron treatment); 5.2  $\mu\text{mol/L}$  (without iron treatment) and ferritin 261.63  $\mu\text{g/L}$ ; III group have iron 11.34 (with iron treatment)  $\mu\text{mol/L}$ ; 4.2  $\mu\text{mol/L}$  (without iron treatment) and ferritin 382.21  $\mu\text{g/L}$  and IV group have iron 10.26  $\mu\text{mol/L}$  (with iron treatment) and ferritin 484.75  $\mu\text{g/L}$ . Inflammation is most common confounder in KD associated hyperferritinemia. The odds ratio for anemia with the use of intravenous iron hydroxide was 0.36 (95% CI: 0.25 to 0.89), i.e., a 2.78-fold higher chance of developing anemia without the use of this medication.

### CONCLUSION

The impaired renal function is directly related to a decline in the value of iron, the development of anemia and an increase value of serum ferritin and creatinine. The anemia predominated in patients with kidney disease; intravenous iron hydroxide use was a protective factor.

Kidney diseases

W036

### **THIOREDOXIN REDUCTASE ACTIVITY, SERUM IL6 , AND NT-PROBNP LEVELS IN DIALYSIS PATIENTS**

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<sup>1</sup>*inonu university turgut özal medical center, malatya*

#### **BACKGROUND-AIM**

Cytokines have a direct stimulating effect on natriuretic peptide secretion. N-terminal probrain type natriuretic peptide (NT-proBNP) is a valuable biomarker for mortality in dialysis patients. The aim of this study was to compare thioredoxin reductase, NT-proBNP, IL6 levels and High Sensitive C reactive protein (HsCRP) in peritoneal dialysis (PD), and hemodialysis (HD) patients and investigate relationship among these parameters.

#### **METHODS**

In age and sex matched 30 HD patients, in 30 PD patients and 20 healthy controls, HsCRP, thioredoxin reductase, serum IL6, and NT-proBNP levels were measured.

#### **RESULTS**

HsCRP, IL6 and NT-proBNP levels in HD and PD patients were significantly higher than in controls ( $p < 0.05$ ). There was no difference according to HsCRP, IL6, and NT-proBNP between HD and PD patients ( $p > 0.05$ ). Thioredoxin reductase was not different in 3 groups ( $p > 0.05$ ). Thioredoxin reductase was not correlated with any parameters studied. HsCRP were positively correlated with BMI ( $r: 0.275, p: 0.034$ ), IL6 ( $r: 0.633, p: 0.000$ ), and NT-proBNP ( $r: 0.277, p: 0.032$ ), and negatively correlated with serum albumin ( $r: -0.425, p: 0.001$ ). Serum IL6 levels were positively correlated with HsCRP ( $r: 0.633, p: 0.000$ ), BMI ( $r: 0.775, p: 0.034$ ) and negatively correlated with albumine ( $r: -0.342, p: 0.007$ ). Serum NT-proBNP levels were negatively correlated with serum albumin ( $r: -0.385, p: 0.002$ ), and positively correlated with age ( $r: 0.315, p: 0.002$ ), HsCRP ( $r: 0.277, p: 0.032$ ), systolic ( $r: 0.421, p: 0.001$ ) and mean blood pressure ( $r: 0.311, p: 0.015$ ). In multiple regression analyses the predictors of HsCRP were IL6 ( $\beta: 0.677, p: 0.000$ ), the predictor of serum IL6 was HsCRP ( $\beta: 0.677, p: 0.000$ ). The predictors of serum NT-proBNP levels were serum albumin ( $\beta: -0.416, p: 0.000$ ) and mean blood pressure ( $\beta: 0.414, p: 0.000$ ).

#### **CONCLUSION**

NT-proBNP levels in dialysis patients are not related with IL6 levels and thioredoxin reductase.

Kidney diseases

W037

**IRON PROFILE AND PARATHYROID HORMONE STATUS IN CHRONIC KIDNEY DISEASE PATIENTS ADMITTED TO TRIBHUVAN UNIVERSITY TEACHING HOSPITAL[TUTH].**

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**BACKGROUND-AIM**

Background: Chronic kidney disease [CKD] is emerging as a new threat in context to Nepal with rise in the incidence of hypertensive and diabetic patients. Nepalese migrant workers coming from gulf countries are also reported to have kidney diseases although its documentation is at a preliminary stage at present. Besides the disturbance to the excretory function, there is also hormonal disturbance in CKD. An increase in parathyroid hormone [iPTH] affecting calcium homeostasis in the second stage of CKD and decrease in erythropoietin leading to anemia in third stage of CKD have been stated. Anemia in CKD is said to be of anemia of chronic diseases type although iron deficiency anemia is also seen. Some studies have stated anemia as a complication of primary hyperparathyroidism. Estimation of PTH in CKD was only recently started in the TUTH hospital. Thus, the objective of this study was to find the status of PTH and iron profile in CKD patients visiting the hospital for check up, admission for dialysis or renal transplant.

**METHODS**

Method: The study was performed during the time period of 3 months [November 2014-January 2015]. Urea, creatinine, uric acid, calcium, phosphorus was analysed by autoanalyzer BT3000. Intact PTH [iPTH] and ferritin was analysed by chemiluminescent immunoassay. Iron was analyzed by ferrozine method.

**RESULTS**

Result: Total of 75 chronic kidney disease patients [males=54, females=21] were enrolled. The mean value and standard deviation were as follows: urea=18.3±6.4mmoles/L, creatinine = 464.3±229 micromoles/L, uric acid=369.1±105.9micromoles/L, calcium=1.8±0.2mmoles/L, phosphorus=1.7 ± 0.54mmoles/L, iPTH=226.7±197.2pg/ml, iron=60±30.8 µg /dl, TIBC=272±77.9 µg/dl and ferritin =412 ±330 µg /L.

**CONCLUSION**

Conclusion: CKD patients in this study showed hyperparathyroidism, low normal iron level with hyperferritinemia (a sign of inflammatory response). Iron deficiency anemia was not significant. Hence, the CKD patients admitted in TUTH needs to be monitored from endocrine perspective along with the monitoring of creatinine level for dialysis.

Kidney diseases

W038

### **CYSTATIN C, MEASURED GLOMERULAR FILTRATION RATE AND IMAGING MARKERS OF VASCULAR DAMAGE IN ESSENTIAL HYPERTENSION**

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#### **BACKGROUND-AIM**

Background: Cystatin C, one of the most sensitive glomerular filtration rate (GFR) biomarkers, may have an important role in evaluating the presence of subclinical target organ damage (TOD) in patients with essential arterial hypertension (HT). We analyzed the correlation between cystatin C and markers of vascular damage (intima-media thickness of common carotid arteries (CIMT) and renal artery resistance index (RRI)) in patients with asymptomatic HT.

#### **METHODS**

Methods: A total of thirty-seven patients with HTA (age 64 (57 – 70) years, 17 male and 20 female) and measured glomerular filtration rate (mGFR) > 60 ml/min/1.73 m<sup>2</sup> and 30 controls (age and sex matched clinically healthy volunteers) were enrolled into the study. Patients were evaluated in relation to the presence of subclinical vascular TOD (defined as CIMT > 0.9 mm). Cystatin C serum concentration was measured by the immunoturbidimetric method, GRF by radioisotopic method (two blood samples after 180 and 240 min, 99m Tc diethylene triamine penta-acetic acid – mGFR), CIMT and RRI by doppler ultrasonography of the carotid arteries and kidneys.

#### **RESULTS**

Results: Median serum concentration of cystatin C was significantly higher in group I - patients with subclinical vascular TOD (n=17) compared to group II - patients without subclinical vascular TOD (n= 20) (1.13 (1.1 – 1.20) mg/l vs. 0.90 (0.88 – 1.07) mg/l, P < 0.01). Considering the median value of RRI there was no significant differences between groups (0.67 (0.65 – 0.71) vs. 0.63 (0.61 – 0.67), P>0.05). We observed significant correlation between cystatin C and mGFR (r = 0.44, P < 0.01) and CIMT (r = 0.43, P < 0.05). No significant correlation was found between cystatin C and RRI. In multivariate regression analyses, serum cystatin C (p < 0.001) was independently of mGFR associated with CIMT.

#### **CONCLUSION**

Conclusion: Cystatin C is associated to CIMT, a imaging marker of subclinical vascular damage in asymptomatic patients with hypertension and mGFR higher than 60 ml/min/1.73 m<sup>2</sup>.

Kidney diseases

W039

**ACCOUNTING FOR INDIVIDUAL BODY SURFACE AREA DOES IMPROVE ESTIMATION OF ABSOLUTE GFR USING THE CKD-EPI EQUATION**J. Chew-harris<sup>4</sup>, P. Chin<sup>2</sup>, C. Florkowski<sup>1</sup>, P. George<sup>1</sup>, Z. Endre<sup>3</sup><sup>1</sup>Clinical Biochemistry Unit, Canterbury Health Laboratories, Christchurch<sup>2</sup>Clinical Pharmacology, Christchurch Hospital, Christchurch<sup>3</sup>Department of Nephrology, Princes of Wales Hospital, Australia<sup>4</sup>Department of Pathology, University of Otago, and Clinical Biochemistry Unit, Canterbury Health Laboratories, Christchurch**BACKGROUND-AIM**

Laboratories report an estimated glomerular filtration rate (eGFR) with every plasma creatinine request which are based on corrections to a body surface area (BSA) of 1.73 m<sup>2</sup>. A local survey revealed approximately 60% may not be fully aware of eGFR units. We therefore compared estimated glomerular filtration rate (eGFR) according to the CKD-EPI equation, with (CKD-EPI, mL/min/1.73m<sup>2</sup>) and without body surface area (BSA)-normalization (CKD-EPI\_BSA, mL/min), against measured Tc-DTPA GFR (mL/min)

**METHODS**

The CKD-EPI and CKD-EPI\_BSA equations were compared in 222 individuals with Tc-DTPA GFR for bias, proportion within 30% of GFR (P30) and area under the receiver-operator curve (ROC) for detecting GFR <90 mL/min. In 80 oncology patients and 78 obese subjects, we also evaluated concordance in relation to carboplatin dosing.

