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Original article

ASSESSMENT OF BONE HEALTH IN ADULTS WITH CYSTIC FIBROSIS IN THE REPUBLIC OF NORTH MACEDONIA

ПРОЦЕНКА НА КОСКЕНО ЗДРАВЈЕ КАЈ ВОЗРАСНИ ПАЦИЕНТИ СО ЦИСТИЧНА ФИБРОЗА ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Abstract

Introduction. The term cystic fibrosis bone disease (CFBD) is used to describe low BMD and /or fragility fractures in CF patients.
Aim. We decided to carry out a bone health screening of adult patients with CF in the Republic of North Macedonia and determine their current status.
Methods. We conducted a prospective study which comprised a sample of 30 individuals with CF above the age of 18 years, of the population of ~50 adults with CF in North Macedonia. Sex, age, height and weight (later converted to BMI kg/m^2) were recorded; blood sample analyzed for serum levels of calcium, free calcium, phosphate, parathyroid hormone (PTH) and 25(OH)D. We conducted an interview with all subjects regarding additional risk factors. All subjects underwent DXA scan by measuring BMD at the lumbar spine and proximal hip.
Results. Approximately one half of the adults with CF had low BMD and about a quarter of them also had osteoporosis. 33.3% of patients had history of fragility fractures, the mean BMI was lower than the recommended values; vitamin D deficiency was found in 60% and continuous use of glucocorticoids was recorded in 30% of subjects. Conclusion: Our findings are in agreement with those of other studies. The most effective method for evaluating BMD in adult CF patients is DXA scanning and regular monitoring.

Keywords: cystic fibrosis, bone density, osteoporosis

Апстракт

Вовед. Терминот Коскена болест при цистична фиброза (Cystic Fibrosis Bone Disease -CFBD) се користи да се опише ниска коскеноминерална густина и/или фрагилни фрактури кај пациентите со цистична фиброза (ЦФ).

Цел. Одлучивме да спроведеме истражување за коскеното здравје на возрасни пациенти со цистична фиброза во Република Северна Македонија и да ја утврдиме сегашната состојба.
Методи. Спроведовме проспективна студија на примерок од 30 индивидуи со ЦФ над 18 години, од популацијата од ~50 возрасни со ЦФ во Северна Македонија. Нотиравме: пол; возраст; висина и тежина (кои ги конвертиравме во индекс на телесна маса); анализиравме крвен примерок за серумски нивоа на калциум, јонизиран калциум, фосфор, паратироиден хормон и 25(OH)D. Спроведовме интервју со сите учесници во студијата за присуство на дополнителни ризик фактори. Кај сите субјекти извршивме снимање на ДЕКСА скен со мерење на коскеноминералната густина во регииите на лумбален рбет и проксимален колк.
Резултати. Околу половина од возрасните пациенти со ЦФ имаат ниска коскеноминерална густина, а околу една четвртина од нив остеопороза. 33,3% од пациентите даваат податок за фрагилна фрактура, средната вредност на индексот на телесна маса е пониска од препорачаната, недостаток на витамин Д најдовме кај 60 %, а континуирана употреба на гликокортикоиди кај 30 % од субјектите.
Заклучок. Овие податоци одговараат на истите од други студии. Најефективен начин за проценка на коскеноминералната густина кај возрасни пациенти со ЦФ е ДЕКСА скенирање и мониторирање.

Клучни зборови: цистична фиброза, коскена густина, остеопороза

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder which is caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and is characterized by chronic respiratory failure, recurrent infections, and pancreatic insufficiency, which often lead to decreased life expectancy. Advances in care and treatment in the last decades have prolonged

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the life expectancy of these patients with more patients reaching adulthood and even their fifties [1]. This has resulted in new complications previously rarely seen in CF, such as reduced bone mineral density (BMD) and increased fracture rates [1-3]. The term cystic fibrosis bone disease (CFBD) is used to describe low BMD and/or fragility fractures in CF patients. CFBD is considered a multifactorial disorder, arising from both direct and indirect contributors from the underlying CF defect, such as poor nutritional status, lung infection, vitamin D insufficiency, a negative calcium balance, pancreatic insufficiency, hypogonadism, delayed puberty, CF-related diabetes, glucocorticoid treatment, reduced levels of weight bearing activity and the effect of CFTR dysfunction on bone cells [3]. Fragility fractures occur following an event which would otherwise not be expected to result in a fracture and are characterized by their association with low bone mineral density (BMD)[4]. These fractures, particularly of the vertebrae and ribs, have the potential to increase morbidity in CF patients as a result of pain and deterioration in respiratory status, prolonged periods of bed-rest and inactivity.

Regular screening of bone health is advised for CF patients by international guidelines [2,3].

In our country, assessment of bone health in adults with CF is performed sporadically and mostly when fragility fractures occur. We decided to carry out a bone health screening of adult patients with CF in the Republic of North Macedonia and determine their current status.

Material and methods

We conducted a prospective study which comprised a randomly selected sample of 30 individuals with CF above the age of 18 years, of the population of ~50 adults with CF in North Macedonia. The aim was to obtain a study sample that was representative of the adult CF population in our country. None of the participants has undergone lung transplantation or received CFTR modulator or antiresorptive treatment. In all group subjects diagnosis of CF and CF genotype were previously obtained and verified. Sex, age, height and weight (later converted to BMI kg/m^2) were recorded. Biochemical data were obtained from a blood sample taken when patients were clinically stable and analyzed for serum levels of calcium, free calcium, phosphate, parathyroid hormone (PTH) and 25(OH)D. We conducted an interview with all subjects regarding vit. D and calcium supplementation, glucocorticoid use, history of fragility fracture and presence of cystic fibrosis related diabetes (CFRD). All subjects underwent DXA scan by measuring BMD at the lumbar spine and proximal hip. All scans were performed on the same device (Hollogic Discovery) and by the same operating technician. BMD values were expressed as Z-scores (Z-score LS for lumbar spine and Z-score PH for proximal hip).

Statistical analysis was performed using STATISTICA 7.1 and SPSS Statistics 23.0. The following statistical methods were used: descriptive statistics for numeric variables, Pearson's Chi-Square (p)/(2-sided) and Fisher's Exact Test (p)/(2-sided).

Results

There were 18 male and 12 female subjects in the study group. Descriptive characteristics (mean, median, minimum, maximum and standard deviation) of the study group (age, BMI and biochemical parameters) are shown in Tables 1 and 2. All examined biochemical values were in the normal range, except for 25 (OH)D where the minimum value recorded was 12.20 ng/ml and values below 30.00 ng/ml were recorded in 18 patients (60%), of which 6 patients had less than 20.00 ng/ml.

Table 1. Descriptive statistics for age, weight, height and BMI (body mass index) of the study group

Variable	Valid N	Mean	Median	Minimum	Maximum	Std. Dev.
Age	30	26.50	25	18	45	7.28
Weight/kg	30	54.5	51.5	29	85	15.53
Height/cm	30	161	162	133	177	9.37
BMI/kg/m^2	30	20.95	19.70	15.80	29.40	3.56

Table 2. Descriptive statistics of biochemical data of the study group

Variable	Valid N	Mean	Median	Minimum	Maximum	Std. Dev.
Ca	30	2.35	2.35	2.19	2.58	0.09
Ca+	30	1.22	1.22	1.17	1.30	0.03
P	30	1.44	1.48	0.98	1.99	0.27
25(OH)D	30	30.81	27.30	12.20	81.30	14.81
PTH	30	39.56	39.05	17.20	65.10	13.34

The results of the interview with all subjects regarding presence of cystic fibrosis-related diabetes (CFRD), history of fragility fracture, glucocorticoid use and vitamin D and calcium supplementation are shown in Table 3. CFRD was present in 33.3% of subjects. The total number of subjects with history of fragility fractures was 10, with 6 subjects experiencing more than 1 fracture (total number of fractures 18). Regular gluco-

corticoid use (peroral or parenteral) was recorded in 30% (total number 9) of subjects. Vitamin D + calcium supplements were declared to be used by all participants with doses of peroral 2000 IU D3 and 1000 mg calcium daily.

The descriptive results for DXA scan measurements of all participants are presented in Table. 4. Table 5 shows the distribution of Z-score (LS and PH)-total number and percentage by gender and for the whole study group. 53.3% of all subjects had Z-score LS total less than-2(33.3% of females, 66.7% of males) and Z-score PH total less than-2 was found in 33.3% (25% of females, 38.,9% of males). Pearson's Chi-Square=3.21 and p>0.05 (p=0.07)/(2-sided) for gender distribution showed no significant difference for Z-score LS. This distribution of Z-score FN total and gender with Fisher's Exact Test p>0.05 (p=0.69)/(2-sided) showed no significant difference. These results indicated that 53.3% of all subjects (both genders) in the present study demonstrated CF-related low BMD.

Table 3. Interview results of additional factors for the study group, CFRD (cystic fibrosis-related diabetes)

Category	CFRD	Fragility fracture	Glucocorticoids	Vit. D +Calcium supplements
Yes	10	10	9	30
No	20	20	21	0
Missing	0	0	0	0

Table 4. Descriptive results of Z-score for PH (proximal hip) and LS (lumbar spine)

Variable	Valid N	Mean	Median	Minimum	Maximum	Std. Dev.
Z-score PH total	30	-1.52	-1.45	-4.30	0.90	1.40
Z-score LS total	30	-1.89	-2.15	-4.40	2.20	1.75

Table 5. Distribution of Z-score for PH (proximal hip) and LS (lumbar spine) by total number and gender

			Z-score LS total < - 2	Z-score LS total > - 1	Z-score PH total < - 2	Z-score PH total > - 1
Gender	Female	Count	4	5	3	5
		%	33.3%	41.7%	25.0%	4,7%
	Male	Count	12	2	7	5
		%	66.7%	11.1%	38.9%	27.8
Total		Count	16	7	10	10
		%	53.3%	23.3%	33.3%	33.3%

Subjects with Z-score <-2 and history of fragility fracture (total number 8; 26.6%) were diagnosed as osteoporotic.

Discussion

Our assessment of bone health in adults with CF was conducted according to the European Cystic Fibrosis Bone Mineralisation guidelines [2] and the American Cystic Fibrosis guidelines [3]. These guidelines recommend all adults above the age of 18 years to have an initial assessment of bone health with DXA scan and laboratory examination of 25(OH) D. Additional risk factors have to be recorded, such as low BMI, fragility fractures, cystic fibrosis related diabetes, glucocorticoid use.

BMI is the generally accepted indicator for monitoring the nutritional status of patients with CF. In adults, weight and height measurements should be converted to BMI (kg/m2) to minimize the influence of short stature [3]. Malnutrition in individuals with CF can negatively affect bone mineralization, hampering the achievement of an optimal peak bone mass which can lead to a precociously reduced bone density in adult life [6]. In adults, the target BMI should be ≥22 for women and ≥23 for men [7]. In our study, BMI (20.95 ±3.6) showed borderline results which indicate moderate to poor nutritional status. But, this was also reported in many other studies [8], which suggests that this is a common problem in adults with CF and it is addressed in the European consensus on nutrition in patients with cystic fibrosis [8].

Our assessment of biochemical data showed values in the reference range for calcium, free calcium, phosphate and PTH. However, serum 25(OH)D demonstrated values (30.81±14.81) below 30.00 ng/ml in 18 patients (60%), of which 6 patients had less than 20.00 ng/ml. This is despite the record of all study subjects for regular vitamin D and calcium supplementation. Wolfenden *et al.* reported that 76% of 185 adults with CF had a 25-hydroxy vitamin D level <30 ng/ml despite routine supplementation [9]. More than 20 other reports indicate that vitamin D insufficiency (low or low-normal 25

(OH)D levels) is common among individuals with CF [10-12]. Studies are lacking to determine the most effective vitamin D supplementation regimen to correct vitamin D deficiency. In adults, the dose should be adjusted aiming to maintain 25(OH) D concentration above the deficiency threshold [2,3].

Additional risk factors present in patients with CF can cause or aggravate low BMD and CFBD. Diabetes develops in ~10% of individuals with CF [2]. In our study, CFRD was present in 33.3% of participants. Although CF-related diabetes may differ from classical type I or II diabetes, it plays a role in reducing BMD [2].

Many studies have found glucocorticoid therapy to be a risk factor of low bone mass in CF [13]. Our interview results indicated continuous glucocorticoid therapy in 30% of subjects. The European treatment guidelines clearly state that, to protect bone health, the use of glucocorticoids should be minimized whenever possible [3].

Fragility fractures are an important contributing factor to establishing the diagnosis of osteoporosis in patients with CF. Even more, the term osteoporosis can be applied to adults with CF who have sustained a low trauma fracture as it is an indicator of increased bone fragility [2,3]. Aris *et al.* [14] reported 54 fractures from subject interviews in 70 patients with over 1410 patient-years of analysis. Fracture rates were approximately 2-fold higher in women with CF, aged 16-34 years, and men with CF, aged 25-45 years, compared to the general population. Donovan *et al.* [11] studied 30 adults with CF and found that vertebral fractures were present in 19% by radiograph review, and 41% had a confirmed history of previous fracture. In the present study, the total number of subjects with history of fragility fractures was 10 (33.3%), with 6 subjects experiencing more than 1 fracture (total number of fractures 18).

DXA is currently the gold standard for measurement of bone mineral content (BMC) and bone mineral density (BMD) in people with CF [2,3]. DXA scans are used for assessment and monitoring of bone health, but also when bisphosphonate therapy is considered. In adults with CF below 50 years of age and premenopausal women, BMD is measured at the lumbosacral spine and proximal hip and expressed as Z-score [2,3].

According to the forementioned guidelines [2,3], the term "CF-related low BMD" may be applied to adults with a BMD Z-score below -2. In younger adults (below 50 and premenopausal women), osteoporosis is defined as having a BMD Z-score <-2 and a significant fracture history. In this study, by following the recommended assessment methods, we found that 53.3% of the study group had low BMD and 26.6% had osteoporosis. This is in line with the conclusions of J. Paccou *et al.* in their systematic review [15], which analyzed data from 10 studies and a total of 888 patients. They found that the prevalence of osteoporosis in adult CF was 23.5% (95% CI, 16.6-31.0). Our results are consistent with their findings.

Conclusion

The present study was conducted on a sample representative of the adult population with CF in North Macedonia and revealed that approximately one half of the adults with CF have low BMD and about a quarter of them also have osteoporosis. 33.3% of patients had a history of fragility fractures; the mean BMI was lower than the recommended values; vitamin D deficiency was found in 60% and continuous use of glucocorticoids was recorded in 30% of subjects. These findings are in agreement with those of other studies. To maintain bone health and quality of life for adults with CF, it is crucial to implement preventive bone disease measures, detect low BMD early and provide treatment where indicated. The most effective method for evaluating BMD in adult CF patients is DXA scanning and monitoring, which offers reliable and reproducible results with a low radiation exposure and is widely acceptable.

Conflict of interest statement. None declared.

References

1. Cystic Fibrosis Foundation Patient Registry. Annual Data Report. Bethesda, Maryland, 2021.
2. Aris RM, Merkel PA, Bachrach LK, *et al.* Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab* 2005; Reviewed 2021; 90(3): 1888-1896.
3. Sermet-Gaudelus I, Bianchi ML, Garabédian M, *et al*. European cystic fibrosis bone mineralisation guidelines. *J Cyst Fibros* 2011; 10 Suppl 2: S16-S23.
4. National Institute for Health and Care Excellence NICE: Clinical Guideline [CG146] - Osteoporosis: assessing the risk of fragility fracture. 2012, updated 2017.
5. Putman MS, Anabtawi A, Le T, *et al*. Cystic fibrosis bone disease treatment: Current knowledge and future directions. *J Cyst Fibros* 2019; 18 Suppl 2: S56-S65.
6. Anabtawi A, Le T, Putman M, *et al*. Cystic fibrosis bone disease: Pathophysiology, assessment and prognostic implications. *J Cyst Fibros* 2019; 18 Suppl 2: S48-S55.
7. Thornton CS. Body Mass Index and Clinical Outcomes in Persons Living With Cystic Fibrosis-Is Bigger Always Better? *JAMA Netw Open.* 2022; 5(3): e220749.
8. Sinaasappel M, Stern M, Littlewood J, *et al*. Nutrition in patients with cystic fibrosis: a European consensus. *J Cyst Fibros* 2002; 1(2): 51-75.
9. Wolfenden LL, Judd SE, Shah R, *et al*. Vitamin D and bone health in adults with cystic fibrosis. *Clin Endocrinol (Oxf)* 2008; 69: 374-381.
10. Ott SM, Aitken ML. Osteoporosis in patients with cystic fibrosis. *Clin Chest Med* 1998; 19: 555-567.
11. Donovan Jr DS, Papadopoulos A, Staron RB, *et al*. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. *Am J Respir Crit Care Med* 1998; 157: 1892-1899.
12. Lark RK, Lester GE, Ontjes DA, *et al*. Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients. *Am J Clin Nutr* 2001; 73: 602-606.