**RESULTS**

Chi-square analysis indicated differences in P30s were larger between CKD-EPI\_BSA and CKD-EPI with increasing BMI; in those with BMI ≥30 kg/m<sup>2</sup> (32%), in those with BMI >25.0-29.9 kg/m<sup>2</sup> (18%) and in those with BMI ≥18.5 – 25.0 kg/m<sup>2</sup> (2%) (P<0.0001). The ROC area under curve (AUC) for CKD-EPI\_BSA equation to detect GFR < 90 mL/min (0.85) and > 125 mL/min (0.81) was greater than for the CKD-EPI (0.80 and 0.71, respectively). Concordance for carboplatin dosing using the CKD-EPI\_BSA equation was 71% and 56% by the CKD-EPI equation (P =0.07) for the cancer patients. For the obese, concordance for carboplatin dosing was 65% using the CKD-EPI\_BSA and 26% by the CKD-EPI (P<0.0001).

**CONCLUSION**

The magnitude of differences between the performances of the equations with and without BSA normalisation in predicting absolute GFR were more evident in the overweight and obese, than the normal-BMI individuals. Estimation of absolute Tc-DTPA GFR using the CKD-EPI equation was improved by removal of BSA normalisation, reflected by higher proportion of results within 30% of GFR and less underestimations of GFR. There are implications of using eGFR without removal of BSA in clinical settings such as in drug dosing.

Kidney diseases

W040

**ESTABLISHMENT OF THE ERM-DA471/IFCC TRACEABLE SERUM CYSTATIN C VALUES USING THE INTERNATIONAL STANDARD REFERENCE MATERIAL**A. Chittamma<sup>2</sup>, P. Meemaew<sup>2</sup>, C. Panthong<sup>2</sup>, K. Katesarin<sup>2</sup>, S. Vanavanant<sup>2</sup>, C. Kitiyakara<sup>1</sup><sup>1</sup>Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand<sup>2</sup>Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand**BACKGROUND-AIM**

It has been previously proposed that a drift in the Siemens cystatin C (CysC) nephelometric assay has occurred over the past decade. With the availability of ERM-DA471/IFCC standard reference material (ERM), clinical laboratories can establish traceable CysC values for optimal results. This study aims to establish ERM traceable CysC values and evaluate the impact of recalibration with traceable CysC on CKD classification in an epidemiology study.

**METHODS**

ERM was reconstituted as instruction. The working standards were prepared by diluting the neat reconstituted reference material into 5 concentrations. Materials were analyzed in duplicate on 2 separate days using the Siemens BN ProSpec analyzer. Correlation of CysC results between observed and target values was calculated by Deming regression. The correction equation was applied to results from samples (n=5,489), obtained from a cross-sectional community-based study in Southeast Asia in which CysC had been analyzed by the manufacturer procedure in 2008. Glomerular filtration rate (eGFR) was estimated by CysC based CKD-EPI equation for non standardized and standardized CysC assays. CKD stages correspond to categories of eGFR values was defined according to K/DOQI.

**RESULTS**

The Deming regression showed a slope of 0.932 (95% confidence interval [CI], 0.911 to 0.953) and intercept of -0.081 mg/L (95% CI, -0.149 to -0.012) with a high R<sup>2</sup> of 0.998, p<0.0001. The average of %recoveries for all of CysC levels were improved from 91.0% to 98.6%. After applying the correction equation to the previous epidemiological results, original CysC values (0.864±0.301 mg/L) were significantly lower than the ERM traceable CysC (1.014±0.300 mg/L), p<0.05. The average bias was -15.3%. The eGFR values from non traceable CysC classified subjects into CKD stage 1, 2, 3, 4 and 5 for 60.0%, 33.6%, 6.1%, 0.2% and 0.1% while the other were 38.0%, 49.7%, 11.6%, 0.6% and 0.1%, respectively. A total of 29.0% of patients was reclassified which demonstrated moderate agreement between both methods (kappa=0.522).

**CONCLUSION**

The accuracy of CysC determination can be easily improved by using of ERM-DA471/IFCC reference material for establishing the traceable CysC values that could provide more reliable eGFR which is an important to investigate CKD in population.



Kidney diseases

W041

### **ASSESSMENT OF D-DIMER AT EARLY STAGES (1-3) OF CHRONIC KIDNEY DISEASE USING A BIOCHIP BASED IMMUNOASSAY**

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#### **BACKGROUND-AIM**

Chronic kidney disease (CKD) is frequently unrecognised and presents with progressive decline of renal function leading to end-stage renal failure and death. The Modification of Diet in Renal Disease (MDRD) classification of renal disease describes the progressive stages of the disease (stages 1-5) with respect to the estimated glomerular filtration rate (eGFR). The complexity in diagnosing a patient with CKD at an early stage has led to most patients not receiving a diagnosis until the disease has progressed to an advanced stage. D-dimer is a product of fibrinolysis and is formed when cross-linked fibrin is degraded by plasmin. It is traditionally employed to diagnose deep vein thrombosis. However, additional applications have been reported. This study investigated the potential of D-dimer as an earlier biomarker of CKD by assessment at stages 1 to 3 on a biochip platform.

#### **METHODS**

D-dimer was assessed in serum samples: 327 CKD patients (137 Stage 1, 109 Stage 2 and 81 Stage 3) and 140 healthy controls. The analysis was performed with a biochip based immunoassay applied to the Evidence Investigator analyser. Statistical analysis was performed using MedCalc v12.5, all data represented as median [95% CI].

#### **RESULTS**

Significant differences in concentration of D-dimer across the disease groups and controls were initially found (Kruskal-Wallis test, significance determined as  $p < 0.0001$ ).

Post-hoc analysis was performed comparing CKD groups with controls using Mann-Whitney (with Bonferroni correction) and D-dimer displayed significantly higher median concentrations at all CKD stages (Stage 1-3) compared to control [(60.9 [49.1-68.9], 56.4 [50.1-71.6], 90.5 [65.1-117.0]) ng/ml respectively ( $p < 0.0006$  for all) compared to control (29.1 [24.4-34.7], ng/ml). Receiver operating characteristic (ROC) curve analysis was conducted to assess diagnostic performance of diseased versus healthy subjects; area under the curve was determined as 0.783 (95% CI: 0.743-0.820).

#### **CONCLUSION**

Application of this biochip based immunoassay to the assessment of D-dimer in serum at early stages (1-3) of CKD showed increased median concentration in CKD patients compared to controls. This indicates the potential utility of D-dimer in diagnosing early stage CKD.

Kidney diseases

W042

### **THE ROLE OF TACROLIMUS IN ERYTHROCYTES' ANTIOXIDATIVE CAPACITY IN LONG - TERM PERIOD AFTER RENAL TRANSPLANTATION**

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#### **BACKGROUND-AIM**

Transplanted kidneys are prone to oxidative stress-mediated injury by pre-transplant and post-transplant conditions that cause reperfusion injury or imbalance between oxidants and antioxidants. Antioxidative erythrocytes' enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR), as well as glutathione (GSH) protect against the harmful effects of free radicals. Tacrolimus (TAC) is a part of the most immunosuppressive regimens, binds extensively to erythrocytes, and in low concentration has antioxidant properties, however in high concentration it resulted in an increase of oxidative stress.

The aim of the study was to analyze the relation between antioxidative erythrocytes' enzymes and immunosuppressive therapies and renal function in kidney transplant patients.

#### **METHODS**

Our study included 72 renal transplant recipients and 62 healthy individuals. All of patients were on triple immunosuppressive regimen, which included tacrolimus, mycophenolate mofetil and prednisone. We measured SOD, GPX and GR activity in erythrocytes as well as concentration of GSH in whole blood.

#### **RESULTS**

Erythrocytes' SOD and GSH were increased, while GPX and GR were decreased in patients compared to controls. Also, SOD correlated positively with GR and negatively with GFR, while erythrocytes' GPX correlated positively with GR. Correlation analysis between tacrolimus daily dose (TDD), tacrolimus trough concentration (TTC), dose adjusted trough concentration (DATC) and erythrocytes' oxidative stress parameters show that TDD correlated positively with GSH as well as negatively with GFR. Also, DATC positively correlated with GFR and negatively with GPX and GSH in erythrocytes.

#### **CONCLUSION**

Tacrolimus may be involved in renal function deterioration in long – term period after transplantation as well independent from oxidative stress mediated reduction in renal function. Regarding our findings higher daily amounts of tacrolimus, which were required for optimal concentration of a drug, increased patients' erythrocytes antioxidative capacity. This might mean that tacrolimus acted as an erythrocytes' antioxidant.

Kidney diseases

W043

**URINARY PROTEIN PROFILE IN PATIENTS WITH CHRONIC KIDNEY DISEASE**M. Dajak<sup>2</sup>, N. Milinković<sup>1</sup>, I. Dragašević<sup>2</sup>, B. Stojimirović<sup>3</sup>, S. Ignjatović<sup>1</sup><sup>1</sup>Center of Medical Biochemistry, Clinical Center of Serbia and Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia<sup>2</sup>Center of Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia<sup>3</sup>Center of Urology and Nephrology, Clinical Center of Serbia and School of Medicine, Belgrade, Serbia**BACKGROUND-AIM**

The extent of protein excretion in the urine is widely recognized as a biomarker of the severity of chronic kidney disease (CKD) and as a predictor of renal function decline. Most guidelines currently recommend the measurement of proteinuria for the detection, diagnosis, prognosis and treatment of kidney disease. The aim of this study was to investigate a panel of low and high molecular weight proteins to determine the relationship to cause and stage of CKD in 108 patients with different stages CKD. We measured total and 6 specific proteins in second morning urine: albumin and immunoglobulin G (IgG), established biomarkers of glomerular permeability, and alpha-1-microglobulin (A1M), beta-2-microglobulin (B2M), cystatin C (CYSC) and beta-trace protein (BTP), established biomarkers of renal tubular function.

**METHODS**

Urinary total protein (turbidimetric method, Abbott) and creatinine (alkaline picrate method, Abbott) were measured on Architect ci8200. Specific proteins were measured by immunonephelometry (BNII, Siemens). All urine protein values were expressed as mg/mmol creatinine.

**RESULTS**

The majority (80%) of urine samples contained pathologic levels of total protein. Urinary levels of individual were also commonly increased (64–92% of samples depending on individual protein), and were significantly correlated (Spearman) with each other (coefficients were: 0.258-0.949;  $p < 0.05$  for all comparisons). The comparison analysis (Mann-Whitney U-test) showed that albumin and IgG were significantly higher, and A1M and BTP significantly lower in the glomerular disease group than in other disease groups. BTP and A1M significantly increased from early to late CKD; the median values for CKD stage 1-5 were: 0.48, 1.45, 6.57, 9.53 and 23.73 mg/mmol creatinine for A1M, and 0.12, 0.17, 0.63, 1.86 and 2.98 mg/mmol creatinine for BTP. Other proteins only showed significant increase in end stage of CKD.

**CONCLUSION**

The quantitative measurement of albumin, IgG, A1M and BTP in urine is useful to differentiate the origin of proteinuria and to determine the cause of CKD. Urinary A1M and BTP are better biomarkers of severity of CKD than other investigated proteins in patients with CKD.

Kidney diseases

W044

### **EASY RECOGNITION OF DECOY CELLS IN URINARY SEDIMENTS OF KIDNEY TRANSPLANTED PATIENTS USING AUTOMATED INTELLIGENT MICROSCOPY.**

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#### **BACKGROUND-AIM**

The examination of the urine sediment provides useful diagnostic information about renal diseases. BK virus (BKV) infections are currently considered one of the most important diseases in kidney transplants recipients, with a prevalence of decoy cells (viral containing shed urothelial cells) between 20% and 60%. The progression of BKV infection to BKV nephropathy (1%-8% of decoy-positive patients), lead to graft loss in up to 80% of affected individuals. Although the definitive diagnosis of BKV nephropathy requires renal biopsy, noninvasive methods to screen the presence of decoy cells in urine could improve the patient management.

#### **METHODS**

We use the IRIS iQ200® analyzer (Iris Diagnostics, Chatsworth CA) for the execution of urine sediment analysis; this system allows a quantitative reporting of the elements present in urine providing visual results that can be reviewed by the laboratory expert. 32 consecutive urine specimens of kidney transplant patients were examined by iQ200 and compared with manual microscopy results. Particular attention was paid to detection and quantitative count of cells with "decoy" appearance.

#### **RESULTS**

Using an automated intelligent microscopy, we well recognize pathological elements. Decoy cells were observed in the urine of 8 patients (25%) and confirmed by phase-contrast microscopy observation. In 3 of them, the images of IQ200 were decisive in recognizing these cells, especially in a context with very few cellular elements. Polymerase chain reaction (PCR) for BK virus (BKV) DNA in urine and plasma confirmed the viral infection in 2 patients.

#### **CONCLUSION**

The clinical utility of urine microscopy in the differential diagnosis and prediction of outcome in kidney transplanted patients may be increased by using an automated system. The iQ200 analyzer has provided accurate results also on very low concentrated urine samples. In our experience the images of decoy cells were clear and convincing. This method, noninvasive and not expensive, could facilitate early diagnosis of BK virus replication, follow-up study of the disease by quantitative count of decoy cells and support decision to modulate or change immunosuppressive therapy. Moreover, the possible long-term storage of images is a helpful tool for the patients' care.

Kidney diseases

W045

### **USEFULNESS OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (UNGAL) AT INTENSIVE CARE UNIT (ICU) ADMISSION.**

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#### **BACKGROUND-AIM**

The acute kidney injury (AKI) is defined as the sudden loss of renal function. AKI in intensive care units remains common and tightly associated with mortality.

Here we estimate the diagnostic accuracy of uNGAL at the admission to an adult general ICU for early detection of AKI, need for renal replacement therapy (RRT) and prediction of 30 days mortality.

#### **METHODS**

We conducted a prospective observational study of 415 consecutive adult patients admitted to a general ICU. The study was approved by the institutional review board and informed consent was obtained from the patient surrogate. AKI was defined by AKIN criteria. Patients were followed up 30 days until their discharge from hospital or death.

Clinical information: Gender, age, cause for admission, APACHE, SAPS index and need for RRT.

Laboratory tests: fungal detection at ICU admission, daily creatinine till 96 h as well as weekly and discharge creatinine.

NGAL was determined by using Standardized Clinical Platform ARCHITECT assay, provided by Abbott Diagnostics.

Statistical analysis: SPSS17 was used. Diagnostic characteristic of uNGAL were evaluated with receiver–operating characteristic curves (ROC) for AKI diagnosis, need of RRT and 30 days mortality. Yeuden test was used to find best sensitivity, specificity, predictive positive value (PPV) and predictive negative value (PNV).

#### **RESULTS**

99 patients (23,9%) developed AKI, 46 (11%) needed RRT and 71 (17,1%) died. AKI patients had higher ICU mortality (29%) than non AKI (7,9%; $p<0.001$ ) as well as longer UCI and hospital length of stay.

The ROC curve for uNGAL at admission and the occurrence of AKI was 0.845 (CI 0.80 to 0.89)  $p<0.001$ . We found for NGAL values  $>60\text{ng/ml}$  78% sensitivity, 78% specificity, 53% PPV and 92% PNV.

The ROC curve describing the relationship between uNGAL at admission and the need of RRT was 0.80 (CI 0.74 to 0.87)  $p<0.001$ . Yeuden test showed 71% sensitivity, 81% specificity, 32% PPV and 96% PNV for uNGAL values  $>156\text{ng/mL}$ .

The ROC curve for uNGAL at admission and 30 days mortality was 0.66 (CI 0.59 to 0.74)  $p<0.001$ . In this case, a 57% sensitivity, 73% specificity, 30% PPV and 89% PNV was obtained for uNGAL values  $>100\text{ng/mL}$ .

#### **CONCLUSION**

Urine NGAL at admission in ICU patients predicts AKI, as well as the need for RRT and 30 days survival.

Kidney diseases

W046

**HIGH CUT-OFF HAEMODIALYSIS AND SERUM FREE LIGHT CHAINS: OPTIMIZING THE TREATMENT OF PATIENTS WITH MULTIPLE MYELOMA AND ACUTE KIDNEY INJURY**J.L. García De Veas Silva<sup>1</sup>, C. Bermudo Guitarte<sup>2</sup>, J.C. Rojas Noboa<sup>2</sup>, P. Menéndez Valladares<sup>2</sup>, R. Duro Millán<sup>2</sup><sup>1</sup>Hospital Universitario Virgen de las Nieves - Granada (Spain)<sup>2</sup>Hospital Universitario Virgen Macarena - Sevilla (Spain)**BACKGROUND-AIM**

Acute kidney injury (AKI) is present in 15-30% of patients with Multiple Myeloma (MM) and the survival of these patients is highly dependent on the recovery of the renal function. Cast nephropathy secondary to MM is the most frequent cause of renal failure in these patients. The effective elimination of serum free light chains (sFLC) with the application of haemodialysis with high cut-off membranes (HCO-HD) alongside with chemotherapy is associated with an improvement in the renal function.

**METHODS**

A 57 years old man diagnosed of kappa light chain MM presented in the initial study an AKI with 12.4 mg/dl of creatinine, 229 mg/dl of urea and an altered sFLC ratio of 75.23 (free kappa=697.4 mg/l and free lambda=9.27 mg/l). The renal biopsy confirmed cast nephropathy. The patient underwent twelve sessions of HCO-HD (sessions of six hours in alternate days) with high cut-off membrane (Theralite, Gambro) to remove sFLC in addition to Bortezomib and Dexamethasone (B/D) treatment. sFLC were measured by turbidimetry using the assay Freelite (The Binding Site, UK). Blood samples were collected pre- and post-HD to determine creatinine and sFLC.

**RESULTS**

During therapy sFLC kappa levels decreased significantly. After twelve cycles of HCO-HD, kappa sFLC clearance was 94% from an initial level of 392.2 mg/l to a final level of 19.90 mg/l. This treatment produced an improvement in the patient's renal function with a decrease of 83% in the creatinine serum levels (from 9.70 mg/dl to 1.65 mg/dl). After HCO-HD, the patient continued on conventional haemodialysis and finished the treatment with B/D achieving a stringent complete response (immunofixation negative, 13.70 mg/l of free kappa, 9.58 mg/l of free lambda, ratio of 1.43, Bence Jones protein negative and absence of plasma cells in bone marrow). The patient became dialysis independent and underwent autologous stem cell transplantation (ASCT).

**CONCLUSION**

A combination of the efficient and direct removal of the nephrotoxic excess of sFLC using HCO-HD with effective chemotherapy with B/D allowed an efficient reduction of the sFLC levels. sFLC determination by Freelite allowed an accurate and rapid evaluation of the rate of sFLCs decrease, proving useful to monitor the efficiency of the therapy adopted.

Kidney diseases

W047

**HOW TO INTERPRET LOW PROTEINURIA (100-350 MG/L) DETERMINED WITH A BENZETHONIUM CHLORIDE METHOD?**N. Gillain-martin<sup>1</sup>, C. Nève<sup>1</sup>, E. Firre<sup>2</sup>, L. Radermacher<sup>3</sup><sup>1</sup>Clinical Laboratory, CHR Citadelle, Liège<sup>2</sup>Infectious diseases, CHR Citadelle, Liège<sup>3</sup>Nephrology, CHR Citadelle, Liège**BACKGROUND-AIM**

Proteinuria detection is essential for management of chronic kidney disease. The KDIGO guidelines of 2012 are based on six glomerular filtration rate and three albuminuria/proteinuria categories. They use the ratio to creatinine concentration and mg/g expression: I: <150, normal or mildly increased; II: 150-500, moderately increased and III: >500, strongly increased, or mg/mmol : <15, 15-50 and >50. Low proteinuria is difficult to interpret but SDS agarose electrophoresis defines proteins profile : albumin, alone or with transferrin, a selective proteinuria with good prognosis, or low and/or high molecular weight (MW) proteins with poorer prognosis. Our purpose is to define protein profile in KDIGO categories when proteinuria is low and to determine similar categories for proteinuria in mg/L when ratio to creatinine is not available.

**METHODS**

762 samples: proteins quantified with benzethonium chloride (Roche) (100 to 350 mg/L), analyzed with Hydragel 5 Proteinurie (Sebia) and their ratio to creatinine; urines from adult patients with renal risk due to diabetes, drug treatment, lithiasis or monoclonal gammopathy.

**RESULTS**

284 samples belong to I (37.27%), 379 to II (49.73%) and 99 to III (12.99%). Albumine alone is detected in 63% of I. This percentage decreases significantly ( $p<0.05$ ) in II in favor of the other profiles. 18% of I show low MW proteins. In III, 4% present isolated albumine and 28%, low and high MW proteins suggesting glomerulotubular damage (significantly higher than in II). Similar patterns are observed for proteinuria in mg/L. We suggest the following ranges for I' : 100-150 (n :330, 43.3%), for II' :150-250 (n :300, 39.37%) and for III' >250 mg/L (n :132, 17.32%). This classification gives comparable (no significant difference) informations on proteins profile and prognosis : good prognosis for 71.12%, 42.11%, 28.28% in I, II and III and 71.51%, 45.33% and 21.96% in I', II' and III'.