13. Conway SP, Morton AM, Oldroyd B, *et al*. Osteoporosis and osteopenia in adults and adolescents with cystic fibrosis: prevalence and associated factors. *Thorax* 2000; 55(9): 798-804.
14. Aris RM, Renner JB, Winders AD, *et al*. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. *Ann Intern Med* 1998; 128(3): 186-193.
15. Paccou J, Zeboulon N, Combescure C, *et al*. The prevalence of osteoporosis, osteopenia, and fractures among adults with cystic fibrosis: a systematic literature review with meta-analysis. *Calcif Tissue Int* 2010; 86(1): 1-7.

Original article

ASSESSMENT OF ENZYMURIA FROM PROXIMAL RENAL TUBULE IN INFLAMMATORY SERONEGATIVE RHEUMATOID ARTHRITIS

PROCENKA NA ENZIMURIJA OD PROKSIMALNA RENALNA TUBULA KAJ VOSPALITELEN SERONEGATIVEN REVMATOIDEN ARTRITIS

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Abstract

Introduction. To determine the effects of non-treated seronegative rheumatoid arthritis (RA) on proximal renal tubule, sensitivity of alanine aminopeptidase (AAP), γ-glutamyltransferase (γ--GT), β2 microglobulin in urine β2M), as well as to determine the relation with rheumatoid factor (RF) and C-reactive protein (CRP), DAS 28 - disease activity score.
Methods. RF was determined by agglutination test (latex RF test, while kinetic methods were used for determination of alanine aminopeptidase (AAP) and γ-glutamyltransferase (γ-GT), as well as MEIA (microparticle enzyme immunoassay) to determine β2 microglobulin in urine. Samples (serum and urine) of 70 participants were examined (35 RA not treated, 35 healthy control group of patients).
Results. In 35 RF negative RA, AAP enzymuria was present in 12 (34.28%) patients, γ-GT was present in 7 patients (20%), while β2 microglobulin was present in 3 patients (8.57%). In the healthy control group, 4 patients showed AAP positivity (11.42%), 2 patients γ-GT positivity (5.71%) and 1 patient showed presence of 2 microglobulin in urine (2.85). RF was not detected in any patient (0%).
Conclusion. AAP has a higher sensitivity of γ-GT and β2 microglobulin in the detection of asymptomatic renal lesions in non-treated seronegative RA.

Keywords: alanine aminopeptidase, (AAP); γ-glutamyl transferase (γ--GT), β2 microglobulin (β2M); rheumatoid arthritis (RA), rheumatoid factor (RF)

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Апстракт

Вовед. Да се одреди ефектот на нелекуваниот серонегативен Реуматоиден артритис (РА) на проксималната ренална тубула, осетливоста на Аланин аминопептидаза (ААП), γ-глутамилтрансфераза (γ-ГТ), β2 микроглобулин во урина β2М, како и поврзаноста со реуматоидниот фактор (РФ) и Ц-Реактивниот протеин (ЦРП), ДАС 28 индексот на активноста на болеста.
Методи. РФ е одреден со тест за аглутинација (Латекс РФ тест, додека кинетичка метода за одредување на Аланин аминопептидаза (ААП) и γ-глутамилтрансфераза (γ-ГТ), како и МЕIA (Microparticle Enzyme Immunoassay) за одредување наβ2микроглобулин во урината. Ииспитани се примероци (серуми и урини) на 70 партиципанти, (35 РА не третирани, 35 контролна здрава група) кај истите партиципанти.
Резултати. Кај 35 РФ негативни РА, ААП ензимурија е присутна кај 12(34,28 %) пациенти, γ-ГТ е присутен кај 7 пациенти,(20%), додека β2 Микроглобулин е застапен кај 3 пациенти (8.57%).
Кај контролната здрава група, 4 пациенти покажа ААП позитивитет (11,42%), 2 пациенти γ-ГТ позитивитет (5,71%) и 1 пациент присуство на β2 Микроглобулин во урина (2.85). РФ покажа застапеност кај ниеден пациент (0%)
Заклучок. ААП има повисока сензитивност од γ-ГТ и β2 Микроглобулин во детекција на асимптоматски бубрежни лезии кај нетретиран серонегативен РА.

Клучни зборови: аланин аминопептидаза, (ААП), γ-глутамил трансфераза (γ-ГТ), β2 микроглобулин

β2M); ревматоиден артритис (РА), ревматоиден фактор (РФ)

Introduction

Enzymes in urine can derive from plasma, glands of the urogenital tract, epithelial cells of the urinary tract, leukocytes, erythrocytes [1] and kidneys. There are about 40 different enzymes [2-6] in the urine that belong to different groups: oxidoreductase, transferase, hydrolase, lyase, while isomerases and ligases are not found in the urine. The occurrence of such a large number of enzymes in the urine indicates the dominant role of kidneys in their excretion.
Examination of the cell membranes of the brush epithelium of proximal tubules confirms the localization of alanine aminopeptidase (AAP) in 90%, alaline phosphatase (AF) in 70% and γ-glutamyl transpeptidase (γ-GT) in 50% of the total activity of these enzymes in the kidney [7-9].

Aim

The aim of this study was to determine the effects of non-treated rheumatoid arthritis on tubular function; AAP, γ-GT and β2M being used as indicators of proximal tubular damage.

Materials and methods

In patients included in this study, disease diagnosis was based on the revised diagnostic criteria for classification of rheumatoid arthritis proposed in 1987 by the American Rheumatism Association (ARA) [10-13]. In order to be included in the RA group, patients had to meet at least 4 of the predicted 7 classification criteria. Criteria from 1 to 4 were present at least 6 weeks. The study included 35 patients (age 28, age 7) who were diagnosed with seronegative RA, as well as 35 patients (age 18, age 17) who served as a healthy control group. The mean age was 48.5 years (±4.13) (37-65 years) in the RA group, 36.2 years (±10.78) (29-65) in the healthy group. The average time of onset of disease in months from the beginning was 14.97 (± 15.23), in the interval of 1-14 months. None of the patients in the study had a history of previous or current renal impairment. The others negated use of other drugs before samples were taken. The samples were collected in one-year period.

Inclusion criteria

The study included patients with RA at the age of 18-65 years, who were not previously treated with NSAIDs or DMARDs.

Exclusion criteria

The study did not include patients with symptoms or conditions that could directly or indirectly affect the results, such as:

1. Patients with a history of gonorrhea, mild to moderate hepatic, renal, hematologic, cardiovascular, neurological diseases, nausea, vomiting, autoimmune disease.
2. Patients with diabetes mellitus, acute infections, malignant neoplasms, febrile conditions.
3. Patients with urinary tract arthritis, urinary tract infections, SLE, mixed connective tissue disease, vasculitis.
4. Patients with a history of blood transfusion, and excessive body weight.
5. Patients who received baseline therapy.
6. Patients with a history of glycemia or increased levels of product degradation in 0 range: serum creatine and urine, serum urea, hypertension, arterial hypertension and hematological and enzyme status.
7. Patients previously treated with salicylates, antibiotics, gold salts, or diuretics.
8. All paticipants voluntarily took part in this study.

Clinical assessment of disease activity

Clinical assessment and interpretation were made by a subspecialist in the given area. The disease activity was assessed using DAS 28 index. (Disease Activity Score - DAS 28) [14]. Indexes utilize mathematical formula by using a unique composite quantitative score consisting of palatable painfully sensitive joints (maximum number 28) and swollen joints (maximum number 28), global assessment of disease activity (0-100 mm Visual Analog Scale VAS), as well as morning stiffness (minutes). DAS 28 index ranges from 0 to 10 and score below 3.2 qualifies the disease as low active.

Laboratory assessment

For clinical assessment of the disease, it is necessary to consider the following laboratory variables: complete blood count (CBC) and differential, acute phase reactants, such as C-reactive protein (CRP), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), alkaline phosphatase (AF), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), urea/serum, creatinine/ serum.
Urine samples were taken not only for routine urinary examination, but also for determination of AAP, γ-GT, β2M.
Serum urea was determined by the Kassirer method..
Reference values: Serum urea (3-7.8 mmol / L).
Creatine in serum and urine and was determined by the Jaffe method.Reference values: Serum creatine 45 - 109 μmol / L; Creatine in urine 7 - 17 mol / dU.

C-reactive protein (CRP) was determined by the agglutination test (latex CRP test)
Reference values: < 6 mg / L CRP in serum
Rheumatoid factor (RF) was determined by the agglutination test (latex RF test)
Reference values: < 8 IU / ml in serum

Determination of alanine aminopeptidase activity (AAP): kinetic method
Reference values: AAP in urine 0.25-0.75 U/mmol creatinine

Determination of γ-glutamyltranspeptidase (γ-GT) activity: Ifcc method
Valuable referents- Reference values?
γ-GT (urine) 0.84-1.80 u / mmol creatinine

Determination of β2 microglobulin (β2M) concentration in urine: by MEIA (microparticle enzyme immunoassay) method
Reference values:
β2 microglobulin (urine) = 0.02-0.19 mg / L

Statistical analysis

For testing the significance of the differences between two arithmetic means, i.e., the corresponding proportions, Student's t-test was used when comparing the mean values of the given number of parameters between two groups, such as Wilcoxon- matched test for independent samples. Sensitivity and predictivity for positive and negative tests of the examined markers was determined with tests for sensitivity and specificity. The P value between 0.05 and 0.1 was considered statistically significant. The data processing was made with the statistical package Statistica 7.0.

Results

In the group of 35 patients with RA, RF seronegative RA, AAP enzyme was present in 12 (34.28%) patients, γ-GT was present in 7 patients (20%), while β2 microglobulin in urine was not present at all (0%).
In the healthy control group, 4 patients showed AAP positivity (11.42%), 2 patients γ-GT positivity (5.71%) and 1 patient presented with β2 microglobulin in urine (2.85). RF was not detected in any patient (0%)

AAP, γ-GT, β2M and DAS 28 Index of Disease Activity

In the group of 35 patients with RA, DAS 28>3.2 was present in 28 patients (80%).
In these 28 patients DAS 2>3.2, AAP positive were 10(35.71%) and their M±SD (1.25±0.43) range (0.85-2.46), γ-GT positive were 5 (17.85%) their M±SD (2.65±0.46) range (0.95-3.45), while β2M was not present in any patient.
In 7 seronegative RF patients with DAS 28 <3.2 (20%), Of these 7 patients, AAP was positive in 2 patients (28.57%) and their M±SD (1.20±0.49) range (0.80-2.30), γ-GT was positive in 2 patients (28.57%) and their M±SD (2.50±1.07), range (0.90-2.20). β2M was not detected in any patient.
1. Seronegative RF patients with DAS 28 >3.2 had higher AAP values than RF seronegative with DAS 28 <3.2. (1.25 (±0.43) *vs* 1.20 (±0.49), that had lower DAS 28 index. Between these 2 groups of AAP, there was no statistical correlation (p = 0.185017).
2. Seronegative RF patients with DAS 28>3.2 had a slightly higher value of γ-GT than RF seronegative with DAS 28<3.2. (2.65±0.46) *vs* (2.50±1.07). Between these 2 groups of γ-GT, there was no statistical correlation (p=0.670077); this group had larger γ-GT induction than seronegative RF patients with DAS 28<3.2. There was no statistical correlation between DAS 28 index in RF negative patients with DAS 28<3.2 and DAS 28>3.2 (p = 0.323).

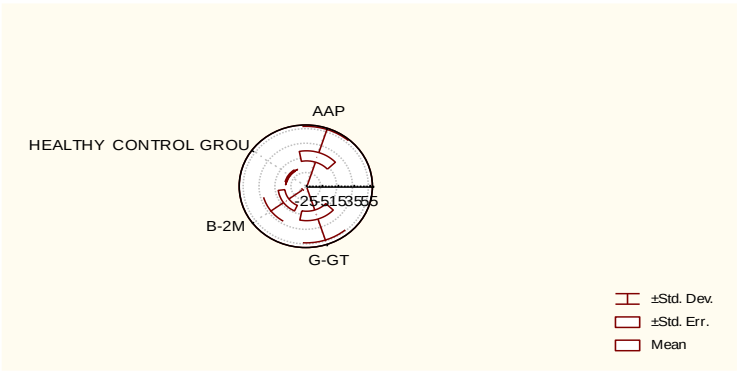

Fig. 1. Distribution of aline aminopeptidase (AAP), γ-glutamyl transferase (γ-GT), β2 microglobulin (urine) in rheumatoid arthritis

1. There was a statistical correlation using Wilcoxon-matched test between AAP in RA and healthy control group for p<0.05 (p=0.026113). Within the RA group,

there was a statistical correlation between AAP and γ-GT for p <0.05 (p=0.000003).
2. There was no statistical correlation using Wilcoxon-matched test between β2M in RA and healthy control group for p<0.05 (p=0.054759).
3. There was a statistical correlation using Wilcoxon-matched test between AAP and γ-GT in RA and age, disese duration in months, CRP, SER, morning stiffness, serum creatine, urine creatine and serum urea in the same group for p<0.05: (AAP *vs* Age, p=0.000000; AAP *vs* Disease duration in months, p=0.000000, AAP *vs*,. CRP, p=0.040620; AAP, γ-GT *vs* SER, p=0.000000; AAP, γ-GT *vs* morning stiffness, p=0.000010; AAP, γ-GT *vs* serum creatine, p=0.000000; AAP, γ-GT *vs* creatine in urine, p=0.000000; AAP, γ-GT, *vs* serum urea, p=0.000000).

Discussion

In standard medical rheumatology, the greatest emphasis is put on rheumatoid arthritis as the most exposed/ common disease. Seronegative RA is a rare form, difficult to be recognized and most often confused with degenerative rheumatism, probably due to its frequency.
Urinary enzyme activity is normally low and increases when renal tubular cells are excreted [15]. Urinary enzymes, especially NAG, AAP, AF are very sensitive indicators of parenchymal renal damage in comparison with functional measurements such as glomerular filtration rate (GFR), creatinine and inulin clearance. The relatively low sensitivity of the GFR can be explained by the large renal functional reserve and its large capacity for compensation [16]. Elevations in urinary enzyme activity may indicate the location of the primary renal tubular damage due to its localization in the brush border area (microsomal AAP) and tubular lysozyme (NAG). They can be used in early diagnosis of acute renal failure because nephrotoxicity is induced by immunosuppressive drugs, contraceptives, antibiotics and cadmium exposure [17-19].
The sensitivity of AAP is higher in comparison with γ-GT and β2M. Other standard routine tests used to assess renal function show low sensitivity: creatine in serum and urine, urea in serum. Seronegativity has an impact on the occurrence of AAP enzymuria. This is also valid for seronegative patients with DAS 28 > 3.2 who have a much larger AAP induction than DAS 28 < 3.2. The statistical correlation of disease duration in months indicates that the non-treated RA affects kidney tissue as one of the visceral manifestations of disease.
Non-treated RA primarily affects tubular brush border area and enzymes that derive from this area have increased sensitivity.

Conclusion

AAP has a higher sensitivity than γ-GT and β2M in the detection of asymptomatic renal lesions in the non-treated seronegative RA. AAP and γ-GT can be used in the everyday clinical practice to diagnose early, asymptomatic renal lesions.

Conflict of interest statement. None declared.

References

1. Chiu JSP. Models used to asses renal function. *Drug Devel Res* 1994; 32: 247-255.
2. .Portman RJ, Kissane JM, Robson AM. The use of beta 2-microglobulin to diagnose tubular injury in pediatric renal disease. *Kidney Int* 1986; 30: 91-98.
3. .Hong CY,Chia KS. Markers of diabetic nephropathy. *J Diabetes Complications*1998; 12: 43-60.
4. Sherman RL, Drayer ED, Leyland-Jones BR, *et al*. N-Acetyl-b-glucosaminidase and b-2 microglobulin. *Arch Intern Med* 1983; 143: 1183-1185.
5. Hultberg B, Ravnskov U. The excretion of N-acetyl-beta-glucosaminidase in glomerulonephritis. *Clin Nephrol* 1981; 15: 33-38.
6. Johnston IDA, Jones NF, Scoble JE, *et al*. The diagnostic value of urinary enzyme measurements in hypertension. *Clin Chim Acta* 1983; 133: 317-325.
7. Sanberg T, Bergmark J, Hultberg B, *et al*. Diagnostic potential of urinary enzymes and beta-2-microglobulin in acute urinary tract infection. *Acta Med Scand* 1986; 219: 489-495.
8. A Wiela - Hojenska, M Hurkacz. The significance of beta 2-microglobulin in diagnosis and therapy. *Postery Hig Med Dosc* 1998: 52(5): 507-514.
9. Wellwood JM, Ellis BG, Price RG, *et al*. Using N-acetil Beta-D-glocosaminidase activities in patients with renal disease. *Brit Med J* 1975; 139: 408.
10. Hofstra JM, Joreon KJ Deegens, Hans L Wilems, Jack FM Wetzels. Beta-2-microglobulin is superior to N-acetyl-beta-glucosaminidase in predicting prognosis in idiopathic membranous nephropathy. *Nephrol Dial Transplant* 2008; 23(8): 2546-2551.
11. Arnett FC, Edworthy Sm, Bloch Da, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-324.
12. Van Gestel AM, Prevoo MLL, van't Hof MA, *et al*. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. *Arthritis Rheum* 1996; 39: 34-40.
13. Prevoo ML, van't Hof MA, Kuper NH, *et al*. Modified disease activity scores that include 28-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-48.
14. Balsa A, Carmona L, González-Álvaro I, *et al*. Value of DAS-28 and DAS 28-3 as compared to ACR-defined remission in rheumatoid arthritis. *J Rheumatol* 2004; 31: 40-46.
15. Prevoo MLL, van Gestel AM, van't Hof MA, *et al*. Remission in a prospective study of patients with rheumatoid arthritis. American Rheumatology Association preliminary remission criteria in relation to the disease activity score. *Br J Rheumatol* 1996; 35: 1101-1105.
16. Maruhn D, Paar D, Bock KD. Lysosomal and brush border membrane enzymes in urine of patients with renal

artery stenosis and with essential hypertension. *Clin Biochem* 1979; 12: 228-230.
17. Johnston IDA, Jones NF, Scoble JE, *et al*. The diagnostic value of urinary enzyme measurements in hypertension. *Clin Chim Acta* 1983; 133: 317-325.
18. Clinical practise guidelines for chronic kidney disease: evaluation,classification and stratification. *Am J Kidney Dis* 2002; 39(Suppl1): S1-S266.
19. Williams RC, Nissen MH, Malone CC. Rheumathoid factor from patient with rheumathoid arthritis react with Des-Lus[58] - beta-2- microglobulin, modified beta-2-microglobulin. *Clin Exp Immunol* 1993; 92: 419-424.
20. Viergever PP, Swaak AJG. Urine-and serum beta-2 microglobulin in patients with rheumathoid arthritis: a stydy of 101 patients without sign of kidney disease. *Clin Rheumatol* 1989; 8: 368-374.