**CONCLUSION**

In a population with renal risk, a mildly increased proteinuria may reveal lesions and requires additionnal testing. We propose the ranges 100-150, 150-250 and >250 mg/L for the three KDIGO categories when creatinine is not available. These values are related to the use of benzethonium chloride, so to a single method.

Kidney diseases

W048

### **CREATININE ASSAYS – GLOBAL PROGRESS ON IMPLEMENTING IDMS TRACEABILITY**

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#### **BACKGROUND-AIM**

In order for laboratories to achieve the best evidence-based laboratory practice in the field of nephrology, major international guidelines recommend the use of serum creatinine assays which provide results traceable to the international reference method, Isotope Dilution Mass Spectrometry (IDMS). To meet this need, manufacturers must produce IDMS-aligned assays, and also describe this traceability in the Information for Use (IFU) to allow laboratories to be sure they have selected the correct assay. We assessed the information available in manufacturers IFUs for serum creatinine on these two issues.

#### **METHODS**

IFUs and other supporting information for serum creatinine assay were obtained via internet searches, direct contact to distributors and manufacturers and from local laboratories. Only English language sources were included. The information was assessed for the following criteria: Category 1 (C1): Clear statement that assay results are traceable to IDMS, C2: Calibrator supplied with traceability information supplied (IDMS not specifically mentioned), C3: Calibrator supplied with creatinine concentration provided but no traceability information, C4: No calibrator supplied with kit, C5: Unable to determine traceability from supplied information (eg single use device without calibrator). Enzymatic and Jaffe creatinine assays have not been analysed separately.

#### **RESULTS**

Information was obtained on 84 creatinine assays from 53 manufacturers from 15 countries covering 5 continents. The assays were classified as: C1: n=12, C2: n=16, C3: n=19, C4: n=10, C5: n=27. We note that an assay in categories 2, 3 and 5 may produce IDMS traceable results when used as intended by the manufacturer, however further information is required by the laboratory for confirmation. With category 4 assays the results are dependent on the selection of a calibrator appropriate for the assay and verification by the laboratory.

#### **CONCLUSION**

IDMS traceable creatinine assays are currently available from many manufacturers. However this small sample indicates two issues. Firstly there are many creatinine assays available that may not be IDMS traceable, and secondly that for assays which may be IDMS traceable, the information supplied does not make this clear to the user. While it is likely that enzymatic assay accuracy may be less affected by non-creatinine chromogens in patient samples or in the calibrator, there remains a need to confirm the traceability and provide this information for end-users.



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**EFFECT OF RENAL TRANSPLANTATION ON SERUM HEPATOCYTE GROWTH FACTOR LEVELS IN HEMODIALYSIS PATIENTS WITH HEPATITIS C VIRUS INFECTION.**

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**BACKGROUND-AIM**

Hepatocyte growth factor (HGF) has mitogenic, motogenic, morphogenic, and anti-apoptotic activities on renal cells. It is one of the cytokines that plays an imperative role in tubular repair and regeneration following renal injury. The purpose of this study was to evaluate the effect of renal transplantation on serum hepatocyte growth factor levels in hemodialysis patients with hepatitis C virus (HCV) infection.

**METHODS**

Seventy four subjects with HCV infection were enrolled in the study, they had normal liver function tests and patients with active hepatic disease were excluded from the study. The 74 subjects were divided into three groups: Control group (n=10), hemodialysis group (HD) (n= 30) and renal transplant recipient group (n=34). Serum HGF determination was performed using quantitative sandwich enzyme immunoassay.

**RESULTS**

Our study revealed that serum levels of HGF in HD patients showed a significant increase as compared to control ( $p < 0.001$ ) and renal transplant groups ( $p < 0.001$ ). In HD patients, serum HGF levels showed a positive correlation with both serum creatinine levels ( $r = 0.874$ ,  $p < 0.001$ ) and urea levels ( $r = 0.559$ ,  $p < 0.001$ ) but did not correlate with ALT levels or duration of HD. Serum HGF values in renal transplant recipients showed no statistically significant changes as compared to controls and did not correlate with either creatinine, urea, ALT and cyclosporine blood levels.

**CONCLUSION**

Our results suggested that elevated serum HGF in HD patients might be attributed to its increased production in response to the chronic renal injury, the effect of heparin, or its reduced removal in CRF patients. Elevated serum HGF values in HD patients return back to normal in renal transplant recipients with good allograft function.

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W050

### **C1Q BINDING HLA ANTIBODIES AFTER KIDNEY TRANSPLANTATION**

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#### **BACKGROUND-AIM**

Several studies have shown that human leukocyte antigen (HLA) donor-specific antibodies (DSA) are associated to lower kidney graft survival and increased risk of rejection. It is also generally agreed that complement-fixing HLA antibodies are a contraindication to solid organ transplant. Their ability to activate complement depends on multiple factors including titer, avidity, isotype and antigen density. Recently, the development of assays to detect complement-fixing antibodies (C1q) on Luminex® platform has provided new insights into the clinical significance of DSAs.

#### **METHODS**

Post-transplant sera of 20 patients were screened for the presence of circulating anti-HLA I and II DSA, using Luminex® Single Antigen test (LSA I and II; One Lambda, CA). Sera DSA were analyzed for the presence of C1q-binding HLA class I and class II antibodies (C1qScreen; One Lambda, CA).

#### **RESULTS**

We analyzed median fluorescence intensity (MFI) values of IgG HLA antibodies (class I and class II) identified by LSA test and their C1q-binding ability by C1q test. Among 20 patients LSA results revealed 40 DSA; all specificities with MFI value  $\geq 8600$  (23%) showed C1q test positivity. The remaining 77% of DSA specificities with MFI value  $\leq 8600$  was divided into two different groups: 23 DSA (74%) were negative to C1q test, while the remaining 26% were positive. Very lower DSA levels (MFI < 500) were C1q positive in one serum. We have observed majority C1q binding HLA class II antibodies (especially DQB1) respect to C1q binding HLA class I antibodies.

#### **CONCLUSION**

Despite the small cohort of patients, data provided new insights regarding the characteristics of clinically relevant DSA. C1q test could be important to define forbidden specificities (C1q-fixing) in kidney transplantation. We observed prevalence of C1q positive HLA class II antibodies, especially DQB1, as expected, since their documented role in shorter graft survival. Literature indicates 10000 MFI as positive reference value; our preliminary data suggest that MFI values, as 8600 and lower, might be associated to C1q positivity, so their monitoring could be useful to identify patients at risk of developing antibody mediated rejection (AMR). Further study is required to better define the prognostic utility of C1q test.

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**EVALUATION OF LEVELS OF PARATHYROID HORMONE AND BIOCHEMICAL MARKERS OF BONE METABOLISM IN EARLY STAGES OF CHRONIC KIDNEY DISEASE**R. Mijovic<sup>1</sup>, B. Ilincic<sup>1</sup>, V. Cabarkapa<sup>1</sup>, N. Curic<sup>1</sup>, S. Nikolic<sup>1</sup>, R. Zeravica<sup>2</sup><sup>1</sup>Center for Laboratory medicine, Clinical Center of Vojvodina, Faculty of Medicine - University of Novi Sad, Serbia<sup>2</sup>Center for Laboratory medicine, Clinical Center of Vojvodina, Novi Sad, Serbia**BACKGROUND-AIM**

Chronic kidney disease (CKD) is accompanied by disturbances of calcium (Ca) and phosphorus (P) metabolism, increased risk for development of secondary hyperparathyroidism and disturbed bone metabolism. The aim of the study was to evaluate levels of parathyroid hormone and biochemical markers of bone metabolism in early stages of chronic kidney disease.

**METHODS**

This pilot study included 40 patients stratified in two groups. I group-20 patients in stage 2 of CKD (CKD2) and II group-20 patients in stage 3 of CKD (CKD3). 15 healthy age and gender matched subjects were included in control group. Glomerular filtration rate (GFR) was determined by isotope clearance method with Diethylenetriamin-Penta-Acetic acid (DTPA) labeled with technetium -99mTc. Markers of renal function (Cystatin C, urea, creatinine) as well as levels of intact parathyroid hormone (iPTH), Ca, P, osteocalcin and CrossLaps were estimated in all study participants. iPTH, osteocalcin and CrossLaps were determined by electrochemiluminescent method on Elecsys 2010, Cobas, Roche.

**RESULTS**

iPTH level was in reference range for the laboratory method in all study groups but there were significantly higher values of iPTH in CKD3 group of patients regarding the controls (50,69±15,63 vs. 38,62±12,37; p<0,05) and CKD2 group of patients (50,69±15,63 vs. 37,75±11,87; p<0,01). Negative significant correlation between iPTH and 99mTc-DTPA values (r=-0.44;p<0,05) was observed in CKD3 group. In both study groups, no significant correlation between levels of iPTH and Ca and P (p>0,05;all) as well as between levels of osteocalcin and CrossLaps and 99mTc-DTPA (p>0,05;all) were found.

**CONCLUSION**

Negative correlation of iPTH with GFR as well as lack of correlation of iPTH with Ca and P might indicate that used immunometric assay measure not only iPTH but also biologically inactive fragments which can overestimate at some point the level of parathyroid hormone in patients with early stages of CKD. This could be important for interpretation of iPTH levels during progression of CKD. Lack of correlation of osteocalcin and CrossLaps with GFR can indicate that these bone markers can be used for bone metabolism evaluation in early stages of CKD.

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**MODIFICATION OF THE CHRONIC KIDNEY DISEASE EPIDEMIOLOGY COLLABORATION EQUATION IN THE KOREAN POPULATION**T. Jeong<sup>1</sup>, D. Ko<sup>1</sup>, E. Cho<sup>1</sup>, W. Lee<sup>1</sup>, S. Chun<sup>1</sup>, W. Min<sup>1</sup><sup>1</sup>Department of Laboratory Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, South Korea**BACKGROUND-AIM**

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which is more accurate than the Modification of Diet in Renal Disease (MDRD) equation, has been introduced in clinical laboratories. The Scr concentration is affected by not only age, sex, and muscle mass, but also ethnicity. However, the CKD-EPI equation was derived from mostly of Caucasian, African American and Hispanic populations. We aimed to develop and validate a Korean version of the CKD-EPI equation.