Original article

TREATED PATIENTS AND COMPLETED HOSPITAL DAYS OF PATIENTS WITH RESPIRATORY DISEASES AND TUBERCULOSIS IN THE YEARS 2020 AND 2021 IN THE SKOPJE REGION

ЛЕКУВАНИ БОЛНИ И ОСТВАРЕНИ БОЛНИЧКИ ДЕНОВИ ЗА БОЛНИТЕ СО РЕСПИРАТОРНИ БОЛЕСТИ И ТУБЕРКУЛОЗА ЗА 2020 И 2021 ГОДИНА ВО СКОПСКИОТ РЕГИОН

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Abstract

Introduction. Hospitals should reflect the needs and values of the communities in and around them, while at the same time being resilient and capable to maintain and scale up services in emergency situations. Although public health approaches against tuberculosis have saved tens of millions of lives, a modest progress has been made to control tuberculosis.
Aim. The aim of this study was to show the number of people suffering from respiratory diseases and tuberculosis in the Skopje region in 2020 and 2021 and the number of days spent at the Institute for Lung Diseases and Tuberculosis - Skopje.
Methods. Data used in this study were derived from the Inpatient Report (form no. 3-21-60A), which is regularly filled out in hospitals for each discharged patient. The statistical-informative method and descriptive analysis were performed.
Results and discussion. A total of 17 people were treated for tuberculosis at the Institute for Lung Diseases and Tuberculosis - Skopje during 2021, of which five were women and 12 men. They completed a total of 405 days of hospitalization or had an average treatment duration of 26.2 days, holing the first place in relation to the others treated in this facility. In 2020, a total of 60 tuberculosis patients were hospitalized. Of them, 44 men and 16 women. They completed a total of 2093 days, or the average treatment duration was 35 days.
Conclusion. The situation with tuberculosis in the Skopje region follows the general condition in the country with a downward trend of 38.3% for those hospitalized at the Institute of Lung Diseases and Tuberculosis in 2021 compared to 2020. This trend follows the number of completed hospital days and the average treatment duration from 35 days in 2020 to 26.2 days in 2021.

Keywords: tuberculosis, hospitalized, average treatment duration

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Апстракт

Вовед. Болниците треба да ги одразуваат потребите и вредностите на заедниците во и околу нив, а истовремено да бидат еластични и способни да одржуваат и да ги зголемат услугите во итни ситуации. Иако јавно-здравствените пристапи против туберкулозата имаат спасено десетици милиони животи, постигнат е скромен напредок за да се контролира туберкулозата.
Цел. Целта на ова истражување е да се прикаже бројот на заболени од респираторни болести и туберкулоза во Скопскиот регион за 2020 и 2021 година и бројот на остварени денови во Институтот за белодробни болести и туберкулоза - Скопје.
Методи. Основен материјал за работа во ова истражување е Извештајот за стационарно лекувано лице (образец бр. 3-21-60А), кој редовно се пополнува во болниците за секој испишан болен. Методот на работа е статистичко-информативен со направена дескриптивна анализа.
Резултати и дискусија. Во Институтот за белодробни болести и туберкулоза - Скопје во текот на 2021 година од туберкулоза се лекувале вкупно 17 лица, од кои пет жени и 12 мажи. Истите остварile вкупно 405 денови на хоспитализација или имале просечно траење на лекување од 26,2 дена. Тие се наоѓаат на прво место во однос на останатите лекувани во оваа установа. Во 2020 година хоспитализирани биле вкупно 60 пациенти од туберкулоза. Од нив 44 мажи и 16 жени. Тие остварile вкупно 2093 денови или просечното траење на лекување било 35 дена.
Заклучок. Состојбата со туберкулоза во Скопскиот регион ја следи општата состојба во државата со тренд на опаѓање од 38,3% за хоспитално лекуваните во Институтот за белодробни заболувања и туберкулоза во 2021 во однос на 2020 година. Овој тренд

го следи бројот на остварени болнички денови и просечното траење на лекување од 35 дена во 2020 до 26,2 дена во 2021 година.

Клучни зборови: туберкулоза, хоспитално лекувани, просечно траење на лекување

Introduction

Respiratory diseases often lead to disability and premature death. They are the reason for high costs in primary and hospital health care, and they also reduce the productivity of patients since they cannot work and die prematurely. The consequences for societies and economies are devastating, but they hit hardest the poor, vulnerable and at-risk populations, who get sick earlier and die earlier than their peers in wealthier societies. Tuberculosis (TB) continues to cause significant morbidity and mortality globally. WHO estimates that 10 million people got infected by tuberculosis in 2017; 8.7 million (87%) of these people live in 30 countries with very high numbers of cases. Among these 10 million infected, only 6.4 million have been diagnosed and officially notified. An estimated 1.3 million people die from tuberculosis each year. Tuberculosis is a disease of poverty. Although most developed countries have an estimated TB incidence of less than 10 per 100,000 population per year, the 30 countries with high TB burden (which are mostly low-developed countries) have an estimated collective TB incidence of 183 per 100,000 population per year, with an incidence of over 400 per 100,000 inhabitants per year in eight countries [1]. In all countries, the burden of tuberculosis is also primarily borne by the poorest people [2]. The global tuberculosis incidence is estimated to be slowly declining by 1.6% per year, well short of the target of 4-5%, according to the WHO's End TB Strategy [5]. Worldwide Disease, Injury and Risk Factors in Tuberculosis data (1990-2016) show that few nations are expected to meet the UN Sustainable Development Goals for eliminating the epidemic by 2030 if current incidence trends persist [3]. Humans and *Mycobacterium tuberculosis* have lived together for thousands of years. Although approximately 1 billion people worldwide are thought to have *Mycobacterium tuberculosis* in them, only some of these individuals will develop active tuberculosis. Beyond the conventional model of distinct latent and active forms of tuberculosis disease, there is a growing acceptance that the pathology of tuberculosis disease falls on a spectrum/is due to complex bacterial and host dynamics. This understanding of the pathophysiology of tuberculosis is still evolving [4]. Individual immunity to TB also appears to change over time, even within a single human host. According to a recent study, immune responses within individual granulomas indicate that systemic immunity and local immune responses at the site of infection play a significant role in preventing tuberculosis infection [5]. In addition, data show that some people exposed to tuberculosis do not get the disease, while others, after even a small contact, go from infection to disease very quickly. According to studies of treatment resistance in tuberculosis, a large number of patients who develop drug-resistant tuberculosis are likely to be infected with drug-resistant strains [6]. According to some data, other factors, such as ineffective serum drug concentrations, drug concentrations in pulmonary tissue, and the presence of drug efflux pumps on the surface of bacteria, may contribute more to the development of acquired drug resistance than nonadherence to the recommended drug regimen with antibiotics [7]. The new studies also demonstrate the temporal evolution of the disease after infection. Although it has long been accepted in the scientific community that individuals newly infected with *M. tuberculosis* are at greatest risk for developing the disease within the first few years of infection, analysis of historical data has confirmed that the incubation period for *M. tuberculosis* is probably shorter than previously believed, of about 24 months [8]. Some biomarkers give hope for locating people most at risk of progression [9]. TB remains one of the world's deadliest infectious killers. Every day, over 4,000 people lose their lives to TB and nearly 30,000 people contract this preventable and curable disease. Global efforts to combat TB have saved an estimated 66 million lives since 2000. For the first time in more than a decade, TB deaths increased in 2020. What is remarkable about tuberculosis and its sister infectious killers - HIV, malaria, and bacterial pneumonia - is that they cause the greatest misery and death among the young, young adults in cases of tuberculosis and HIV and children in cases of malaria and bacterial pneumonia. In countries with a high prevalence of tuberculosis, premature mortality has a disproportionately negative effect on family life, social structure, community institutions and the economic output of nations. WHO estimates that infectious diseases account for 52% of all years of life lost (premature mortality) in the world, while non-infectious diseases constitute solely 34%22.

The most common source of infection is the sputum of a tuberculosis patient. The percentage of infected and the degree of illness depends on the number and amount of bacilli in the air and the length of exposure (in the home permanently or temporarily, outside the home). Although the described cases of acquired infection are in a very short time interval of contact with the patient, a significantly longer time of exposure is still required for the infection to occur.

Aim

The aim of this study was to show the number of people suffering from respiratory diseases and tuberculosis in the Skopje region for 2020 and 2021 and the number of days spent at the Institute for Lung Diseases and Tuberculosis - Skopje.

Material and methods

Data used in this study was the Inpatient Report (form no. 3-21-60A), which is regularly filled out in hospitals for each discharged patient. The statistical-informative method and descriptive analysis were performed.

Results and discussion

According to the results obtained, in 2020, a total of 145 people suffering from chronic lower respiratory diseases were treated at the Institute for Lung Diseases and Tuberculosis, coming first on the list. Second were patients with influenza and pneumonia, or 76 patients who completed a total of 833 days and an average treatment duration of 11 days. Third on the list were patients hospitalized with tuberculosis, a total of 60, fourth - patients with diseases of the pleura (24) and in the fifth place were neoplasms of uncertain or unknown cause [10]. Patients with neoplasms spent a total of 84 days and stayed in the hospital for an average of 7.6 days. A total of 26 Skopje residents suffered from tuberculosis and were treated at the Institute for Lung Diseases and Tuberculosis in 2020. They stayed in the hospital as inpatients for an average of 40 days.
Tuberculosis of the respiratory system was prevalent in the entire period of analysis with 75% to 88% of the total number of cases. In a much smaller percentage, tuberculosis was diagnosed on meninges, urogenital organs, glands, bone-joint system, etc.

Table 1. Treated patients and completed hospital days according to disease blocks, sex and facility

Block code	Disease block	Total			Skopje residents			Citizens of Macedonia			Citizens of other countries		
		LB	BD	PTL	LB	BD	PTL	LB	BD	PTL	LB	BD	PTL
J40-J47	***Chronic lower respiratory diseases***												
	Men	85	1.169	13.75	66	644	9.76	19	525	27.63	0	0	0
	Women	60	553	9.22	50	450	9	10	103	10.30	0	0	0
	Total	145	1722	11.88	116	1094	9.43	29	628	21.66	0	0	0
J10-J18	***Influenza and Pneumonia***												
	Men	39	407	10.44	23	262	11.39	16	145	9.06	0	0	0
	Women	37	426	11.51	24	301	12.54	13	125	9.62	0	0	0
	Total	76	833	10.96	47	563	11.98	29	270	9.31	0	0	0
A15-A19	***Tuberculosis***												
	Men	44	1.666	37.86	20	869	43.45	24	797	33.21	0	0	0
	Women	16	427	26.69	6	173	28.83	10	254	25.40	0	0	0
	Total	60	2093	34.88	26	1042	40.08	34	1051	30.91	0	0	0
J90-J94	***Other pleura diseases***												
	Men	18	189	10.50	3	33	11	15	156	10.4	0	0	0
	Women	6	64	10.67	6	64	10.67	0	0	0	0	0	0
	Total	24	253	10.54	9	97	10.78	15	156	10.4	0	0	0
D37-D48	**Neoplasms of uncertain and unknown reason**												
	Men	6	38	6.33	2	14	7	4	24	6	0	0	0
	Women	5	46	9.20	1	11	11	4	35	8.75	0	0	0
	Total	11	84	7.64	3	25	8.33	8	59	7.38	0	0	0

Source Center for Public Health – Skopje

In 2021, a total of 17 tuberculosis patients were treated at the Institute for Lung Diseases and Tuberculosis, coming first on the list. Second were influenza and pneumonia, with a total of 15 treated patients and 176 completed days. In the third place were chronic lower respiratory diseases, a total of 9, while two patients were treated with malignant neoplasms of the respiratory and interthoracic organs. Fourth were symptoms and signs related to the circulatory and respiratory system, but with an undefined diagnosis and with two treated patients. A total of 12 Skopje residents suffered from tuberculosis and were hospitalized in 2021. They stayed in the hospital for an average of 29 days.

In general, the occurrence of this chronic infectious disease depends on many factors, mostly on the living conditions. Therefore, population controls are regularly carried out in detention and rehabilitation centers, psychiatric hospital wards and among the Roma population. Accordingly, the following number of cases were registered:

- 2 cases in the detention and rehabilitation centers in 2019;
- 3 cases in psychiatric hospital wards;
- among the Roma population (both in 2018 and 2019), a total of 16 cases were registered.

Table 2. Treated patients and completed hospital days according to disease blocks, sex and facility

Block code	Disease block	Total			Skopje residents			Macedonia residents			Other countries residents		
		LB	BD	PTL	LB	BD	PTL	LB	BD	PTL	LB	BD	PTL
A15-A19	***Tuberculosis***												
	Men	12	337	28.08	8	257	32.13	4	80	20	0	0	0
	Women	5	108	21.6	4	91	22.75	1	17	17	0	0	0
	Total	17	445	26.18	12	348	29	5	97	19.4	0	0	0
J10-J18	***Influenza and Pneumonia***												
	Men	9	112	12.44	8	100	12.5	1	12	12	0	0	0
	Women	6	64	10.67	4	43	10.75	2	21	10.5	0	0	0
	Total	15	176	11.73	12	143	11.92	3	33	11	0	0	0
J40-J47	***Chronic lower respiratory diseases***												
	Men	5	48	9.6	4	34	8.5	1	14	14	0	0	0
	Women	4	35	8.75	4	35	8.75						
	Total	9	83	9.22	8	69	8.63	1	14	14	0	0	0
R00-R09	***Symptoms and Signs regarding the Circulation and Respiratory System***												
	Men	1	11	11	1	11	11	0	0	0	0	0	0
	Women	1	2	2	1	2	2	0	0	0	0	0	0
	Total	2	13	6.5	2	13	6.5	0	0	0	0	0	0
C30-C39	***Malign Neoplasms of the Respiratory and Intrathoracic Organs***												
	Men	2	19	9.5	2	19	9.5	0	0	0	0	0	0
	Women	0	0	0	0	0	0	0	0	0	0	0	0
	Total	2	19	9.5	2	19	9.5	0	0	0	0	0	0

Source Center for Public Health – Skopje

The Republic of North Macedonia is among the countries with a low tuberculosis incidence rate compared to the countries in the European region. The number of active tuberculosis patients shows a decreasing trend. Over the years, a significant decrease in the prevalence of tuberculosis has been observed in RNM, which means that the state campaigns fighting this disease are successful. In 2010, there were a total of 592 people sick with tuberculosis in the country and the prevalence rate was 28.8 per 100,000 inhabitants. In the coming years, the prevalence of sick people dropped significantly, so in 2019 it was10.4 per 100,000 inhabitants, which was a decrease of 2.8 times.

Conclusion

1. The number of treated tuberculosis patients in the analyzed period decreased by 38.3%.
2. The average treatment duration of tuberculosis patients decreased by 25.1%.
3. Tuberculosis is mainly concentrated in conditions of poverty and other social and economic challenges and in the most vulnerable populations. Poverty, malnutrition, poor living and working conditions, among others, affect how people get sick, develop TB and cope with treatment demands (including medical, financial and social) and affect the health outcomes they face.
4. Coordinated action by all sectors is required in order to stop tuberculosis onset, by providing right services, support and enabling a safe environment in the right place, at the right time.
5. It is necessary to reduce the percentage of people dying from this disease, from the predicted 15% in 2015 to 6.5% by 2025.
6. Procurement of first-line antituberculosis drugs to treat newly registered cases of tuberculosis. This activity aims to prevent the spread of tuberculosis by treating newly detected cases of tuberculosis. Procurement of second-line antituberculosis drugs for the treatment of patients with resistant forms of tuberculosis. This activity aims to prevent the spread of resistant forms of tuberculosis, the treatment of which is time-consuming, expensive and often unsuccessful.