**METHODS**

A total of 1,121 subjects aged 18 years old and older who underwent a chromium-51-ethylenediaminetetraacetic acid GFR measurement were enrolled. The study subjects were randomly divided into two groups which was development cohort (n=897, 80%) and validation cohort (n=224, 20%). Statistical analysis was done by SAS 9.4 (SAS Institute Inc., NC) program. To evaluate the performance of Korean CKD-EPI equation, bias (estimated GFR – measured GFR) was calculated. The  $\pm 10\%$  (P10) and  $\pm 30\%$  (P30) accuracies and root mean square error (RMSE) were compared. And the prevalence of CKD was also compared.

**RESULTS**

In development cohort (n=897), the mean GFR was  $70.3 \pm 35.2$  mL/min/1.73m<sup>2</sup> and the number of female subjects was 340 (38%). The Korean CKD-EPI equation by non-linear mixed effect model was as follows. Male, Scr  $\leq 0.9$  mg/dL, GFR =  $141 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$ ; male, Scr  $> 0.9$  mg/dL, GFR =  $141 \times (\text{Scr}/0.9)^{-1.065} \times (0.993)^{\text{Age}}$ ; female, Scr  $\leq 0.7$  mg/dL, GFR =  $144 \times (\text{Scr}/0.7)^{-0.420} \times (0.993)^{\text{Age}}$ ; female, Scr  $> 0.7$  mg/dL, GFR =  $144 \times (\text{Scr}/0.7)^{-1.391} \times (0.993)^{\text{Age}}$ . The mean bias (mL/min/1.73m<sup>2</sup>) of original CKD-EPI equation was  $-1.9 \pm 16.9$  and those of Korean CKD-EPI equation was  $-1.5 \pm 16.7$ . The median bias (mL/min/1.73m<sup>2</sup>) of original and Korean CKD-EPI equation was  $-1.1$  and  $0.0$ , respectively. The P10 and P30 of original CKD-EPI equation were 35.7% and 82.6% and those were 36.6% and 84.4% for Korean CKD-EPI equation. The RMSE of original and Korean CKD-EPI equations was 17.0 and 16.7. The prevalence of CKD stage 3 was 25.0% for 51Cr-EDTA GFR, 26.3% for original CKD-EPI equation and 25.4% for Korean CKD-EPI equation.

**CONCLUSION**

The Korean CKD-EPI equation showed lower bias and higher accuracy and precision than that of the original CKD-EPI equation. The Korean CKD-EPI equation might be more useful than original CKD-EPI equation in Korean population.

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W053

**DOCTOR**

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**BACKGROUND-AIM**

Advanced Glycation End-products are uremic toxins that accumulate progressively in hemodialysis patients. The aim of this study was to assess the one year increase of Skin Autofluorescence (AF), a measure of Advanced Glycation End-products accumulation and plasma markers, as predictors of mortality in hemodialysis patients.

**METHODS**

169 Hemodialysis patients were enrolled in this study. Skin Autofluorescence was measured twice, one year apart using AGE Reader. Beside routine blood chemistry, additional plasma markers including Superoxide Dismutase, Myeloperoxidase, Inter-Cellular Adhesion Molecule 1, C-Reactive Protein, Heart-type Fatty Acid Binding Protein, and von Willebrand Factor were measured at baseline. The mortality of hemodialysis patients was followed for 36 months.

**RESULTS**

Skin Auto fluorescence values of the hemodialysis patients at the two time points were significantly higher ( $p < 0.001$ ) than those of healthy subjects of the same age. Mean one year AF of hemodialysis patients was  $0.16 \pm 0.06$  which was around 7-9 folds higher than one year AF in healthy subjects. Multivariate Cox regression showed that age, hypertension; one year AF, C-reactive protein, Inter-Cellular Adhesion Molecule 1, and Heart-type Fatty Acid Binding Protein were independent predictors of overall mortality. Hypertension, one year AF, C-Reactive protein, and Heart-type Fatty Acid Binding Protein were also independent predictors of cardiovascular mortality.

**CONCLUSION**

One year AF and plasma Heart-type Fatty Acid Binding Protein used separately and in combination, are strong predictors of overall and cardiovascular mortality in hemodialysis patients.

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W054

**THE EFFECT OF ADIPONECTIN ON BONE TISSUE IN RELATION WITH 1,25 DIHYDROXY VITAMIN D3 IN PATIENTS WITH CHRONIC RENAL FAILURE**S. Ozdem<sup>1</sup>, V.T. Yilmaz<sup>3</sup>, S.S. Ozdem<sup>2</sup>, L. Donmez<sup>4</sup>, G. Suleymanlar<sup>3</sup>, F.F. Ersoy<sup>3</sup><sup>1</sup> Akdeniz University Medical School, Department of Medical Biochemistry, Antalya, Turkey<sup>2</sup> Department of Clinical Pharmacology<sup>3</sup> Department of Internal Medicine, Division of Nephrology<sup>4</sup> Department of Public Health**BACKGROUND-AIM**

Renal osteodystrophy leads to osteoporosis and bone fractures in chronic renal failure (CRF). Adiponectin is an adipokine derived from adipose tissue. It exerts important roles in energy homeostasis and insulin sensitivity and correlates negatively with obesity. It has been shown that adiponectin affects osteoblasts through its receptors expressed in these cells. In the present study, to investigate the possible mechanisms of action of adiponectin on bone tissue we determined the levels and the relation of adiponectin with biochemical markers of bone turnover in CRF patients on hemodialysis.

**METHODS**

The study included 27 CRF patients on hemodialysis (10 women, 17 men, mean age: 50.9 ± 16.5 years, mean weight: 68,66±14,37) and 41 healthy control subjects (22 women, 19 men, mean age: 46.3 ± 11.5 years, mean weight: 68,97±9,66). Serum adiponectin, total ALP (TALP), bone ALP (BALP), N-mid osteocalcin (OC), beta CrossLaps (CTX), procollagen amino terminal propeptide (PINP), 1,25 dihydroxy vitamin D3, calcium and phosphorus levels were measured in CRF patients and control subjects.

Relations of serum adiponectin level with biochemical markers of bone turnover were also investigated using Spearman correlation test.

**RESULTS**

Serum levels of adiponectin (34676 ± 33429 vs. 9703 ± 4599 ng/mL, p<0.0001), TALP (317.11 ± 204.44 vs. 197.51 ± 55.96 U/L, p<0.001), BAP (38.87 ± 34.71 vs. 12.33 ± 5.06 µg/L, p<0.0001), OC (424.52 ± 634.61 vs. 31.73 ± 44.12 ng/mL, p<0.001), CTX (3.04 ± 2.27 vs. 0.24 ± 0.14 ng/mL, p<0.0001), PINP (788.76 ± 710.39 vs. 49.72 ± 19.21 ng/mL, p<0.0001) and phosphorus (4.67 ± 1.97 vs. 3.81 ± 0.53 mg/dL, p<0.01) were all significantly high, whereas 1,25 dihydroxy vitamin D3 levels (2,52±1,18 vs 28,43±11,71 pg/mL, p<0,0001) were significantly low in CRF patients as compared to control subjects. Adiponectin levels correlated negatively with age (r=-0.492, p<0.01) and weight (r=-0.394, p<0.05), but positively with 1,25 dihydroxy vitamin D3 (r=0.561, p=0.002) levels in CRF patients on hemodialysis.

**CONCLUSION**

The finding of a significant relation between adiponectin and 1,25 dihydroxy vitamin D3 levels supported the view that dihydroxy vitamin D3 was involved in the effects of adiponectin on bone metabolism in CRF patients.

Kidney diseases

W055

### **EVALUATION OF A NEW URINARY AND CSF ALBUMIN ASSAY ON THE BECKMAN COULTER AU5800® CLINICAL CHEMISTRY SYSTEM**

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#### **BACKGROUND-AIM**

Mildly increased urinary albumin excretion (30-300 mg/L) is considered a clinically important indicator of progressive renal disease, atherosclerotic disease and cardiovascular mortality. It is used to predict the development of diabetic nephropathy as this protein tends to appear ahead of other serum proteins in urine during the course of renal glomerular damage. Screening for urinary albumin is therefore recommended by the American Diabetes Association and other guidelines for all diabetic patients.

Beckman Coulter has developed a new sensitive albumin assay for the quantitative measurement of albumin in urine and CSF. The performance of this assay was evaluated within the clinical laboratory on the Beckman Coulter AU5800® Clinical Chemistry System.

#### **METHODS**

This method was compared with the current Beckman Coulter Microalbumin assay and the Siemens BN ProSpec by running  $\geq 100$  Urine and  $\geq 40$  CSF samples. Additionally the precision was assessed over 5 days following CLSI guideline EP15-A2, the linear range was assessed following CLSI EP-06A and the high dose hook effect was evaluated.

#### **RESULTS**

The new assay is traceable to IRMM DA470k/IFCC whereas the current Beckman Coulter Microalbumin assay is traceable to a purified albumin standard. Therefore there was approximately a 10% bias when comparing these two methods; Deming regression of  $y = 1.12x + 2.8$  mg/L and  $y = 1.10x - 0.5$  mg/L for Urine & CSF respectively. The new assay compared well with the Siemens BN ProSpec with Deming regression of  $y = 0.96x - 1.6$  mg/L and  $y = 0.97x - 0.3$  mg/L for Urine & CSF respectively.

The precision study gave estimates of total precision with three urine pools with albumin concentrations of 14, 29 & 202 mg/L of 4.6 %CV, 2.7% CV and 2.0%CV respectively. The assay was shown to be linear up to 450 mg/L and there was no high dose hook effect up to 20,000 mg/L.

#### **CONCLUSION**

The results of this study demonstrate that the new Beckman Coulter Urine/CSF assay is reliable, accurate and precise, and that patient sample results show good agreement with existing assays.

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**ASSESSMENT OF NUTRITIONAL AND BIOLOGICAL STATUS IN MOROCCAN HEMODIALYSIS PATIENTS**

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**BACKGROUND-AIM**

The impact of malnutrition on prognosis is important in chronic renal failure. Early recognition of malnutrition is essential to improve the outcome of these patients. The aim of our study is to assess the nutritional and biological status of hemodialysis patients.

**METHODS**

Cross-sectional study involving 110 patients (60 women/50 men) recruited at the department of Nephrology-dialysis-kidney transplantation UHC Ibn Rochd, Casablanca. The Medical Ethical Committees approved the study and all participants gave their written informed consent before inclusion in the study. Patients were divided into two groups depending on the number of hemodialysis sessions/week: Group A (twice per week) and Group B (thrice per 3/week). Nutritional status was determined based on (1) a dietary survey, (2) anthropometric data: body mass index (BMI), fat mass (FM), muscle mass (MM) and (3) biochemical tests including protein catabolism rate (nPCR), albumin, lipid profile and hemoglobin (HbA1c).

**RESULTS**

Data showed that 97% of patients consumed cereals daily, 26% of patients did not eat meat, and 25% did not consume dairy products. Weekly dialysis time was  $11,18 \pm 1.78$  hrs. According to biological data, we found 17% of our patients with hypoproteinemia, 56% with albumin  $\leq 40$  g/l, 74,60% presented serum prealbumin  $< 300$  mg/l, 46% with total cholesterol  $\leq 1,5$  g/l, 50% have low HDL-C and 22% of our patients have nPCR  $< 1$  g/kg/j. Responders to the nutritional questionnaire analysis, 84,12% of patients reported an intake  $< 25$  kcal/kg/day and 78,57% reported a protein intake  $< 0,8$  g/kg/day. Anthropometric data showed an average weight of  $58 \pm 14$  kg with a BMI  $\leq 23$  kg/m<sup>2</sup> in 70% of cases. Low indexes of MM and FM were found in 82% and 17% of patients, respectively. In univariate analysis using Epi info, both groups A and B were comparable in age, sex ratio, HbA1c levels, and weight. However in group A, the lipid profile, nPCR and MM were significantly higher compared to group B ( $P < 0,001$  for all).

**CONCLUSION**

In this work we confirm the presence of high prevalence of malnutrition in our hemodialysis patients (70%) with significant biological and/or clinical consequences. Dietary counseling associated with adequate dialysis are imperative to optimize their nutritional status.



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### **NEPHROTIC SYNDROME AND ANTI-PHOSPHOLIPASE A2 RECEPTOR ANTIBODIES**

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#### **BACKGROUND-AIM**

Membranous nephropathy is one of glomerular diseases most frequently associated with nephrotic syndrome in adults. M-type phospholipase A2 receptor (PLA2R) has been identified as an antigen in the membranous nephropathy. PLA2R is expressed on the surface of the podocytes serving as targets for circulating antibodies, which lead to the formation of immune complexes in situ, to the complement activation and proteinuria.

#### **METHODS**

Case report of male 70 years old. Ex-smoker. Arterial Hypertension. Dyslipemia. Gouty arthritis. Progressive increase of edema of the lower, initially treated with diuretics without improvement, proteinuria in the nephrotic range and normal renal function.

#### **RESULTS**

On examination important edema of the lower limbs, facial region and arms. Circulating antibodies: anti-PLA2R antibodies tested positive by indirect immunofluorescence. The renal biopsy: diffusely thickened capillary presence. No thrombus deposition substances. Tubular atrophy and interstitial fibrosis of 20-25%. Inflammation interstitial focal. Mild interstitial edema. Focal tubular necrosis. Sporadic tank Tamm-Horsfall protein in tubular lights. Absence of vasculitis and arteriolo-hyalinosis. Mild intimal hyperplasia. Reduplication of elastic. Direct immunofluorescence: with intense granular deposition of IgG and C3. In less intensity and similar distribution of Kappa and Lambda. Studies in glomerulus (IgA, IgM, C1q and fibrinogen) negatives. The diagnostic was membranous glomerulonephritis (stage I) with focal tubular necrosis and tubular atrophy/interstitial fibrosis.

#### **CONCLUSION**

The identification of anti-PLA2R antibodies may aid in the diagnosis and treatment in patients with membranous nephropathy and should be secured in conducting studies aimed at optimizing therapy, although these circulating anti-PLA2R antibodies not detected in approximately 30% of patients with membranous nephropathy, but their presence helps to support the diagnosis of renal biopsy; studies on the presence anti-PLA2R antibodies indicate the need for new technologies that can test the prognostic biomarkers in glomerulopathy and especially in those cases where the renal biopsy is not possible.

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### **URINE SEDIMENT EXAMINATION AS RELIABLE DIAGNOSTIC TEST IN DAILY PRACTICE**

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#### **BACKGROUND-AIM**

To determine the urinalysis as effective diagnostic tool in various diseases of the urinary tract in daily practice.

#### **METHODS**

The patient, 70-year-old man, coming in our laboratory for 8 months with symptoms of lower urinary tract disorder (frequent urination, sometimes pain during urination or feeling the need to urinate without being able to do that), with microhematuria with duration for two weeks. Freshly collected urine were analysed on LabUMat automated urine strip reader and UriSed automated microscopic analyser by cuvette based microscopy (CBM) technology (77 Elektronika Kft, Budapest, Hungary). The instrument fills native urine into a cuvette. After centrifugation, the cuvette is forwarded to the microscope table where a built-in camera takes digital images, performing automatic focusing at different positions and saving a well-focused image of each field, and evaluates the images automatically by Auto Image Evaluation Module (AIEM). In order to confirm particles identification they were reevaluate by manual microscopy prepared according to the European Urinalysis Group Guidelines.

#### **RESULTS**

The results obtained by deep stick test demonstrate positive results in each urine sample.

In previous microscopic examination we found only a few isomorphic erythrocytes (2- 5Erc/ $\mu$ L) with rare transitional epithelial cells from superficial layers without any change in their morphology. In last urine samples (2 months later) we found, either isolated or in cluster ovoid and tailed cells with increased nuclear-cytoplasmic ratio and increased number of nuclei.

#### **CONCLUSION**

Hematuria is the main symptom of many urologic diseases and its diagnostic array is wide, ranging from benign pathologies such as infections or stones to neoplasia. Microhematuria also requires a distinct work-up, especially when there are risk factors for the urothelial neoplasia. In order not to overlook early symptoms of malignant and relevant benign diseases, urinalysis must be the first approach in differential diagnosis therefore well-educated microscopist must be able to recognize even doubtful particles. The importance of this test seems more serious in countries of developing world with restricted human and financial resources.

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### COMPARISON BETWEEN TWO METHODS OF ANALYSIS FOR QUANTITATIVE PARAMETERS IN URINE SEDIMENT

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#### BACKGROUND-AIM

Urine sediment is a highly important part in the evaluation of renal and urinary tract diseases. Recently, a wide variety of automated urine microscopy analyzers has been introduced to laboratories, thereby improving accuracy, precision and throughput.

The aim of this study was to compare the performance of the Cobas u701 with Sysmex UF1000i for the quantitative parameters erythrocytes (RBC) and leukocytes (WBC).

#### METHODS

Assays were performed on the same day of collection with Cobas u701 and Sysmex UF1000i of Roche Diagnostics with urine samples which provenance from routine and emergency labs. Cobas u701 centrifuges urine samples onto a slide that is then processed by laser scan imaging. The particles in the images are electronically classified. UF-1000i uses advanced flow cytometry technology with hydrodynamic focusing and specific fluorescent dyes for bacteria and sediment.

The Passing and Bablok regression analysis was used for method comparison study. Linear equations, as well as Pearson's Correlation Coefficient were calculated. For calculation of sensitivity and specificity the lab internal reference values were: for cobas u 701 RBC: < 7 p/μL, WBC: < 10 p/μL; and for UF 1000i: RBC: < 15 p/μL; WBC: < 25 p/μL.

#### RESULTS

The study consisted of 418 data pairs for RBC and 392 data pairs for WBC. Passing-Bablok-Regression, Linear regression and Pearson's Correlation Coefficient (R) were calculated for the following methods: RBC (Cobas=-1.33+0.543 UF1000i; Cobas=-5.38+0.783 UF1000i, R=0.768) and WBC (Cobas=-1.50+0.943 UF1000i; Cobas=-4.52+0.991 UF1000i, R=0.985). Considering Cobas u701 as reference method, we obtained 64% of specificity and 61% of sensitivity for RBC, and 72% of specificity and 98% of sensitivity for WBC.

#### CONCLUSION

In conclusion, our results indicate that in quantitative parameters is possible to use either Sysmex UF1000i or Cobas u701 to perform total cell count. Nevertheless, this study revealed that UF1000i has frequently higher counts of erythrocytes than Cobas u701. According to the leukocytes, the correlation between the methods compared was successful.

Is important to mention that laboratories should select their convenience internal reference values to obtain the major diagnostic accuracy according to the method system used.

Kidney diseases

W060

### **SEMI-QUANTITATIVE PARAMETERS IN URINE SEDIMENT: COMPARISON BETWEEN AUTOMATIC OPTICAL MICROSCOPY AND FLOW CITOMETRY**

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#### **BACKGROUND-AIM**

In the last decades, the use of automated systems for the analysis of urine sediment have offered a significant labor savings, thus allowing the operators to dedicate more time to the pathologic findings.

The aim of this study was to compare the diagnostic performance of the Cobas u701 with Sysmex UF1000i for the semi-quantitative parameters: bacteria (BAC), epithelia cells (EC, squamous epithelial cells (SEC) and non-squamous epithelial cells (NEC)), hyaline (HYA) and pathological (PAT) casts.

#### **METHODS**

Assays were performed on the same day of collection with Cobas u701 and Sysmex UF1000i of Roche Diagnostics with urine samples which provenance from routine and emergency labs. Cobas u701 centrifuges urine samples onto a slide that is then processed by laser scan imaging. The particles in the images are electronically classified. UF-1000i uses advanced flow cytometry technology with hydrodynamic focusing and specific fluorescent dyes for bacteria and sediment. The tests were performed within two hours after collection.

For calculation of sensitivity and specificity the diagnostic cut-off values were: for cobas u 701: BAC 80/ $\mu$ L, NEC 1 p/ $\mu$ L, SEC 12 p/ $\mu$ L, HYA 1 p/ $\mu$ L, PAT 1 p/ $\mu$ L; and for UF 1000i: BAC 120 / $\mu$ L, NEC 1 p/ $\mu$ L, SEC 32 p/ $\mu$ L, HYA 1 p/ $\mu$ L, PAT 1 p/ $\mu$ L.