Conflict of interest statement. None declared.

References

1. Lonnroth K, Jaramillo E, Williams BG, *et al*. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. *Soc Sci Med* 2009; 68: 2240-2246.
2. GBD Tuberculosis Collaborators. The global burden of tuberculosis: results from the Global Burden of Disease Study 2015. *Lancet Infect Dis* 2018; 18: 261-284.
3. GBD Tuberculosis Collaborators. Global, regional, and national burden of tuberculosis, 1990-2016: results from the Global Burden of Diseases, Injuries, and Risk Factors 2016 Study. *Lancet Infect Dis* 2018; 18: 1329-1349.
4. Torrelles J, Schlesinger L. Integrating lung physiology, immunology and tuberculosis. *Trends Microbiol* 2017; 25: 688-697. 18 S.
5. Shah NS, Auld S, Brust J, *et al.* Transmission of extensively drug-resistant tuberculosis in South Africa. *N Engl J Med* 2017; 376: 243-253.
6. Dheda K, Lenders L, Magombedze G, *et al.* Drug penetration gradients associated with acquired drug resistance in patients

with tuberculosis. *Am J Respir Crit Care Med* 2018; 198: 1208-1219.
7. Behr M, Edelstein P, Ramakrishnan L. Revisiting the time table of tuberculosis. *BMJ* 2018; 362: K2736.
8. Zak DE, Penn-Nicholson A, Scriba TJ, *et al*. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet* 2016; 387: 2312-2322.
9. Saunders MJ, Wingfield T, Tovar MA, *et al*. A score to predict and stratify risk of tuberculosis in adult contacts of tuberculosis index cases: a prospective derivation and external validation cohort study. *Lancet Infect Dis* 2017; 17: 1190-1199.

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Original article

VACCINE BREAKTHROUGH INFECTIONS IN COVID-19 PATIENTS - SINGLE CENTER STUDY IN THE REPUBLIC OF NORTH MACEDONIA

КОВИД-19 ИНФЕКЦИИ НА ПРОБИВ КАЈ ВАКЦИНИРАНИ ПАЦИЕНТИ - СТУДИЈА ОД ЕДЕН ЦЕНТАР ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

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Abstract

Our study evaluates vaccine breakthrough infections in Coronavirus Disease 2019 (COVID-19) patients who presented for medical examination at a tertiary care hospital in Skopje, Republic of North Macedonia. We retrospectively evaluated medical files of 249 completely vaccinated patients who presented at the hospital since June 2021 till October 2021, with a clinical picture of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. The average time from complete vaccination to symptom onset was 79.8±41.8 days. Out of 249 patients, 158(63.45%) were treated as outpatients, and 91(36.55%) were hospitalized. From the hospitalized patients, 61(67.03%) were discharged and 30(32.97) died. Breakthrough infections occurred in the Sinopharm vaccine group in 45.78%, Sinovac in 20.08%, Pfizer in 14.86%, AstraZeneca in 10.84% and Sputnik in 7.23%. The highest mortality was found in patients vaccinated with mRNA1273 vaccine, followed by inactivated virus containing vaccine and with non-replicating viral vector vaccine, while the lowest mortality was found in those vaccinated with either BNT162b2 vaccine or human adenovirus vector-based COVID-19 vaccine. Male gender (p=0.006), age over 65 years (p=0.002) and presence of comorbidities (p=0.006) were major contributing factors for a poor outcome in vaccinated hospitalized patients with COVID-19. Due to the uneven distribution of the samples in our patient cohort it would be misleading to look at breakthrough cases, disease severity and outcome by vaccine brand due to different representation of vaccine brands. Breakthrough infection, hospitalization, and death from COVID -19 could differ across different vaccination profiles.

Keywords: COVID-19, SARS-CoV-2, vaccination, breakthrough infection, outcome

Апстракт

Студијата ги евалуира инфекциите на вакцинален пробив кај Корона вирусната болест 2019 (КОВИД-19) кај пациенти кои се појавиле на преглед во терциерна болница во Скопје, Република Северна Македонија. Ретроспективно се евалуирани медицинските истории на 249 комплетно вакцинирани пациенти кои дошле на преглед на Клиниката за инфективни болести и фебрилни состојби, Скопје во периодот јуни 2021 до октомври 2021 година со клиничка слика на акутен тежок респираторен коронавирус 2 синдром (САРС-КоВ-2 инфеција). Средното време после комплетна вакцинација до појава на симптоми на болеста изнесуваше 79.8±41.8 дена. Од вкупно 249 пациенти, 158(63.45%) беа третирани амбулантски, додека 91 (36.55%) беа хоспитализирани. Од хоспитализираните пациенти 61(67.03%) оздравеа, додека (32.97%) починаа. Кај пациентите вакцинирани со Sinopharm инфекции на вакцинален пробив имало кај 45.78%, со Sinovac кај 20.08%, Pfizer кај 14.86%, AstraZeneka кај 10.84%, со Sputnik кај 7.23%. Највисока смртност имале пациентите вакцинирани со mRNA1273 вакцината, потоа вакцинираните со вакцини кои содржат инактивиран вирус и не-репликативна вирусна векторна вакцина, додека најмала смртност е утврдена кај пациентите вакцинирани или со BNT162b2 вакцината или со хумани аденовирус базирана КОВИД-19 вакцината. Машкиот пол (p=0.006), возраст над 65 години (p= 0.002) и присуство на коморбидитет (p=0.006) се главни фактори за смртен исход кај вакцинираните, хоспитлизирани пациенти со КОВИД-19. Како резултат на нееднаквата распределност на примерокот кај пациентите, би било погрешно да се евалуира појавата на вакцинален пробив, тежината и исходот на болеста според типот на вакцината, поради различната застапеност на истите. Инфекциите на вакцинален пробив, хоспитализацијата и смртноста поради КОВИД-19 можат да бидат различни кај различни профили на вакцини.

Клучни зборови: КОВИД-19, САРС-КоВ-2, вакцина-

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ција, инфекции на вакцинален пробив, исход

Introduction

With the appearance of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, first detected in Wuhan, China causing highly infectious Coronavirus Disease 2019 (COVID-19), a new global pandemic was unleashed spreading worldwide [1]. Numerous global efforts had been undertaken since the beginning of the pandemic in order to reduce the virus transmission and mortality via different measures including social distancing, wearing facemasks, hand hygiene and restricting interpersonal contact to outdoor settings; widespread testing to identify individuals infected with the virus; different governmental actions including school and workplace closures, bans on public gatherings, travel restrictions and stay-at-home orders in order to mitigate the pandemic [2]. Despite all the measures and efforts, SARS-CoV-2 continues to spread causing very high morbidity (above 225 million confirmed cases) and mortality (more than four and a half million deaths) worldwide as of September 15, 2021 [3]. The development of safe and efficacious vaccine against SARS-CoV-2 was the only possible way to fight the virus and to prevent its spread, together with effective therapy for COVID-19 patients [4]. Recently published meta-analysis of eight COVID-19 vaccines, that have published the data of phase 3 randomized controlled trials (RCTs), reported excellent efficacy (pooled Risk Ratio (RR) to prevent symptomatic disease of 0.17; 95% Confidence Interval (CI): 0.09-0.32)[5]. People who are fully vaccinated against COVID-19 have a significantly reduced risk of severe illness but despite the high level of vaccine efficacy some hospitalizations and deaths have been reported even in fully vaccinated people with breakthrough COVID-19 infections [6-11]. In the Republic of North Macedonia, the vaccination campaign started in February 2021. Vaccination process stared with the vaccination of medical personnel, elderly, as well as immunocompromised individuals as priority target groups [12]. The first vaccines used against SARS-CoV-2 infection in the Republic of North Macedonia were Bnt162b2 (Pfizer/BionTec) vaccine, COVID-19 Vaccine (Vero Cell), Inactivated/Coronavac™ (Sinovac), SARS-CoV-2 Vaccine (Vero Cell), Inactivated (lnCoV) (Sinopharm) followed by Sputnik V and AZD1222 Vaxzevria (AstraZeneca). At the time when this study was conducted, October 2021, the distribution of the vaccines was as presented in Table 1 [13]. By October 2021, only 38% of the population (2.083 million by the census of year 2020) were completely vaccinated, 35% had received only one dose of vaccine, and 53% of individuals over 40 years of age were completely vaccinated, whereas 33% of the population aged 18-39 years were vaccinated with one dose of vaccine [14]. The aim of this study was to evaluate vaccine breakthrough infections and outcome in patients with SARS-CoV-2 breakthrough infection.

Table 1. Number of vaccinated persons in Republic of North Macedonia

Vaccine	First dose	Second dose	Third dose	Grand Total
ChAdOx1-S recombinant, AZD1222 - AstraZeneca COVID-19 Vaccine	66.142	61.000		127.142
COVID-19 vaccine (Vero Cell), Inactivated (Sinovac)	247.227	235.541		482.768
Gam-COVID-VAC (Sputnik V)	22.912	22.549		45.461
Pfizer - BioNTech COVID-19 Vaccine	309.511	285.073	894	595.478
SARS-CoV-2 Vaccine (Vero Cell), Inactivated (Sinopharm)	150.606	149.337	1	299.944
Grand Total	796.398	753.500	895	1.550.793

Material and methods

A retrospective cohort study was undertaken in the period between June 2021 till October 2021 at the University Clinic for Infectious Diseases and Febrile Conditions, Skopje, Republic of North Macedonia. The study included 249 patients with breakthrough infections that had come for a medical checkup at the Clinic due to symptoms of COVID-19 disease (fever, sore throat, headache, fatigue, cough, nasal congestion, ageusia or anosmia). All participants were fully vaccinated with one of the available vaccines in the Republic of North Macedonia with two doses in an interval period as recommended by the manufacturer: Bnt162b2 (Pfizer/BionTec) vaccine, COVID-19 Vaccine (Vero Cell), Inactivated/Coronavac™ (Sinovac), SARS-CoV-2 Vaccine (Vero Cell), Inactivated (lnCoV) (Sinopharm), Sputnik V and AZD1222 Vaxzevria (AstraZeneca). Patients were considered fully vaccinated if the final dose of the vaccine was administered at least 14 days

before symptom onset or a positive RT-PCR (reverse transcriptase-polymerase chain reaction) test for SARS-CoV-2. Breakthrough infections were detected by nasopharyngeal swabs, obtained at any point if a patient had suggestive symptoms for COVID-19 as described above. The swabs were obtained by trained physicians and RT-PCR test for COVID-19 detection was done using either one of the following test: 2019-nCoV "Allplex™, 13BGI, 14 Nucleic Acid Diagnostic Kit"-Sansure Biotech, 15, Charite-Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR,16 RealTime SARS-CoV-2"-EUROIMMUN, 17 TaqMan 2019-nCoV Assay Kit v1,18SARS-CoV-2 Fluorescent PCR"-Maccura 19 "TaqPath™ COVID-19 CE-IVD RT-PCR Kit", 20 SARS-CoV-2/Influenza Multiplex DNA-Technology, 21 Genrui SARS-CoV-2 Detection Kit RT-PCR22, according to manufacturers' protocol.
Inclusion criteria for the patients were evidence for complete vaccination against SARS-CoV-2 infection, positive nasopharyngeal swabs for SARS-CoV-2 infection and age of 18 years or older. Complete vaccination was defined as a period of at least two weeks after receiving two doses of a given vaccine in a time period as described by the manufacturer. Exclusion criteria: pregnancy and age bellow 18 years.
Demographic data, chronic medical conditions, vaccine type, severity of COVID-19 and outcome of the patient were recorded.

Statistical analysis

Kolmogorov-Smirnov test was used to verify the normality of distribution of continuous variables. Categorical variables were expressed as numbers and percentages and analyzed using the chi-square and Fisher exact test when necessary. Normally distributed variables are presented as mean (SD) and non-normally distributed variables as median and range. Difference testing between groups was performed using the Student's t-test when data were normally distributed. When normality was rejected, nonparametric Mann-Whitney U-test was used for independent groups. Data were analyzed with SPSS 24.0 software (SPSS, Chicago, IL).

Results

We evaluated the medical files of 249 completely vaccinated patients who presented with a clinical picture of SARS CoV-2 infection, confirmed by a positive nasopharyngeal swab at the University Clinic for Infectious Diseases, in Skopje, Republic of North Macedonia, since June 2021 until October 2021. This is the only tertiary care hospital for infectious diseases in the whole country. The average time from complete vaccination to symptom onset was 79.8±41.8 days. Out of 249 completely vaccinated patients with breakthrough infection 123(49.4%) were male, and 126(50.6%) were female. In our cohort, 125 patients (50.2%) were younger than 65 years, and 124(40.8%) were 65 years and older. In terms of comorbidities, 172 patients (69%) had one and/or more comorbidities, and 77 patients (31%) were without any comorbidity. General characteristic of analyzed patients are presented in Table 2.

Table 2. General characteristics of analyzed patients

Variable	All patients n=249	Survivors n=219 (87.9%)	Nonsurvivors n=30 (12.1%)	P value
Male, n (%)	123(49.4)	101(82.1)	22(17.9)	0.006
Female, n (%)	40(48.2)	118(93.7)	8(6.3)	
Age (years)				
<65, n (%)	125(50.2)	118(94.4)	7(5.6)	0.002
≥ 65, n (%)	124(49.8)	101(81.5)	23(18.5)	
Without comorbidity n (%)	77(33)	74(33.8)	3(10.0)	
With comorbidity n (%)	172(69)	145(66.2)	27(90.0)	0.006

According to the vaccine brand, most vaccine breakthrough infections occurred in the Sinopharm (45.78%) and Sinovac group (20.08%), followed by those vaccinated with Pfizer (14.86%), AstraZeneca (10.84%) and Sputnik (7.23%) (Table 3). Although at the time of this study the Republic of North Macedonia did not have mRNA-1273 (Moderna) vaccine, the three patients vaccinated with Moderna vaccine and included in our study were vaccinated in third EU countries and had come during the summer holidays for visiting their relatives in North Macedonia when illness occurred. The absolute number and percentages of breakthrough infection by vaccine brand are as presented in Table 3.

Table 3. Breakthrough infections of SARS CoV-2 by different vaccine brand

Vaccine brand	Frequency	Percent
Sinopharm	114	45.8
AstraZeneca	27	10.8
Pfizer	37	14.9
Sputnik	18	7.2
Sinovac	50	20.1
Moderna	3	1.2
Total	249	100.0

After examination and admission to the hospital, patients were categorized according to COVID-19 disease severity classification as per the WHO definition [15].

From the 249 vaccinated patients who were examined, 53(21.3%) had severe/critical form of COVID-19, 65 (26.1%) had moderate and 131(52.6%) had mild form of the disease. Among those with severe and/or critical illness, the mean age was 71.2±9.8 years. In the group of patients with severe and/or critical illness 27(50.94%) patients survived and 26 (49.06%) died. Distribution of disease severity according to vaccine brand is presented in Table 4.

Table 4. Disease severity in breakthrough infection of SARs CoV-2 according to vaccine brand

		Sinopharm	AstraZeneka	Pfizer	Sputnik	Sinovac	Moderna	Total	P value
Disease severity	Mild, n (%)	53(40.5)	13(9.9)	31(23.7)	10 (7.6)	24 (18.3)	0	131(52.6)	P=0.001
	Moderate, n (%)	31(47.7)	9(13.8)	4(6.2)	6 (9.2)	15 (23.1)	0	65(26.1)	
	Severe/ Critical, n (%)	30(56.6)	5(9.4)	2(3.8)	2 (3.8)	11(20.8)	3(5.7)	53(21.2)	
Total	n (%)	114(45.8)	27(10.8)	37(14.9)	18 (7.2)	50(20.1)	3(1.2)	249(100)	

As presented in Table 4, there was a significant statistical difference between the type of vaccine and the severity of the clinical picture in vaccinated patients who had a breakthrough infection, but due to the uneven distribution of the samples this significance cannot be taken reliably.

Of the 249 patients, 158 patients (63.45%) were treated as outpatients, and 91(36.55%) were hospitalized. Of the 91 hospitalized patients, 30 patients (32.97%) died, and 61(67.03%) were discharged. In the group of patients who died, 22 patients were male (17.9%) and 8(6.3%) were female (Table 2). There was a statistical significance between genders and outcome of COVID-19 disease in vaccinated patients with breakthrough infection; namely, male gender had a statistically greater chance for fatal outcome compared to female (P=0.006). Although there was almost equal number of patients <65 and ≥65 years of age, 125(50.2%) and 124(49.8%), respectively, only 7(5.6%) patients died in the age group younger than 65 years and 118(94.4%) recovered, while in the patient group 65 years and older 23(18.5%) died, and 101(81.5%) survived. (Table 2). In terms of age, there was a statistically significant difference for negative clinical outcome for patients older than 65 years, who despite the vaccination had acquired COVID-19, compared to patients younger than 65 years (P= 0.002). Regarding comorbidities, the majority of our patients, 172(69.07%) had comorbidities, and 77(30.92%) were without any comorbidity (Table 2). In the patients' group with comorbidities, majority of patients had at least two or more comorbidities. The most common comorbidities were hypertension, diabetes mellitus, bronchial asthma, cardiovascular disease, chronic obstructive pulmonary diseases, cerebrovascular diseases and hypothyreosis. In the patients' group with comorbidities, 27(90.0%) died, whereas in the patients' group without comorbidities only 3(10.0%) died. Concerning comorbidities, there was a statistical significance for negative clinical outcome of COVID-19 breakthrough infection in the patients' group with comorbidities (P= 0.006). The mean age of the deceased was 71.27±9.79 years, and of the survived 58.83±15.3 years. There was a statistical significance between the age of the deceased and survived patients in vaccine breakthrough COVID-19 infection. Patients older than 71 years had statistically higher chances for lethal outcome compared to patients younger than 59 years (P=0.0001). We tried to evaluate if there was a connection between the disease outcome and the type of vaccine. Table 5 demonstrates the association between the disease outcomes in terms of mortality according to vaccine brand.

Table 5. COVID-19 disease outcome in vaccinated patients with breakthrough infection and type of vaccine

			Vaccine brand						
			Sinopharm	Astra Zeneka	Pfizer	Sputnik	Sinovac	Moderna	P value
Outcome	Deceased	N(%)	15(50.0)	6(20.0)	0(0.0)	0(0.0)	8 (26.7)	1(3.3)	P=0.031
	Survived	N(%)	99(45.2)	21(9.6)	37(16.9)	18(8.2)	42(19.2)	2 (0.9)	
Total		N(%)	114(45.8)	27(10.8)	37(14.9)	18(7.2)	50(20.1)	3(1.2)	

As presented in Table 5, there was a statistical signifycance between the clinical outcome and the vaccine type, but due to the uneven distribution of the samples, this significance cannot be taken reliably.

Discussion

Vaccination against COVID-19 was the most promising prospect of putting the pandemic under control and bringing it to an end. With the emergence of the SARS-CoV-2 virus in a relatively short period of time, various vaccine platforms were established and different vaccines were produced, with different vaccine efficacy [5,16]. Despite the great progress in the science, a

perfect vaccine has not yet been found, a vaccine which would be 100% effective in 100% of the time and in 100% of the population. In our cohort of patients, majority of vaccine breakthrough infections occurred in the Sinopharm and Sinovac groups, followed by those vaccinated with Pfizer, AstraZeneca and Sputnik (45.8; 20.1%, 14.9%, 10.8% and 7.2%, respectively). The three Moderna vaccinated (1.2%) and evaluated patients were vaccinated abroad. In the literature, the mRNA vaccines led to the notable finding of ~95% efficacy for prevention of symptomatic COVID-19 two months after the second dose, which is more than in our cohort of patients. Adenovirus vectored vaccines showed a lower protection against infection with SARS-CoV-2, but achieved >90% protection against severe disease, while Sinopharm showed 79% efficiency against symptomatic disease and hospitalization [17]. According to WHO, in those vaccinated with Sinovac [18], it has an efficacy of 51% for preventing symptomatic disease and 100% for preventing hospitalization, contrary to the findings in our study. It has to be noted that according to the vaccine campaign plan of the government of North Macedonia [12] and the available vaccine brands at the time [13], individuals over 65 years of age had priority for vaccination and were mostly vaccinated with Sinovac and Sinopharm vaccines; therefore the difference of breaktrough infection in different vaccine groups in our cohort of patients might be simply a representation of the majority of vaccinated people. Additionaly, the vaccination campaign in the Republic of North Macedonia started in March 2021 and first to be vaccinated with the available vaccines were health care workers, people necessary for maintaining critical infrastructure, individuals over 65 years of age, people with comorbidities and those at a high risk of developing severe/critical illness [12]. As mentioned in some reports [19], the clinical waning of immunity after the first 2 months is particularly notable in people over 60 years of age, in whom susceptibility increased for both symptomatic infections and hospitalizations. Thus, it has to be further evaluated whether this larger occurrence of breakthrough infections in Sinopharm and Sinovac vaccinated group in our patient cohort was due to the vaccine (in)efficacy, waning of immunity or simply was a corresponding share of the total vaccine representation. The average time from complete vaccination to symptom onset in our cohort was 79.8 ±41.8 days, similar to the findings in other studies [19], which can also be explained with the expected waning of immunity after two months of vaccination but it still has to be further explored. Our study found that male gender, age ≥65 years and presence of comorbidities were major contributing factors for poor outcome in vaccinated hospitalized patients with COVID-19 vaccine breakthrough infection. Similar to our findings, other studies indicated that male gender was a risk factor for serious COVID-19 disease, which is explained by the differences in immunity response, the role of sex hormones, and gender-related behavior [20,21]. Similar to the study of Scobie [22], in our study older age despite vaccination still represented a risk factor for hospital admission or death compared to younger people. The mean age of patients with breakthrough infections and lethal outcome in our study cohort was 71.2±9.8 years, as indicated in other clinical reports that age of the patients is an independent risk factor significantly associated with severe COVID-19 outcomes [23,24]. Similar to the findings in the literature, our study showed that more vaccine breakthrough infections occurred in patients with comorbidities compared to patients without comorbidities, and there was a statistically significant difference in mortality of patients with and without comorbidities (90.0% and 10.0%, respectively). Namely, studies show that patients with comorbidities are more susceptible to infection with SARS-CoV-2 *per se* [25]. Also, it has been shown in different studies that individual characteristics of patients including older age, immunosuppression, comorbidities such as chronic cardiovascular, pulmonary, renal, liver and neurological diseases, advanced pregnancy, and heavy smoking are associated with a higher incidence of severe illness infected with SARS-CoV-2 infection [22,26]. In our cohort hospitalization rate due to SARS-CoV-2 breakthrough infections (36.55%) was higher compared to other studies, as shown through Case Investigation and Reporting of the Centers for Disease Control and Prevention (CDC) [27], which might be a result of different vaccine brands used for immunization, as well as the lower overall health status of our population in general. A study by O'Driscoll [28], accentuates that the risk for SARS-CoV-2 infection grows proportionally with age, and older individuals are at disproportionately higher risk of developing severe COVID-19, and patients over 65 are responsible for 80% of COVID-19 hospitalizations and suffer from a 20-fold higher COVID-19 fatality rate compared to those less than 65 years old. Similar to these findings and other findings in the literature [28-30], in our study vaccinated patients older than 65 years of age had statically significant higher chances for negative clinical outcome compared to patients younger than 65 years.

Our study showed that there was a significant difference between the type of vaccine and the severity of COVID-19 in the vaccinated patients with a breakthrough infection and that the highest mortality was found in patients vaccinated with inactivated virus containing vaccine, followed by a non-replicating viral vector vaccine and the lowest mortality was found in those vaccinated with either mRNA vaccine or human adenovirus vector-based COVID-19 vaccine. In both cases, due to the uneven distribution of the samples, this significance cannot be taken reliably into account. There are several meta-analyses that have compared

vaccine efficacy and presented different findings [31-34]. Unlike the findings in these studies, due to the uneven distribution of the samples in our cohort it would be misleading to look at breakthrough cases by vaccine brand since we received and administered more of some brands than others. Some vaccine profiles had only a small number of participants which might overestimate the outcome if the outcome occurred. These factors make it difficult to directly compare numbers of breakthrough cases, the severity of clinical picture and disease outcome among vaccine brands used for vaccination in our patients. Nevertheless, the differences in the incidence of breakthrough cases based on vaccine type are of interest and will need further investigation.
Our study had several limitations: firstly, some of the vaccines were not available or very not equally represented at the moment of the study, hence they were not included in the analysis. Secondly, the causative variants of SARS-CoV-2 were not determined in each COVID-19 case, but according to the SARS-CoV-2 variant surveillance report by the Institute of Public Health of R.N. Macedonia [35], the delta variant had prevailed in the region during the study period. Another limitation is that the vaccine may mitigate the symptoms of the SARS-CoV-2 infection; therefore, some asymptomatic people escaped from the COVID-19 screening and were not included in the study as vaccine breakthrough infections. Additionally, comparing the severity outcomes with unvaccinated individuals warrants further investigation.

Conclusion

Our study revealed a difference in vaccine breakthrough infection with SARS-CoV-2 in terms of vaccine types, as well difference in the severity of the clinical picture and outcome, but due to the uneven representation of the samples and different vaccine representation it would be misleading to draw any conclusion. Nevertheless, our investigation showed that gender, age and comorbidities are associated with the severity and negative clinical outcome of SARS-CoV-2 infection even in vaccinated patients. Therefore, boosting immunity for vulnerable patient groups in addition to maintaining and promoting preventive measures are essential to prevent severe cases of breakthrough infections of COVID-19. At the same time identifying subgroups of high-risk patients for severe breakthrough infections can help prioritizing early preventive treatment or prophylaxis for SARS-CoV-2 infection.

Conflict of interest statement. None declared.

Reference

1. Zhu N, Zhang D, Wang W, *et al*. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; 382(8): 727-733.
2. Impact of COVID-19 in North Macedonia https://www.oecd-ilibrary.org/sites/bc8382fc-en/index.html?itemId=/content/component/bc8382fc-en.
3. WHO Coronavirus (COVID-19) Dashboard WHO Coronavirus (COVID-19) Dashboard With Vaccination Data. https://covid19.who.int/.
4. Vaccine Development, Testing, and Regulation. History of Vaccines. https://www.historyofvaccines.org/content/articles/vaccine-development-testing-and-regulation.
5. Cheng H, Peng Z, Luo W, *et al.* Efficacy and Safety of COVID-19 Vaccines in Phase III Trials: A Meta-Analysis. Vaccines (Basel) 2021; 9(6): 582.
6. Baden LR, El Sahly HM, Essink B, *et al*..COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021; 384 (5): 403-416.
7. Sadoff J, Gray G, Vandebosch A, *et al*. ENSEMBLE Study Group. Safety and efficacy of single-dose Ad26. COV2.S vaccine against COVID-19. *N Engl J Med* 2021; 384(23): 2187-2201.
8. Thompson MG, Burgess JL, Naleway AL, *et al*. Interim Estimates of Vaccine Effectiveness of BNT162b2 and mRNA-1273 COVID-19 Vaccines in Preventing SARS-CoV-2 Infection Among Health Care Personnel, First Responders, and Other Essential and Frontline Workers - Eight U.S. Locations, December 2020-March 2021. *MMWR Morb Mortal Wkly Rep* 2021; 70(13): 495-500.
9. Tenforde MW, Olson SM, Self WH, *et al*. IVY Network; HAIVEN Investigators. Effectiveness of Pfizer-BioNTech and Moderna Vaccines Against COVID-19 Among Hospitalized Adults Aged ≥65 Years-United States, January-March 2021. *MMWR Morb Mortal Wkly Rep* 2021; 70(18): 674-679.
10. Tande AJ, Pollock BD, Shah ND, *et al*. Impact of the Coronavirus Disease 2019 (COVID-19) Vaccine on Asymptomatic Infection Among Patients Undergoing Preprocedural COVID-19 Molecular Screening. *Clin Infect Dis* 2022; 74(1): 59-65.
11. Swift MD, Breeher LE, Tande AJ, *et al*. Effectiveness of Messenger RNA Coronavirus Disease 2019 (COVID-19) Vaccines Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in a Cohort of Healthcare Personnel. Clin Infect Dis. 2021; 73(6): e1376-e1379.
12. НАЦИОНАЛЕН ПЛАН ЗА COVID-19 ВАКЦИНАЦИЈА ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА. http://zdravstvo.gov.mk/wp-content/uploads/2021/02/Natsionalen-plan-za-imunizatsija.pdf.
13. Data from Moj Termin http://mojtermin.mk/.
14. http://zdravstvo.gov.mk.
15. World Health Organization: Therapeutics and COVID-19: living guideline, 24 September 2021. World Health Organization 2021.https://www.who.int/publications-detail-redirect/clinical-management-of-covid-19.
16. Yewdell JW. Individuals cannot rely on COVID-19 herd immunity: Durable immunity to viral disease is limited to viruses with obligate viremic spread. *PLoS Pathog* 2021; 17(4).
17. Evidence Assessment: Sinopharm /BBIBP COVID-19 vaccine. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/2_sage29apr2021_critical-evidence_sinopharm.pdf.

18. Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac. https://www.who.int/sinovac/coronavac.
19. Gupta RK, Topol EJ. COVID-19 vaccine breakthrough infections. *Science* 2021; 374(6575): 1561-1562.
20. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, *et al*. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol Sex Differ* 2020; 11(1): 29.
21. Butt AA, Yan P, Shaikh OS, *et al*. Rate and Risk Factors for Severe/Critical Disease Among Fully Vaccinated Persons With Breakthrough Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in a High-Risk National Population. *Clin Infect Dis* 2022; 75(1): e849-e856.
22. Scobie HM, Johnson AG, Suthar AB, *at al*. Monitoring Incidence of COVID-19 Cases, Hospitalizations, and Deaths, by Vaccination Status - 13 U.S. Jurisdictions, April 4-July 17, 2021. *MMWR Morb Mortal Wkly Rep* 2021; 70(37): 1284-1290.
23. Kang SJ, Jung SI. Age-Related Morbidity and Mortality among Patients with COVID-19. *Infect Chemother* 2020; 52(2): 154-164.
24. Heald-Sargent T, Muller WJ, Zheng X, *et al*. Age-Related Differences in Nasopharyngeal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Levels in Patients With Mild to Moderate Coronavirus Disease 2019 (COVID-19). *JAMA Pediatr* 2020; 174(9): 902-903.
25. CDC. Coronavirus (COVID-19): symptoms of coronavirus. Centers for Disease Control and Prevention. 2020, https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html.
26. Lipsitch M, Krammer F, Regev-Yochay G, *et al*.. SARS-CoV-2 breakthrough infections in vaccinated individuals: measurement, causes and impact. *Nat Rev Immunol* 2022; 22(1): 57-65.
27. COVID-19 Vaccine Breakthrough Infections Reported to CDC - United States, January 1 April 30, 2021. *MMWR Morb Mortal Wkly Rep* 2021; 70: 792-793.
28. O'Driscoll M, Ribeiro Dos Santos G, Wang L, *et al*. Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* 2021; 590(7844): 140-145.
29. Kim PS, Schildhouse RJ, Saint S, *et al*.. Vaccine breakthrough infections in veterans hospitalized with coronavirus infectious disease-2019: A case series. *Am J Infect Control* 2022; 50(3): 273-276.
30. Wang SY, Juthani PV, Borges KA, *et al*. Severe breakthrough COVID-19 cases in the SARS-CoV-2 delta (B.1.617.2) variant era. Lancet Microbe. 2022; 3(1): e4-e5.
31. Rotshild V, Hirsh-Raccah B, Miskin I, *et al*. Comparing the clinical efficacy of COVID-19 vaccines: a systematic review and network meta-analysis. *Sci Rep* 2021; 11(1): 22777.
32. Iheanacho CO, Eze UIH, Adida EA. A systematic review of effectiveness of BNT162b2 mRNA and ChAdOx1 adenoviral vector COVID-19 vaccines in the general population. *Bull Natl Res Cent* 2021; 45(1): 150.
33. Teerawattananon Y, Anothaisintawee T, Pheerapanyawaranun C, *et al*. A systematic review of methodological approaches for evaluating real-world effectiveness of COVID-19 vaccines: Advising resource-constrained settings. *PLoS One* 2022; 17(1): e0261930.
34. Li XN, Huang Y, Wang W, *et al*. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. *Emerg Microbes Infect* 2021; 10(1):1751-1759.
35. Weekly Epidemiological Report on COVID-19. https://www.iph.mk/en/.

Case report

CLINICAL RECOGNITION OF RETT SYNDROME

КЛИНИЧКО ПРЕПОЗНАВАЊЕ НА РЕТ СИНДРОМ

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Abstract

Rett syndrome is a rare genetic disorder that affects the development of brain, which results in severe mental and physical impairment. It is estimated that it affects one of 10,000 females born every year [1]. On rare occasions, it is also manifested in males.
The most prominent symptoms are neurological, including epileptic attacks, motory deficits, neurogenic apnea and belated or absent speech. Rett syndrome includes periphery pathologies, such as osteopenia, scoliosis, gastrointestinal dysfunction and general growth deficit. It is characterised by normal early growth and development followed by tardiness, loss of voluntary movement of the hands, stereotypical movements of the hands, slow growth of the brain and head, walking problems, epileptic attacks and intellectual disabilities.
The patient in the presented case report is a girl, aged 5 years, investigated due to developmental issues, with a mutation in the MECP2 gene that codes the methyl-CpG-binding protein 2 (MeCP2) suggesting Rett syndrome [1].