#### **RESULTS**

The study consisted of 667 data pairs for all the semi-quantitative parameters performed. Considering Cobas u701 as reference method, we obtained 96.9% of specificity and 42.5% of sensitivity for BAC, 91.6% of specificity and 81.0% of sensitivity for SEC, 94.9% of specificity for NEC, 96.1% of specificity and 75.7% of sensitivity for EC, 99.5% of specificity for HYA and 99.6% of specificity for PAT.

#### **CONCLUSION**

In conclusion, the comparison of the semi-quantitative parameters between u701 on the one hand and UF1000i on the other is overall good.

Due to a low number of positives samples for NEC and the importance of their identification for screening of cancer, it would be recommended the confirmation by manual microscopy in these cases. In addition, pathological casts represent different disease states, for this reason their identification is crucial in the management of kidney diseases too.

The most convenience cut-off values for semi-quantitative parameters in the routine and emergency labs should be established.

Kidney diseases

W061

**ASSOCIATION BETWEEN INFLAMMATORY MARKERS AND SUBCLINICAL TARGET ORGAN DAMAGE IN MALE PATIENTS WITH ESSENTIAL HYPERTENSION**M. Đerić<sup>1</sup>, B. Ilinčić<sup>1</sup>, V. Čabarkapa<sup>1</sup>, I. Radosavkić<sup>2</sup><sup>1</sup>Center for laboratory medicine, Clinical Center of Vojvodina, Faculty of Medicine, University of Novi Sad, Serbia<sup>2</sup>Center for laboratory medicine, Clinical Center of Vojvodina, Serbia**BACKGROUND-AIM**

Background: Inflammatory processes are important participants in the pathophysiology of subclinical target organ damage (TOD) in patients with essential hypertension (HT). We evaluated the association between high sensitive C-reactive protein (hsCRP), neutrophil lymphocyte ratio (NLR), carotid intima-media thickness (CIMT) and carotid plaque in patients with hypertensive subclinical renal TOD (glomerular filtration rate (GFR) from 30 to 60 ml/min/1.73 m<sup>2</sup> or urinary albumin excretion 30-300 mg/day).

**METHODS**

Methods: In fifty male patients median age 65 (60 - 70 years), with long lasting ( $\geq 10$  years) HT, imaging markers of vascular damage (CIMT and carotid plaque) was assessed by B-mode carotid ultrasound, GRF was measured by radioisotopic method (two blood samples after 180 and 240 min, 99m Tc diethylene triamine penta-acetic acid – mGFR), blood and urine biomarkers were measured by standard laboratory methods. Patients were divided in group I - patients with CIMT > 0.9 mm and/or carotid plaque (n=22) and group II - patients without presence of carotid plaque and CIMT  $\leq 0.9$  mm (n=28).

**RESULTS**

Results: Hypertensive patients in group I compared to group II, had significantly higher median serum concentration of hsCRP (3.63 (2.4 - 4.0) vs. 1.7 (1.3 - 3.5) mg/L,  $P < 0.05$ ) and NLR (2.3 (1.7 - 2.5) vs. 1.42 (1.1 - 1.78),  $P < 0.05$ ). Univariate analysis showed that hsCRP significantly correlated with CCIMT ( $r = 0.39$ ,  $P = 0.01$ ) and presence of carotid plaque ( $r = 0.35$ ,  $P = 0.01$ ), while NLR significantly correlated with mGFR ( $r = -0.413$ ,  $P = 0.001$ ) and CCIMT ( $r = 0.396$ ,  $P = 0.01$ ). No correlation was found between inflammatory biomarkers and albuminuria. Multivariate regression analyses suggested that independent determinant of hsCRP was CCIMT ( $P = 0.001$ ), while mGFR ( $P = 0.0056$ ) was independently associated with NLR.

**CONCLUSION**

Conclusion: In male patients with long lasting HT and subclinical renal TOD, hsCRP is associated with CIMT and NLR is associated with GFR.

Kidney diseases

W062

### **COMPARISON OF THE BCG, BCP AND NEPHELOMETRY METHODS USED FOR THE ALBUMIN TESTING FOR THE DIALYSIS PATIENTS.**

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#### **BACKGROUND-AIM**

It has been reported that serum albumin (Alb) level less than 4.0 g/dL correlates well with an increased risk of death in dialyzed patients. Conversely, albumin level > 4 g/dL is required by guidelines regulating standard of care for these patients. This requirement was developed for the bromocresol green (BCG) method, which is known to overestimate albumin levels by non-specific binding of the BCG dye to the globulin. In contrast, bromocresol purple (BCP) method, measures Alb without a positive bias in healthy persons; but it possess negative bias in uremic patients due to inhibitory action of the 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), which is produced by uremic patients. The requirement for albumin to be >4g/dL creates a market for labs testing albumin with the BCG method, despite known positive bias. To define (proportional, constant or random) and to assess the degree of bias for both BCG and BCP methods we compared them to the nephelometry (Neph), interference free method.

#### **METHODS**

Plasma albumin levels were tested for twenty dialyzed patients using BCG, BCP and nephelometry methods. The bias was defined by comparison results obtained from BCG and BCP with ones from Neph method.

#### **RESULTS**

The average Alb concentrations were significantly different for all three methods (BCG: 3.9 g/dL; BCP: 3.1 g/dL; Nep: 3.4 g/dL). The concentrations measures by BCG, were higher than these from BCP by 0.2 to 1.1 g/dL. The concentrations measures by BCP were lower than ones from Neph by 0.3 to 0.7 g/dL. The differences among these three methods has been randomly distributed, that no constant or proportional bias were found.

#### **CONCLUSION**

1. The BCG method, currently used for dialyzed patient's overestimates Alb level to degree that cannot be predicted (random bias). To meet the "standard of care" criteria for albumin >4g/dL, dialysis clinics intentionally send their specimen to the lab using BCG for Alb testing.
2. Even the Centers for Medicare and Medicaid Services, US, recommended the change in target albumin concentration to 3.5 g/dL for BCG and to 3.2 g/dL for BCP, the uremic patients should not be tested by these method, due to random positive and random negative bias respectively.
3. Nephelometry method for albumin measure does not possess known interference that should make it a method of choice for dialysis patients. But because lack of correlation between Neph and BCG methods, a new guidelines based on Neph testing should be developed.

Kidney diseases

W063

**HOMOCITRULLINE: NEW BIOMARKER OF ACUTE RENAL FAILURE?**A. Desmons<sup>1</sup>, S. Jaisson<sup>1</sup>, P. Rieu<sup>3</sup>, A. Wynckel<sup>2</sup>, P. Gillery<sup>1</sup><sup>1</sup>Laboratory of Pediatric Biology and Research, University Hospital of Reims, France. Laboratory of Biochemistry and Molecular Biology, UMR CNRS/URCA N° 7369, Faculty of Medicine, Reims, France.<sup>2</sup>Nephrology unit, University Hospital of Reims, France.<sup>3</sup>Nephrology unit, University Hospital of Reims, France. Laboratory of Biochemistry and Molecular Biology, UMR CNRS/URCA N° 7369, Faculty of Medicine, Reims, France.**BACKGROUND-AIM**

Carbamylation is a nonenzymatic post-translational modification characterized by the irreversible addition of isocyanic acid to amino groups of proteins. Because isocyanic acid mainly originates from the spontaneous dissociation of urea, carbamylation rate is highly increased during renal failure. This reaction leads to the formation of carbamylation-derived products (CDPs), such as carbamylated albumin or carbamylated hemoglobin. The aim of the study was to evaluate homocitrulline (HCit), which results from the carbamylation of  $\epsilon$ -amino groups of lysine (Lys) residues, in acute renal failure (ARF) and to determine if it could be useful for differentiating acute from chronic renal failure (CRF).

**METHODS**

213 patients with renal failure referred to the nephrology unit of the university hospital of Reims were included in this study. Patients were classified into three groups: patients with ARF (ARF group, n=39), patients with CRF complicated with ARF (A/CRF group, n=29) and patients with CRF (CRF group, n=145). Serum total HCit concentrations were determined by LC-MS/MS and expressed as  $\mu\text{mol}$  of HCit per mol of Lys. Kinetic profiles of HCit and urea concentrations were studied in patients suffering from ARF. An HCit threshold between ARF and CRF was investigated.

**RESULTS**

HCit concentrations increased in ARF patients reaching a peak generally delayed compared to the urea concentration peak. HCit concentrations were positively correlated with urea concentrations ( $r=0.51$ ) and with the time elapsed since the estimated onset of ARF ( $r=0.57$ ). Serum HCit were significantly higher in CRF ( $p<0.05$ ) group compared to ARF group. The receiver operating characteristic curve analysis showed that HCit concentrations below  $289 \mu\text{mol/mol}$  Lys were predictive of ARF with a sensitivity of 83 % and a specificity of 72 % and an area under the curve equal to 0.856.

**CONCLUSION**

Our results demonstrate that HCit is a promising biomarker for distinguishing between ARF and CRF patients.

Kidney diseases

W064

### LEAN MASS AND AGE ARE STRONG DETERMINANTS OF GLOMERULAR FILTRATION RATE IN HEALTHY MEN

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#### BACKGROUND-AIM

Understanding determinants of glomerular filtration rate (GFR) is important in aiding prediction and interpretation of kidney function. Body composition is known to affect GFR, but is not included in current screening of kidney disease. We investigated the association between GFR and body composition in healthy young men with differing body mass but without known diabetes or kidney injury.

#### METHODS

Three age-matched groups were recruited: normal BMI (n = 22) < 25 kg/m<sup>2</sup>, muscular (n = 23) with BMI > 30 kg/m<sup>2</sup> and a screened bioelectrical impedance (BIA) body fat < 20%, and obese (n = 22) with BMI > 30 kg/m<sup>2</sup> and a screened BIA body fat > 30%. Dietary analyses, GFR by clearance of 99m Tc-DTPA, and body composition by dual-energy X-ray absorptiometry (DEXA) were measured in all participants.

#### RESULTS

Muscular men had higher GFR (mean 186.4 mL/min; 95% CI 171.7 to 201.1) than normal BMI and obese groups (P = 0.0007). Fat mass protein intake, and smoking status were not associated with GFR; whereas lean mass had the strongest association with GFR. In all subgroups, skeletal muscle mass correlated significantly with GFR (P = 0.04). In multi-variate models, variables with the strongest associations with GFR were age (P = 0.0009) and lean mass (P = 0.0001). A final derived multiple regression equation was; GFR = 38.3 – 0.997 (age) + 2.34 (total lean mass).