Keywords: Rett syndrome, neurodevelopmental disorder, MECP2

Апстракт

Рет синдромот е ретко генетско нарушување кое влијае на развојот на мозокот, што резултира со тешка ментална и физичка попреченост. Се проценува дека се јавува кај околу 1 од 10.000 девојчиња родени секоја година, а поретко се забележува и кај момчињата.
Најистакнатите симптоми се невролошки, вклучувајќи епилептични напади, моторни дефицити, неврогени апнеи и доцнење или отсуство на говорот. Рет синдромот вклучува и периферни патологии како што се остеопенија, сколиоза, гастроинтестинална дисфункција и општ дефицит на раст. Се карактеризира со нормален ран раст и развој проследен со забавување на развојот, губење на волевото користење на рацете, стереотипни движења на рацете, забавен раст на мозокот и главата, проблеми со одење, епилептични напади и интелектуална попреченост.
Пациентот во случајот кој е прикажан е девојче старо 5 години иследувано поради развојни нарушшувања, а потврдената мутација во MECP2 генот што го кодира метил-CpG-врзувачкиот протеин 2 (MeCP2) укажува на Рет синдромот [2].

Клучни зборови: Рет синдром, невро-развојно нарушување, MECP2

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Introduction

Rett syndrome is a severe neurodevelopmental disorder, which is characterized by a normal development until 6-18 months, followed by a regression with loss of speech and stereotypical hand movement in the affected girls [3]. Autism, epileptic attacks, acquired microcephaly and ataxic gait are also common traits of the condition. This condition was first described by Andreas Rett in 1966, and was recognized as a separate entity when it was further described by Hagberg *et al.* in 1983. The estimated prevalence is 1 in 10,000 [2]. Rett syndrome is diagnosed by fulfilling certain diagnostic criteria or an identified mutation in the MECP2 (methyl-CpG-binding protein) gene in females. Mutations of this gene make up to 90% of Rett syndrome. With the increasing accessibility of molecular diagnostics, the clinical spectrum has broadened to include girls that do not meet the clinical criteria for Rett syndrome, as well as men with severe mental retardation and/or encephalopathy.
We present the clinical findings of a female, aged 5 years, presenting an atypical Rett syndrome, where the prominent early characteristic was hypotonia.

Case report

The patient is a 5-year-old girl, born as a third child from a fourth pregnancy in 38th gestational week, with a birth weight of 2030 grams. SGA with a caesarean section and APGAR score of 3/6. The child was incubated for 3 days. During the perinatal period, an inability to adapt to feeding occurred, as well as weight loss, hypotrophy and hypotony. The neurological examination and CNS ultrasound were clean. During a regular check-up, a hypochromic anemia was detected, treated with iron supplements.

At 11 months, slight axial hypotonia, as well as strabismus of the right eye were present, and she was referred to a specialist in neurology.

At 18 months, the child came in for an examination, due to loss of balance and walking and tremors. Upon admission, there was postural instability with extrapyramidal symptomatology, visible tremors, spasmic tonus on the left side and the child only waddled with assistance. Laboratory testing presented clean TORCH serology and Wilson's disease testing. Developmental testing found a delay of motor and intellectual levels (age of 10 months). MRI of the brain revealed a dilation of the occipital horn of the left lateral ventricle, with no other focal lesions or abnormalities.

Upon further examinations, the patient was conscious, maintaining visual contact. One-sided facial expression-like a frown, with no interest in interaction, with ataxic gait, walked only with assistance and had unstable posture. Tendon reflexes were present, normal and symmetrical with normal muscle strength and tone.

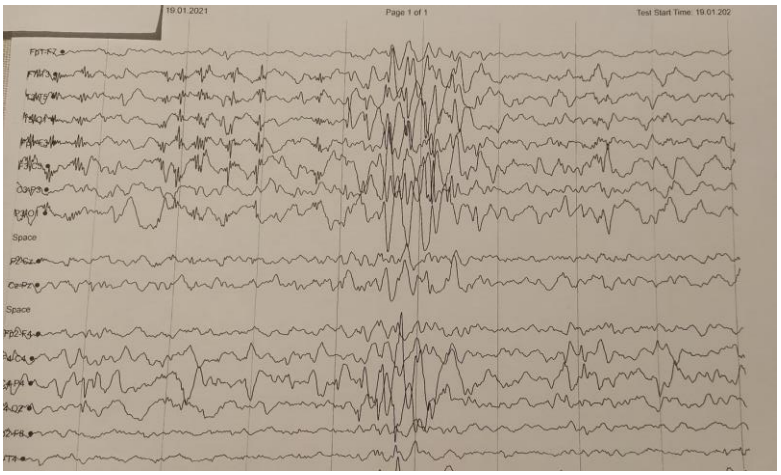

Fig. 1. EEG characteristic for Rett syndrome

At age of 4, the patient presented epileptic attacks, and EEG occurrences of a central spike with low amplitude, characteristic for Rett syndrome (Figure 1). Therapy with valproic acid was prescribed. Genetic investigation of the disease was performed, during which a mutation of the MECP2 gene was found, on the third exon, variation name c.401 C>G (pS134C), which is pathognomonic for Rett syndrome.

The patient came in for an examination six months after the established genetic diagnosis, receiving Depakene therapy. EEG found focus in stabilisation. Upon examination, hypomimia, scoliosis, postural ataxia, unstable gait with assistance and absence of speech were present (Figura 2).

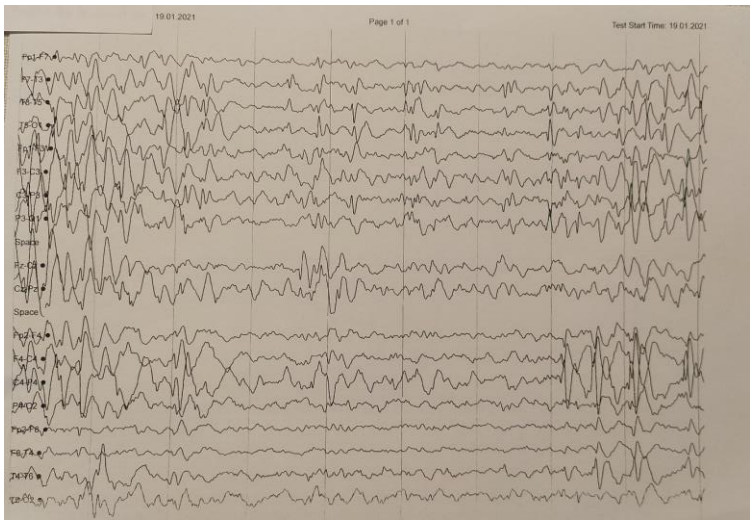

Fig. 2. EEG Rett syndrome

Discussion

We have analyzed the diagnostic criteria by which typical and atypical forms of Rett syndrome are being diagnosed [9,14]. Out of the necessary criteria that the Syndrome accordingly was diagnosed with, our patient possessed the following: normal peri- and postnatal period, with a head circumference in the physiological margins, and normal psychomotor development in the first 6 months [4]. This period was followed by loss of gained abilities, as well as hypotonia, delayed motor and intellectual abilities, ataxic gait and stereotypic hand movements.

From the additional criteria noted for diagnosing Rett syndrome, EEG abnormalities, epileptic attacks and neurogenic scoliosis were present in our case, with no other noted additional criteria.

Seizures are common in children with Rett syndrome, especially in early childhood. Up to 85% of children that are affected will report seizure occurrence during their lifetime [8]. Seizures occur in the majority of patients, with rates ranging from 50% to 90%. Seizure onset is usually toward the end of the regression period or after the regression period [7]. The most common types of seizures are tonic-clonic, tonic, myoclonic, and focal seizures with impaired awareness. An Italian retrospective study analyzed seizure medications in Rett syndrome and found that lamotrigine, sodium valproate, and carbamazepine can be used as drugs of first choice [13]. One study observed different effectiveness of antiseizure medications based on age, and it suggested that clinicians consider age-dependency when prescribing appropriate antiseizure medications in the Rett population. Valproic acid was reported as the most effective anti-seizure medication in younger girls (in 40% of patients younger than 5 years of age and in 19% of girls aged 5 to 9 years), and carbamazepine was reported as the most effective in patients 15 years of age or older [15].

Additionally, girls with Rett syndrome will often have extrapyramidal disturbances, including bruxism (97%), excessive drooling (75%) oculogyric crises (63%), hypomimia (63%), dystonia (59%), rigidity (44%), bradykinesia (41%), and proximal myoclonus (34%) [10].

Some conditions as metabolic and neurodegenerative diseases, organomegaly and deposition diseases which lead to autistic spectrum disorders, perinatal trauma and infections are not noted or are excluded by the laboratory analyses, imaging, TORCH infection serology and Wilson's disease testing. The only diagnostic criterium that belongs in the conditions excluded and occurs in the case presented is an intrauterine growth delay.

All of this has given an indication for further genetic testing, which showed a mutation of the MECP2 gene, which confirmed the Rett syndrome diagnosis. It is well established that mutations in MECP2, located on Xq28, account for 95% of typical Rett and 73.2% of atypical Rett syndrome [6]. More than 99% of female Rett syndrome incidences are *de novo* mutations in the MECP2 gene or possibly from a parent who has germline mosaicism. Approximately 600 mutations have been detected so far within the MECP2 gene [3,4].

Conclusion

Clinical recognition of Rett syndrome is a long-lasting process, due to the early psychomotor development and growth being normal and subsequently followed by a few phases of disease with characteristic criteria. Diagnosing Rett syndrome is further complicated mainly because of the wide variety of diseases that intertwine with Rett syndrome in differential diagnostics, as well as the existence of atypical forms of the Syndrome.

The necessary criteria, key to establishing a diagnosis are: female gender, less than 10 years of age, mental retardation with unknown etiology and present 3 of the 6 criteria [5]. From the additional criteria, at least 5 of 11 should be present.

Patients with a found mutation of the MECP2 gene, do not require any further diagnostic testing.

Conflict of interest statement. None declared.

References

1. Bhat S, Ming X, Dekermenjian R, Chokroverty S. Continuous spike and wave in slow-wave sleep in a patient with Rett syndrome and in a patient with Lhermitte-Duclos syndrome and neurofibromatosis 1. *J Child Neurol* 2014; 29(12): 176-80.
2. Bienvenu T, Philippe C, De Roux N, *et al*. The incidence of Rett syndrome in France. *Pediatr Neurol* 2006; 34(5): 372-375.
3. Christodoulou J, Grimm A, Maher T, Bennetts B. RettBASE: The IRSA MECP2 variation database-a new mutation database in evolution. *Hum Mutat* 2003; 21(5): 466-472.
4. Christodoulou J, Weaving LS. MECP2 and beyond: phenotype-genotype correlations in Rett syndrome. *J Child Neurol* 2003; 18(10): 669-674.
5. Dolce A, Ben-Zeev B, Naidu S, Kossoff EH. Rett syndrome and epilepsy: an update for child neurologists. *Pediatr Neurol* 2013; 48(5): 337-345.
6. Ehrhart F, Sangani NB, Curfs LM. Current developments in the genetics of Rett and Rett-like syndrome. *Curr Opin Psychiatry* 2018; 31(2): 103-108.
7. Glaze DG, Percy AK, Skinner S, *et al*. Epilepsy and the natural history of Rett syndrome. *Neurology* 2010; 74(11): 909-912.
8. Glaze DG, Schultz RJ, Frost JD. Rett syndrome: characterization of seizures versus non-seizures. *Electroencephalogr Clin Neurophysiol* 1998; 106(1): 79-83.
9. Fehr S, Bebbington A, Nassar N, *et al*. Trends in the diagnosis of Rett syndrome in Australia. *Pediatr Res* 2011; 70: 313-319.
10. FitzGerald PM, Jankovic J, Percy AK. Rett syndrome and associated movement disorders. *Mov Disord* 1990; 5(3): 195-202.
11. Moog U, Smeets EE, van Roozendaal KE, *et al*. Neurodevelopmental disorders in males related to the gene causing Rett syndrome in females (MECP2). *Eur J Paediatr Neurol* 2003; 7(1): 5-12.
12. Nomura Y, Segawa M. Natural history of Rett syndrome. *Journal of Child Neurology* 2005; 20(9): 764-767.
13. Pintaudi M, Calevo MG, Vignoli A, *et al*. Antiepileptic drugs in Rett syndrome. *Eur J Paediatr Neurol* 2015; (4): 446-452.
14. Trevathan E, Moser HW. for the Rett Syndrome Diagnostic Criteria Work Group. Diagnostic criteria for Rett syndrome. *Ann Neurol* 1988; 23: 425-428.
15. Vignoli A, Savini MN, Nowbut MS, *et al*. Effectiveness and tolerability of antiepileptic drugs in 104 girls with Rett syndrome. *Epilepsy Behav* 2017; 66: 27-33.

Case report

A NEGLECTED CASE OF ANENCEPHALY

ЗАНЕМАРЕН СЛУЧАЈ НА АНЕНЦЕФАЛИЈА

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Abstract

Introduction. Anencephaly is a neural tube defect (NTD) that occurs when the cephalic end of the neural tube fails to close. With five cases per 10,000 births, it is the second most common NTD worldwide.
Case report. A 23-year-old woman with third pregnancy was hospitalized at the Pathological pregnancy department, University Clinic for Obstetrics and Gynecology in Skopje. Her pregnancy course had been iregularrly controlled, without taking any folic acid in preconception or in the first trimester. The patient had not attended PRISCA 1 or 2, vaginal smear or microbiological examination, ultrasound examination or other obstetric examination of any kind up until her 20th week of pregnancy. The very screening of fetal structural anomalies showed acrania and anencephaly. Termination of pregnancy had been suggested, nontheless the patient had decided to carry on with the pregnancy. After passing her term, she underwent induction of labor. Vaginal delivery was without any complications. The infant was in a clinically difficult condition, with hypotony, cyanosis and major congenital absence of the cranial bones. Regardless of the post-partum palliative treatment, the newborn died at H23 of life.
Conclusion. The root of the possibilities of early detection of anencephaly, detailed examination and opportunity for optimal perinatal management lays in the sonographic technology and its advances. That aside, as physicians, we had to respect our patient's decision to take any desired action considering her circumstances.

Keywords: fetal anomalies, anencephaly, neural tube defects, ultrasound

Апстракт

Вовед. Аненцефалија претставува дефект на невралната туба (ДНТ) што се јавува кога цефаличниот крај на невралната туба не успева да се затвори. Со пет случаи на 10000 раѓања, е втор најчест ДНТ во светот.
Приказ на случај. 23-годишна жена со трета бременост беше хоспитализирана на Одделот за патолошка бременост. Нејзиниот тек на бременоста бил нередовно контролиран, таа не користела фолна киселина во предконцепцијата ниту во првиот триместар. Пациентката не направила PRISCA 1 или 2, вагинален брис или микробиолошки преглед, ултразвучен преглед, како и друг вид акушерски прегледи до нејзината 20-та недела од бременоста. Самиот скрининг на структурните аномалии на фетусот во дваесеттата недела од бременоста, покажал акранија и аненцефалија. Било предложено прекинување на бременоста, но сепак пациентката одлучила да ја продолжи истата. По завршувањето на ВТР, се направи индукција на породувањето. Вагиналното породување помина без никакви компликации. Новороденото беше во клинички тешка состојба, со хипотонија, цијаноза и големо вродено отсуство на кранијалните коски. Без оглед на постпарталниот палијативен третман, новороденчето почина во 23-от час од животот.
Заклучок. Сонографската технологија и нејзиниот напредок претставува суштинска можност за рано откривање на аненцефалија, детален преглед и овозможува оптимален перинатален менаџмент. Имајќи ги в предвид нејзините околности, како лекари, ние мора да ја почитуваме одлуката на пациентката во однос на преземањето било што.

Клучни зборови: фетални аномалии, аненцефалија, дефекти на неврална туба, ултразвук

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Introduction

Anencephaly is a neural tube defect (NTD) that manifests as the lack of a significant section of the brain, skull, and scalp when the cephalic (head) end of the neural tube fails to seal, typically between the 23rd and 26th days of pregnancy. Overall estimate of the

prevalence of anencephaly worldwide is 5.1 per ten thousand births [1].

Although earlier research revealed that anencephaly is a multi-factorial process largely regulated by genes and a variety of environmental factors, there is now undeniable proof that folic acid supplementation prior to pregnancy can significantly prevent anencephaly [2]. One study from the United States of America has demonstrated that daily folate intake for all women of childbearing age through cereal grains with 0.4 mg of folate per day has shown 16% reduction in anencephaly prevalence in the population after just one year [3].

The etiological factors of anencephaly include:

- Environmental conditions (nutritional factors: folic acid deficiency, exposure to nitrates, pesticides, organic solvents, anticonvulsant use, excess of vitamin A intake, socioeconomic status, fever/hyperthermia),
- Genetics of both population and familial ancestry (*MTHFR 677C-T* and *1298A-C* polymorphisms, *PDGFRA* frameshift variant and missense variant, *VANGL1 & 2*, *Pax3*, *CELSR1*, *AMT* and *GLDC* mutations, SNPs in *PARD3*, *PCMT1* polymorphisms),
- Maternal condition (obesity, pre-gestational/gestational diabetes, ethnicity), and
- Fetal condition (female gender) [4].

The mechanism of development of anencephaly as one type of upper NTDs essentially means that the fetal cranial neuropore does not close during neural tube closure in the fourth week of embryogenesis. This type of anomaly leads to failure in the development of the brain, lamina terminalis, and bony cranium. Human anencephaly has been divided into two types: meroacrania (in which mainly rostral brain is affected) and holoacrania (in which posterior brain and skull are affected) [5].