#### CONCLUSION

Age and lean mass were strong determinants of GFR in healthy men of various body compositions. We estimate that GFR decreases by 1 mL/min/year of age and increases 2.3 mL/min/kg of lean mass in healthy men.



Kidney diseases

W065

### **VARIABILITY OF URINARY N-ACETYL-BETA-D-GLUCOSAMINIDASE ACTIVITY DURING STRESS TOLERANCE TEST IN INDIVIDUALS WITH ORTHOSTATIC PROTEINURIA**

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#### **BACKGROUND-AIM**

Background: Orthostatic proteinuria is a type of asymptomatic proteinuria and represent a common condition in school-age children and teenagers, related with a change in position-supine/standing. The aim of this study was to assess the variability of N-acetyl-beta-D- glucosaminidase activity (beta-NAG) as a sensitive marker of tubular damage, during stress tolerance test in young individuals with diagnosed orthostatic proteinuria.

#### **METHODS**

Methods: The evaluation of the changes in qualitative and quantitative composition of urinary proteins, with SDS-PAG electrophoresis, in young individuals 7-24 years old, enabled us detection of subjects with orthostatic proteinuria. Five urinary samples excreted during stress tolerance test were used: two samples of first morning urine, two samples of daily urine and one sample of urine excreted after physical effort. Horizontal thinlayer 4-22% gradient SDS-PAG electrophoresis was conducted, using Coomassie Blue R 250 staining technique. The activity of the enzyme beta-NAG was determined in all five urinary samples in 30 individuals with and 20 without orthostatic proteinuria, aged matched. Beta-NAG activity and creatinine concentration in urine samples were determined using spectrophotometric methods. Enzyme activity was expressed in U/g creatinine.

#### **RESULTS**

Results: In subjects with and without orthostatic proteinuria, the highest mean values for beta-NAG activity were detected in first morning urinary samples and the lowest mean values were detected in samples excreted after physical effort. Besides variations in beta-NAG activity in five samples urine excreted during stress tolerance test, the activity of beta-NAG in all individuals were within the reference intervals.

#### **CONCLUSION**

Conclusion: The results lead to conclusion that there is no significant tubular damage in individuals with orthostatic proteinuria.

Kidney diseases

W066

### **COST MODULATION IN MEASUREMENT OF URINE CREATININE CONCENTRATION FULFILLING THE REQUIREMENTS OF ANALYTICAL QUALITY**

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#### **BACKGROUND-AIM**

In recent years, based on the filtered urine creatinine (uCR) at a nearly constant speed, have published various clinical practice guidelines (CPG) that recommend the measurement of its concentration for comparison with other analytes in urine (albumin protein, calcium, etc.) and determine whether they are excreted at a normal speed. However, it is recommended that when measured as part of creatinine clearance using the same method as in serum. The acceptance of these CPG has resulted in our laboratory a significant increase in demand, which is measured by the method of creatinine amidohydrolase (uCR-E) with a price 4:1 than the kinetic Jaffé method (uCR-J).

Aim: Check if the uCR-J can substitute the uCR-E with the quality assurance for measuring the concentration of UCR to optimize resources

#### **METHODS**

Imprecision (CVa), inaccuracy (ESa) and total analytical error (ETa) was calculated and checked whether they met the objectives of desirable analytical grade, obtained from biological variability and analytical coefficient of variation (CVa <5.5%; ESa <6.4%; ETa <15.5%). CVA, sera from two different concentrations of certain controls. The ESa, by external quality control, calculating the percentage change compared to the group average of uCR-E for 1 year. The ETa, by adding the imprecision with the inaccuracy multiplied by 1.65, confidence level of 95%. The interference study was conducted comparing the results by uCR-J and uCR-E, previously corrected the bias found 5.35% in 453 patients. A clinically relevant interference exists when the variation between methods was greater than 10%.

#### **RESULTS**

CVa, ESa and ETa were less than desirable analytical quality objectives. The CVa in controls sera was 2.2 and 2.5% for uCR-J and 1.4% and 0.9% for uCR-E. The uCR-J and uCR-E inaccuracy was 2.9% and 3.8%, and ETa of 8.9% and 8.6%. Only a clinically relevant interference was observed, increase of 19%, in a polypharmacy patient (1/453; 0.22%). The annual cost of the uCR-E is 48,000 €, and the uCR-J would save a 75%.

#### **CONCLUSION**

uCR-J meets the requirements of quality assurance for measuring the concentration of uCR, is comparable with the uCR-E about to interference and has advantages of cost which will allow optimizing resources.

Kidney diseases

W067

**KIDNEY FUNCTION EVALUATION IN PATIENTS TREATED WITH DABIGATRAN: COMPARISON OF GLOMERULAR FILTRATION RATE ASSESSED BY USING CREATININE AND CYSTATINE.**

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**BACKGROUND-AIM**

Background: Dabigatran (DAB) is 80% renally excreted, glomerular filtration rate (GFR) estimation is recommended to evaluate the kidney function. In this paper we report some preliminary results about evaluation of creatinine (CRE) and CYS cystatine (CYS) GFR in a group of patients evaluated before DAB treatment.

**METHODS**

Materials and Methods: We considered 77 patients, 36 males and 41 females, with age between 54 and 85. In these patients we performed a basal evaluation of kidney function using CRE and CYS based GFR KDIGO prediction equations. CRE was measured using a IDMS traceable dry chemistry enzymatic method. CYS was measured using an immunochemistry IFCC traceable immune assay. After GFR calculation these patients were classified as recommended by KDIGO guidelines. In patients with discordant classification a creatinine clearance was performed.

**RESULTS**

Results: We observed a relatively weak correlation between CRE GFR and CYS GFR ( $R^2=0.54$ ) The mean CRE GFR was  $58\pm 17$  mL/min, the mean CYS GFR was  $51\pm 21$  mL/min this difference was statistically significant ( $p=0.03$ ). Bland-Altman elaboration confirmed that CYS GFR was lower than CRE GFR. Following KDIGO criteria, patients classification performed by using CRE GFR or CYS GFR was concordant in 44 subjects and discordant in 23 (29%). In 21/23 discordantly classified patients the creatinine clearance confirmed classification performed by using CYS GFR.

**CONCLUSION**

Conclusions: CRE GFR is influenced by muscle mass, age, sex and concomitant diseases. Moreover in elder patients CRE GFR demonstrated some reliability problems. CYS GFR is relatively independent of body composition. In this group of 77 patients evaluated before treatment with Dabigatran we observed: a relatively weak correlation between CRE GFR and CYS GFR, CYS GFR was lower than CRE GFR. These differences in the estimation of GFR resulted in a different classification in 29% of considered patients. In these 23 patients, we performed a creatinine clearance which confirmed the classification performed according to CYS GFR in 21 cases (91%). Results obtained in this study, although preliminary and in need of confirmation, would seem to suggest that, in this particular subset of patients, the determination of CYS GFR can be a better renal function indicator than CRE GFR.

Kidney diseases

W068

### **EVALUATION OF URINARY PROTEINS IN WOMEN WITH PREECLAMPSIA BY SDS PAGE**

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#### **BACKGROUND-AIM**

Proteinuria is one of the cardinal features of preeclampsia, which is a common and potentially severe complication of pregnancy. The aim of this study was to compare the concentration of total urinary proteins in women with normal pregnancy and preeclampsia and to determine the most common types of proteinuria in preeclampsia using sodium dodecyl sulfate polyacrylamide gel electrophoresis - SDS PAGE.

#### **METHODS**

In this study were included two groups: first group (n=42) women with preeclampsia and second group (n=20) women with normal pregnancy. The average age of women with normal pregnancy was 33.6±4.1, while in women with preeclampsia was 30.7±5.6. Urinary samples were obtained and the following tests performed: chemical analyses of urine with dipsticks, determination of total urinary proteins by turbidimetric method with sulfosalicylic acid and electrophoretic separation of urinary proteins by horizontal gradient (4-22%) SDS PAGE according to Görg.

#### **RESULTS**

Concentration of total urinary proteins was significantly higher in the women with preeclampsia than in women with normal pregnancy (p<0,05). Electrophoretic patterns of urinary proteins in all women with normal pregnancy were normal (only albumin fraction). All women with preeclampsia showed abnormal electrophoretic patterns. In 9,1% of women with preeclampsia was found high molecular weight proteins (glomerular type of proteinuria), in 2,9% was found low molecular weight proteins (tubular type of proteinuria), in 35,7% was found postrenal proteinuria and in 52,3% was found high and low molecular weight proteins, corresponding to mixed proteinuria (glomerular and tubular type of proteinuria).

#### **CONCLUSION**

The present study shows that SDS PAGE of urinary proteins is high sensitive method for detection of urinary proteins in pregnant women. Screening for proteinuria is essential in detection of preeclampsia in antepartum care of pregnant women. Early detection of proteinuria is important for well-timed treatment and reduction of complications in pregnancy.

Kidney diseases

W069

## COMPARISON OF SEMI-QUANTITATIVE AND QUANTITATIVE METHODS FOR THE MEASUREMENT OF MICROALBUMINURIA

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### BACKGROUND-AIM

Urine Albumin is an early marker of chronic kidney disease (CKD) in diabetic patients. The excretion of recent urine albumin greater than 20 mg/L (microalbuminuria) is considered as a predictor of diabetic CKD. The aim of this study was comparison of semiquantitative and quantitative methods for the measurement of microalbuminuria in diabetic patients.

### METHODS

Recent urine microalbuminuria of diabetic patients were determined by two methods:

1. Semi-quantitative: Colorimetric method using the strip H13 in DIRUI H-800 PLUS (DIRUI®). The content of microalbuminuria is inversely proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000.
2. Quantitative: microalbuminuria was measured by immunoturbidity in COBAS C311 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the quantification of microalbuminuria: positive (microalbuminuria > 20 mg/L) and negative (microalbuminuria < 20 mg/L). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC) using the software MEDCALC®.

### RESULTS

We analyzed 469 diabetic patients between 27 and 85 y.o. (mean age = 56.3), 82 patients (17.5%) had a positive microalbuminuria and 387 patients (82.5%) were negative. The AUC of color scale by the test strip for diagnosis of positive microalbuminuria was 0.985 ( $p < 0.0001$ ) and optimal cut-off value was 1305 exhibiting 100% sensitivity and 86.3% specificity.

### CONCLUSION

The semi-quantitative method by test strip, can be used for the measurement of microalbuminuria in diabetic patients.