The differential diagnosis of anencephaly includes osteogenesis imperfecta, amniotic band syndrome, and microcephaly [6].

The importance of early diagnostic approach has been settled in the recent years, mainly with first-trimester ultrasound, between the 11th and 14th week of pregnancy, amniocentesis, analysis of alpha-fetoprotein levels and blood tests. The prognosis of this fetal condition is extremly poor. If the newborn is not stillborn, then he or she usually dies within a few hours or days after birth.

Case report

A 23-year-old woman presented to our Department of pathological pregnancy, with no previous medical or surgical history, no notion of consanguinity, both of her parents with arterial hypertension, her mother with diabetes melitus type 2. Gravida 3, para 1. Her G1 was by vaginal delivery of a live born female of 3035 g (2018). Fifteen months ago, in 2021, the patient's G2 ended as a missed abortion in the 20th week of pregnancy. G3 was the current pregnancy estimated at 40 weeks and 1 day by her last menstruation period. The pregnancy course had been iregularrly controlled and without taking any folic acid in preconception or in the 1st trimester. The patient had not attended PRISCA 1 or 2, vaginal smear or microbiological examination, ultrasound examination or other obstetric examination of any kind up until her 20th week of pregnancy. She had not done OGTT.

The very screening of fetal structural anomalies, at 20 weeks of pregnancy, showed acrania and anencephaly. Termination of pregnancy had been suggested, nontheless the patient had decided to carry on with the pregnancy. She was accepted at the Department of pathological pregnancy; a complete medical and obstetrical check-up had been done. The patient was clinically stable; height-157 cm, weight-70 kg, T-36.5 C, BP-121/76 mmol/L, HR-80 bpm. Obstetric examination: active uterine contractions, 30 cm uterine height, the fetus was in *situs labilis*, with an active fetal heart beat, on vaginal examination a soft median cervix dilated to 3 cm, unruptured amniotic sac. The ultrasound revealed a single fetal pregnancy with a positive fetal cardiac activity, AC-310 mm, FL-72 mm, the cranial bones were not visable (Figure 1). Placenta was with hypoechogenic zones, on the rear uterine wall.

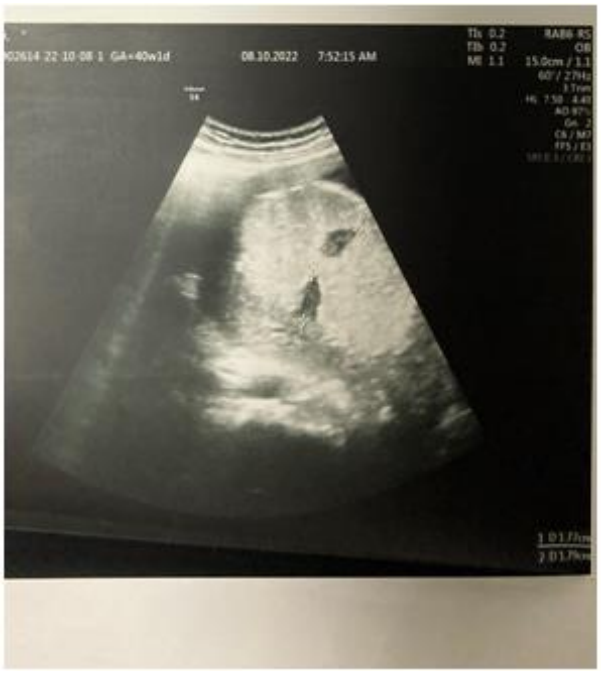

Fig. 1. Ultrasound image showing *anencephaly and absence of cranial bones*

After obtaining a signed informed consent, we started induction of labor with Vag. Prostin E2.

The patient was transferred to the delivery room with ongoing monitoring. After 4 hours and 40 minutes of labor, a newborn female was delivered vaginally. The delivery underwent without any complications. Birth

weight 2585 g, birth length 47 cm and Apgar score 6-6 at first and fifth minutes. Umbilical pH-7.12. Generally, the infant was in a clinically difficult condition, with hypotony, cyanosis and major congenital absence of the cranial bones (Figures 2 and 3). Regardless of the post-partum palliative treatment, the newborn died at H23 of life.

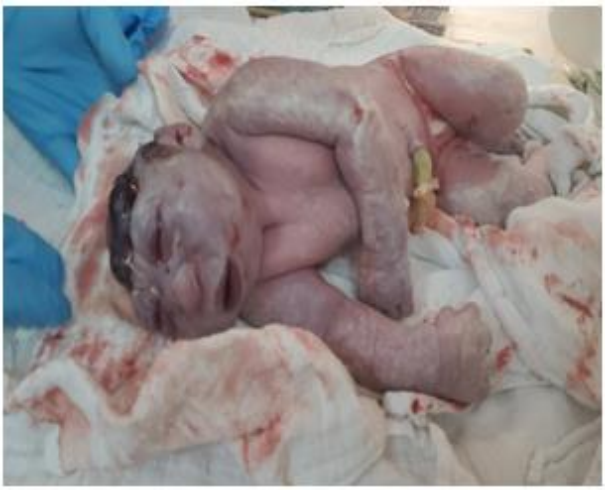

Fig. 2. Newborn with anencephaly (img. No I)

Fig. 3. Newborn with anencephaly (img. No II)

Discussion

The condition of anencephaly raises major attention in clinical, legal, ethical, religious and social aspects of various debates. The main reason for these aspects and for presenting this case report has been raising awareness about the importance of early detection of anencephaly, through high-resolution sonography, in the first trimester of pregnancy. In addition, our crucial focus was on the neglected approach as an issue that every society should work on. Lastly, holistic approach and individualization of every patient condition is paramount.

Neural tube defects, out of all anomalies, are the simplest to recognize prenatally. Prior to targeted ultrasound scanning, amniocentesis and maternal serum fetoprotein (FP) screening were primary methods to detect NTDs [7]. In recent years that has changed as a result of the implementation of routine ultrasound screening for fetal anomaly in most centers in the world.

The prenatal ultrasonographic visualization of anencephaly in the first trimester is different from the familiar second-trimester signs. The cerebral hemispheres are present and exposed to the surrounding amniotic fluid. The ultrasound appearances in the coronal section of the head are best described as "Mickey Mouse face" [8].

In the postnatal period, the clinical diagnosis is established by physical examination that meet four required criteria: 1. No calvarium present, 2. Absence of scalp, 3. External presence of a hemorrhagic, fibrous mass or tissue, 4. Lack of cerebral hemispheres [9]. Overall, the rates of detection of structural anomalies in first-trimester ultrasound vary from 46.1% to 76.1%. The detection of head and neck anomalies is 51%, which is still an unsatisfactory percentage [10]. This proves that we need to raise awareness about the importance of early detection of fetal anomalies.

Prenatal diagnosis of fetal anomalies enables the best perinatal management, giving expectant parents the chance to have additional imaging and genetic tests done as well as information about the prognosis and treatment choices [10].

Conclusion

Fetal structural anomalies are detected in around 3% of all pregnancies. These days, roughly 50% of all major structural anomalies can be detected in the first trimester, including acrania/anencephaly [10]. The root of the possibilities of early detection of anencephaly, detailed examination and optimal perinatal management lays in the sonographic technology and its advances. High-resolution ultrasonography is a reliable method in prenatal diagnosis of anencephaly. The ultrasound diagnosis is made on the basis of absence of the upper portion of the cranial vault. Above the level of the orbits, where the cerebral hemispheres are typically seen, there is either no tissue or an ill-defined mass of heterogeneous density [11]. This imaging method can provide important information about deciding on diagnosis and treatment of a variety of diseases and conditions. That aside, as physicians, in our case, we had to respect the patient's decision to take any desired action considering her circumstances.

Conflict of interest statement. None declared.

References

1. Salari N, Fatahi B, Fatahian R, *et al*. Global prevalence of

congenital anencephaly: a comprehensive systematic review and meta-analysis. *Reprod Health* 2022; 19(1): 201.
2. Atta CA, Fiest KM, Frolkis AD, *et al*. Global Birth Prevalence of Spina Bifida by Folic Acid Fortification Status: A Systematic Review and Meta-Analysis. *Am J Public Health* 2016; 106(1): e24-e34.
3. Frey L, Hauser WA. Epidemiology of neural tube defects. *Epilepsia* 2003; 44 Suppl 3: 4-13.
4. Munteanu O, Cîrstoiu MM, Filipoiu FM, *et al*. The etio-pathogenic and morphological spectrum of anencephaly: a comprehensive review of literature. *Rom J Morphol Embryol* 2020; 61(2): 335-343.
5. Ravi KS, Divasha, Hassan SB, *et al*. Neural tube defects: Different types and brief review of neurulation process and its clinical implication. *J Family Med Prim Care* 2021; 10(12): 4383-4390.
6. Moore L. Anencephaly. *Journal of Diagnostic Medical Sonography* 2010; 26(6): 286-289.
7. Boyd PA, Wellesley DG, De Walle HE, *et al*. Evaluation of the prenatal diagnosis of neural tube defects by fetal ultrasonographic examination in different centres across Europe. *J Med Screen* 2000; 7(4): 169-174.
8. Chatzipapas IK, Whitlow BJ, Economides DL. The 'Mickey Mouse' sign and the diagnosis of anencephaly in early pregnancy. *Ultrasound Obstet Gynecol* 1999; 13(3): 196-199.
9. Tafuri SM, Lui F. Embryology, Anencephaly. [Updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545244/.
10. Edwards L, Hui L. First and second trimester screening for fetal structural anomalies. *Semin Fetal Neonatal Med* 2018; 23(2): 102-111.
11. Medical Task Force on Anencephaly. The infant with anencephaly. *N Engl J Med* 1990; 322(10): 669-674.

Case report

FEMALE GENITAL MUTILATION IN THE REPUBLIC OF NORTH MACEDONIA - AN ISOLATED CASE OR THE BEGINNING OF A BAD PRACTICE

МУТИЛАЦИЈА НА ЖЕНСКИ ГЕНИТАЛНИ ОРГАНИ ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА- ИЗОЛИРАН СЛУЧАЈ ИЛИ ПОЧЕТОК НА ЛОША ПРАКСА

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Abstract

Introduction. Female genital mutilation is a "practice" not unknown to the world. In the Republic of North Macedonia there has not yet been reported a case of female genital mutilation.
Case report. A 41-year-old woman of Albanian ethnic origin with Muslim religious beliefs and practices was admitted to the emergency gynecology department for exploratory curettage. The condition of her external genitalia was consistent with type III mutilation, i.e., infundibulation. The patient reported a reduced need for sexual intercourse, absence of responsive desire and arousal and failure to achieve orgasm. She did not perceive her sexual dysfunction as a problem, but as a "normal" difference in the quality of sexual life between men and women.
Conclusion. It is necessary to alert the professionals about the possibility that this case is not isolated. The absence of victim's perception of sexual dysfunction imposes the need of conducting studies to determine the attitude of the local population towards this phenomenon and sexuality in general. Health workers have to play an active role in presenting all the consequences that this act of violence against women has on their reproductive and sexual health.

Keywords: female genital mutilation, infundibulation, sexual dysfunction

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Апстракт

Вовед. Мутилација на женските генитални органи е „пракса" која не му е непозната на светот. Република Северна Македонија досега не објавила случај на мутилација на женски гениталии.
Приказ на случај. 41 годишна жена од албанско етиничко потекло со муслиманска вероисповест е примена на оддел за ургентна гинекологија за експлоративна киретажа. Стаусот на нејзините надворешни гениталии одговараше за мутилација тип III, односно инфундибулација. Пациентката пријави намалена потреба за сексуални односи и отсуство на возбуда и непостигување на оргазам при истите. Присутната сексуална дисфункција, пациентката не ја доживуваше како проблем, туку како „нормална" разлика во квалитетот на сексуалното живеење меѓу мажите и жените.
Заклучок. Потребно е алармирање на стручната јавност за можноста овој случај да не е изолиран. Отсуството на перцепција за сексуалната дисфункција од страна на жртвата, наметнува потреба од студии за утврдување на односот кој локалното население го има кон оваа појава и сексуалноста воопшто. Потребна е активна улога на здравствените работници во предочување на сите последици кои овој акт на насилство врз жената ги носи по нејзиното репродуктивно и сексуално здравје.

Клучни зборови: мутилација на женски генитални органи, инфундибулација, сексуална дисфункција.

Introduction

Female genital mutilation (defined as the partial or total removal of female genitalia for non-medical reasons), as a "practice" of raising female children, is a phenomenon not unknown to the world, especially to 30 countries in Africa and Asia. In Africa, the prevalence of female genital mutilation ranges from 1% in Zambia and Cameroon, through 38% in Chad, 65% in Ethiopia, to a staggering 87% in Egypt, and 98% in Somalia [1,2]. Asian countries, the Borha community in India, have a prevalence of 75% [3], Indonesia has a national prevalence of 49%, and in Malaysia 83-85% of Muslim female children will be mutilated [4].

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Transcontinental population migration will lead to the emergence of this "practice" in European countries as well. The risk of an immigrant from a country with a tradition of female mutilation being subjected to it in a European country varies depending on the specific country: 5-8% Portugal, 7-12% Ireland, 11-19% Sweden, but more often in the countries near N. Macedonia: 15 -24% in Italy, 25-42% in Greece [5]. This trend was the reason for the creation of the Istanbul Convention (2011) to combat violence against women, of which our country is also a signatory [6]. So far, a case of verified mutilation has not yet been published in the Republic of North Macedonia, nor has this country been the subject of a study to assess the risk of mutilation in view of migration and the changing religious structure of the population.

Case-report

In the Specialized Hospital for Gynecology and Obstetrics "Mother Teresa"-Skopje, a patient was admitted to the emergency gynecology department under the diagnosis: meno-metrorrhagia for exploratory curettage.

The patient was a 41-year-old woman, of Albanian ethnic origin with Muslim religious beliefs and practices. Her history did not disclose any information about diseases or interventions of the female reproductive tract. The obstetric history listed three pregnancies, all three ended by caesarean section, with the reasoning that "she cannot give birth vaginally", without insisting on the reason. After examination performed in a lithotomy position, a disturbed anatomy of the external genital organs was found:

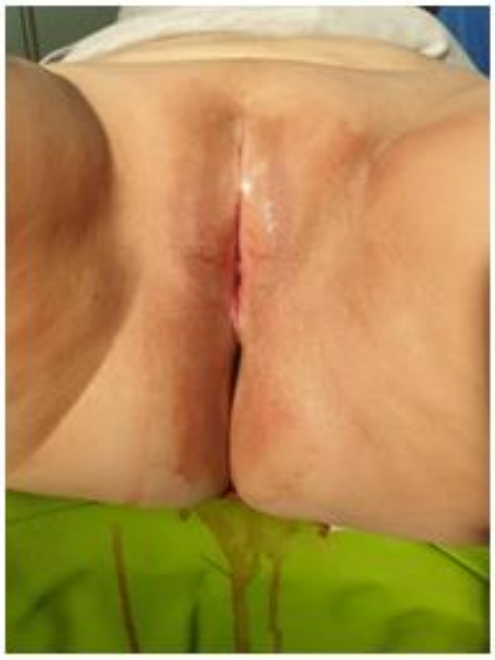

Fig. 1. Anterior half labia majora fusion

On inspection, the labia majora were fused with a visible thin cicatrix along the line of fusion starting from the anterior commissure along the first half of the labia majora. Under it, the vestibule of the vagina could be seen discreetly without other elements of the vulva being noticed (Figrue 1).

After introducing the patient to a short-term intravenous anesthesia and relaxing the muscles of the pelvic floor, pulling the labia majora to the side revealed their approximation and fusion up to the external mouth of the urethra along with complete obliteration of the clitoral region. Towards the posterior commissure, the labia majora were separated continuing towards

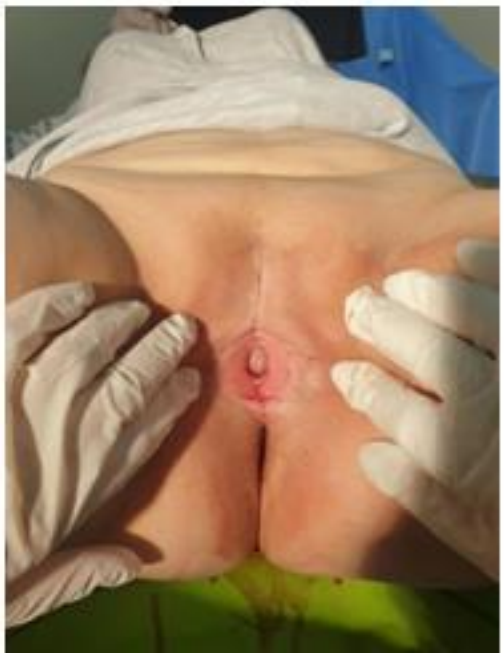

Fig. 2. Infundibulation

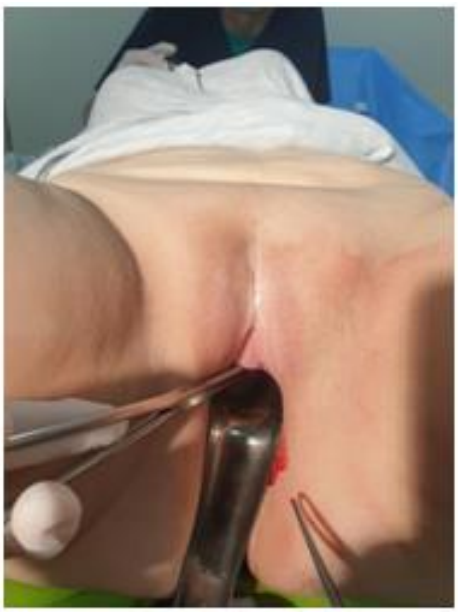

Fig. 3. Adequate vaginal space

the vagina, without the presence of labia minora. The entire status of the patient's external genitalia corresponded to a female genital mutilation of type III, i.e., infundibulation. Spreading the lips to the side, "opened" a small narrow vestibule of the vagina into which the external mouth of urethra protruded (Figure 2).

With posterior ecarter entry and downward traction, a vaginal space with an adequate size was revealed. After accepting the cervix, its normal anatomy was determined, after which the intervention was performed without problems (Figure 3).

The data obtained by an additional and purposeful history showed that the patient was completely unaware of the appearance of her vulva until getting married and discussing it with her husband, which indicated that the intervention was performed in early childhood. Asked about the quality of sexual life, the patient stated a reduced personal desire for intercourse, and reportd absence of sexual arousal and failure to reach orgasm. At the same time, the patient did not perceive this clear sexual dysfunction as a problem, but rather as a "normal" difference between the quality of sexual life of men and women.

Conclusion

This case report of a female genital mutilation in the Republic of North Macedonia should alert the professional and general public to the possibility that this event is not isolated. The victim's absence of awareness of the problem and the acceptance of the poor quality of sexual life (which we saw in this case) indicates the need of conducting pilot studies to determine the attitude of the local population towards this phenomenon and the need to sensitize the public for an adequate defining of this phenomenon from a religious rite to an act of violence against women. A proactive role of health workers is necessary in raising awareness of a woman's inalienable right to possess her own body and a clear presentation of all the consequences that this act of mutilation has on the victim's reproductive, sexual, and thus on mental and emotional health.

Conflict of interest statement. None declared.

Reference

1. (UNICEF) UNCsF. Female genital mutilation/cutting: a statistical overview and exploration of the dynamics of change. New York, United States of America: Statistics and Monitoring Section, Division of Policy and Strategy, 2013.
2. Female genital mutilation/cutting; A global concern UNICEF, New York, 2016.
3. A resource guide to best practice for sensitive and effective reporting on FGC/M: With special reference to Khatna in the Dawoodi Bohra community: Sahiyo Khatna survey 2015.
4. Asia Network to End Female Genital Mutilation/Cutting (FGM/C) Consultation report, 2020.
5. Estimation of girls at risk of female genital mutilation in the European Union, European Institute for gender equality, 2018.
6. Council of Europe Convention on preventing and combating violence against women and domestic violence, Council of Europe Treaty Series - No. 210, Istanbul 2011.

Case report

RESISTANT HYPERTENSION WITH CORONARY ARTERY DISEASE

РЕЗИСТЕНТНА ХИПЕРТЕНЗИЈА СО КОРОНАРНА АРТЕРИСКА БОЛЕСТ

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Abstract

Resistant hypertension is encountered in around 30% of hypertension population and it requires at least four antihypertensive medications for treatment. It is associated with cardiovascular (CV) events. Resistant hypertension often occurs as a comorbidity with coronary artery disease, so quantification of total CV risk, which is best done with the SCORE system, is important for risk stratification of hypertensive patients in order to implement therapy to reduce the cardiovascular risk.
In this study, we report clinical findings of a 66-year-old woman presenting with resistant hypertension and coronary artery disease, her hospital treatment and recommendation for home treatment after being discharged from hospital. Further we discussed the recent guidelines diagnostic and treatment algorithms.
In conclusion, hypertension is the most common risk factor in patients with coronary vascular disease and its regulation, especially in the resistant form, is one of the most important factors for reducing the prevalence of coronary artery disease as well as the degree of major adverse cardiovascular events in patients with existing coronary artery disease.

Keywords: resistant hypertension, coronary artery disease

Апстракт

Резистентната хипертензија е присутна кај околу 30% од популацијата со хипертензија, при тоа потребни се најмалку четири антихипертензивни лекови за нејзино лекување и е асоцирана со кардиоваскуларни настани. Резистентната хипертензија често се јавува како коморбидитет со коронарната артериска болест, така што квантификацијата на вкупниот кардиоваскуларен ризик, најподобен е SCORE системот, е битна за проценка на ризикот и спроведување на терапијата за намалување на истиот.
Во оваа студија ги презентираме клиничките наоди на 66-годишна жена со резистентна хипертензија и коронарна артериска болест, нејзиното болничко лекување и препораките за домашно лекување по отпуштањето. Понатаму во дискусијата се наведени скорешните упатства и алгоритми за дијагноза и третман.
Како заклучок: хипертензијата е најчест ризик фактор кај пациентите со коронарна артериска болест и лекувањето на истата, особено резистентната хипертензија, е еден од најважните фактори за намалување на преваленцата на коронарната артериска болест, како и степенот на несакани кардиоваскуларни настани кај пациенти со постоечка коронарна артериска болест.

Клучни зборови: резистентна хипертензија, коронарна артериска болест

Introduction

Hypertension (HT) affects one third of the world population. Resistant hypertension (Res-HT) is a challenging clinical problem present in around 30% of hypertension population and is associated with cardiovascular (CV) events. Patients with Res-HT have 1.2-3-fold increased CV risk compared to hypertension population with controlled HT (treatment responsive hypertension) [1,2]. However, data regarding the impact of Res-HT on CV events in coronary artery disease (CAD) patients are insufficient.

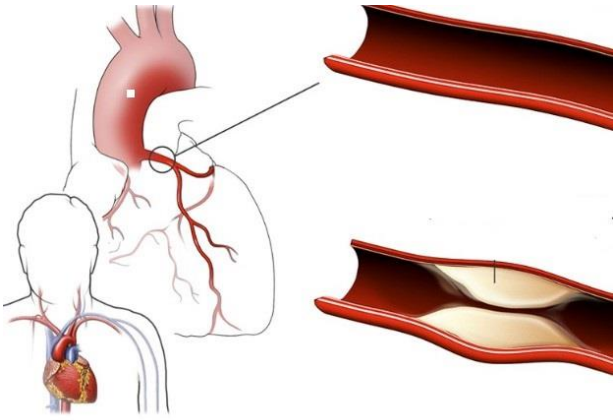

Fig. 1. Difference between a healthy coronary artery and CAD

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Coronary artery disease is a heart disease that affects larger coronary arteries which cannot deliver enough oxygen-rich blood to the heart (Figure 1). The causes of CAD are atherosclerosis and arteriosclerosis.

Resistant hypertension requires at least 4 antihypertensive blood pressure (BP) drugs for BP control.

Table 1. Classification of blood pressure and hypertension grade

Category	Systolic (mmHg)		Diastolic (mmHg)
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension[b]	≥140	and	<90

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Aantihypertensive therapy for regulation of hypertension is required, but also habits and lifestyle changes are necessary. That includes:

1. Reduction of salt intake under 5 gr per day;
2. Increased intake of fruits and vegetables;
3. Maintenance of BMI between 20-25 kg/m^2;
4. Keeping waist circumference for women under 80 cm and for men under 94 cm;
5. Practicing daily physical activity for 30 minutes;
6. Reducing alcohol intake and complete cessation of smoking.

Besides HT, humans may have concomitant diseases like diabetes and hypertension, hypertension and chronic kidney disease, CAD and/or CVD and hypertension. Their interference creates circulus vitiosus and causes difficulty in HT control. Table 2 presents HT with concomitant diseases and their systolic/diastolic BP threshold under antihypertensive treatment.

Table 2. Blood pressure levels, concomitant disease - antihypertensive treatment

Age group	Office SBP treatment threshold (mmHg)					Office DBP treatment threshold (mmHg)
	Hypertension	+ Diabetes	+ CKD	+ CAD	+ Stroke/TIA	
18 - 65 years	≥140	≥140	≥140	≥140[a]	≥140[a]	≥90
65 - 79 years	≥140	≥140	≥140	≥140[a]	≥140[a]	≥90
≥80 years	≥160	≥160	≥160	≥160	≥160	≥90
Office DBP treatment threshold (mmHg)	≥90	≥90	≥90	≥90	≥90	

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With an aim to adjust/harmonize antihypertensive therapy worldwide, experts have given recommendations and antihypertensive therapy guidelines as follows:

1. Two antihypertensive drugs are recommended as an antihypertensive therapy by starting preferably one tablet with two active substances (for example ACE/ARA with diuretic or ACE/ARA + calcium antagonist),
2. Target values for BP: under 140/90 mmHg or as close as possible to 130/80 mmHg,
3. At high normal blood pressure 130-139/85-89 mmHg, antihypertensive therapy will be started at those who have increased cardiovascular risk or established CAD.

Assessment of hypertension and cardiovascular risk is necessary for timely initiation of appropriate therapy to prevent additional complications. So, quantification of total CV risk is important for risk stratification of hypertensive patients in order to see if statin therapy or antiplatelet therapy is necessary to reduce CV risk. CV

risk assessment is best done with the SCORE system, especially in patients with hypertension and confirmed CAD, diabetes, renal disease or elevated blood cholesterol level. Correlation between high blood pressure and cardiovascular and renal events is continuous, hence the need to distinguish normal blood pressure from hypertension.

Hypertension is often associated with other risk factors such as dyslipidemia and glucose intolerance, which on the other hand give a multiplying effect in the development of coronary artery disease.

Table 3. Classification of hypertension according to BP level, presence of BP risk factors, target organ damage and comorbidities

Hypertension disease staging	Other risk factors, HMOD, or disease	BP (mmHg) grading			
		High normal SBP 130–139 DBP 85–89	Grade 1 SBP 140–159 DBP 90–99	Grade 2 SBP 160–179 DBP 100–109	Grade 3 SBP ≥180 or DBP ≥110
Stage 1 (uncomplicated)	No other risk factors	Low risk	Low risk	Moderate risk	High risk
	1 or 2 risk factors	Low risk	Moderate risk	Moderate to high risk	High risk
	≥3 risk factors	Low to Moderate risk	Moderate to high risk	High Risk	High risk
Stage 2 (asymptomatic disease)	HMOD, CKD grade 3, or diabetes mellitus without organ damage	Moderate to high risk	High risk	High risk	High to very high risk
Stage 3 (established disease)	Established CVD, CKD grade ≥4, or diabetes mellitus with organ damage	Very high risk	Very high risk	Very high risk	Very high risk

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Case report

The patient was a 66-year-old woman, who required a cardiology consultation for her frequent headaches and high unregulated blood pressure and chest pain. She had frequent attacks of headache and frequent outpatient internist and cardiologist consultations due to high and unregulated blood pressure levels. She had grade 3 hypertension, diabetes type 2 that has lasted for 10 years, and hyperlipidemia. Chronic kidney disease was discovered 5 years ago as a result of nephrolithiasis. She has been smoking for 20 years. She denied diseases of interest in the family history and food and drug allergy.

The patient was admitted to the hospital in a serious general condition with significantly high blood pressure (250/120 mm/Hg). Because she had signs of left heart failure with signs of incipient heart failure, she was admitted in the intensive care unit. During hospital stay, she was treated with intensive diuretic therapy, therapy with nitro-medications, calcium antagonists and ACE inhibitors.

After stabilization, she was transferred to the department unit for further investigation and additional therapy.

Upon further hospital examinations, ECG presented sinus rhythm with a heart rate of 78/min, biphasic T waves in D2 and aVL. System status was with normal function.

From the laboratory tests: glycemia 13.5 mmol/l, HgA1c 10.4%, triglycerides 5.15 mmol/l, total cholesterol 6.0 mmol/l, LDL 2.5 mmol/l, HDL 0.9 mmol/l. Elevated levels of creatinine and urea in serum were noted (creatinine 444. 410..456 μmol/L and urea 19. 4..20. 2..21.6 mmol/L). Holter blood pressure monitoring was performed with a finding of a high unregulated day-night blood pressure variations and echocardiographic findings presented a regular dimension of the LV with preserved EF and hypokinesia based on the lower wall. Carotid Doppler findings were with normal flow velocities, but thickened BMI and 50% stenosis in ACI lat. dexter were observed. Peripheral Doppler presented grade 1 of circulatory insufficiency in the legs and diabetic angioneuropathy. When coronarography was performed, two-vessel coronary artery disease (mLAD 70%, mRCA 85%) was found and percutaneous coronary intervention (PCI/RCA) was made. Ophthalmological examination presented grade 2 of hypertonic with non-proliferative diabetic retinopathy.

A nephrology specialist was consultant who gave advice on hydration and a proposal for further therapy.

During the hospital stay, an antihypertensive treatment was initiated upon the newest guidelines (Figure 2 and 3).

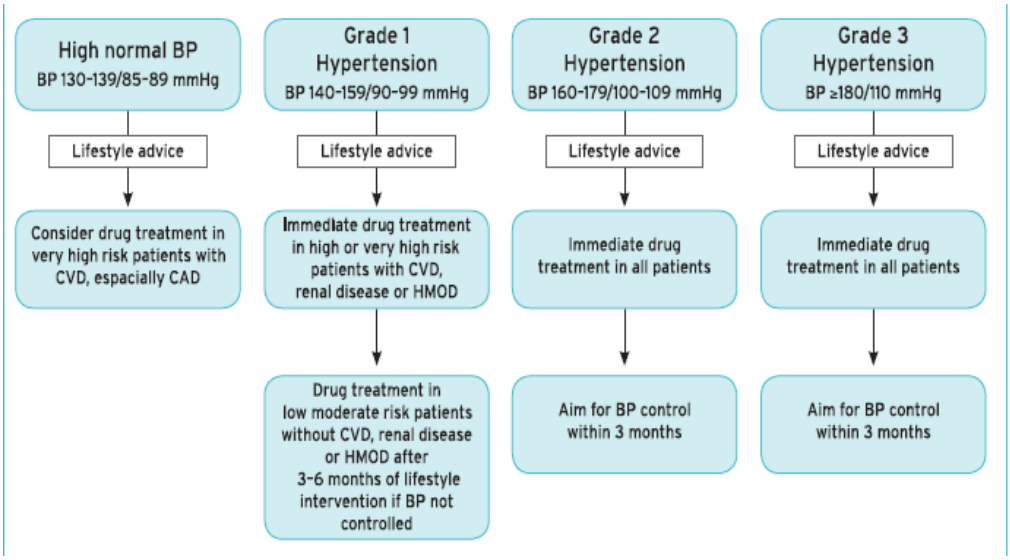

Fig. 2. Algorithm for initiation of antihypertensive treatment depending on BP level
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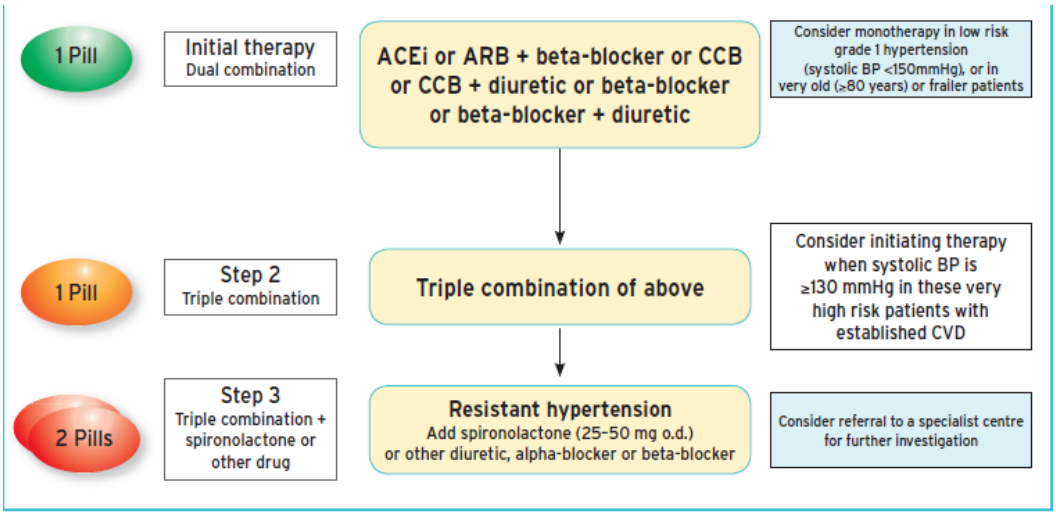

Fig. 3. Drug treatment in hypertension and CAD
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On the day of hospital discharge, antihypertensive, antiaggregation and antilipemic therapy was prescribed (Tabl. Aspirin a 100 mg 1x1, Tabl. Clopidogrel a 75 mg 1x1, Tabl. Rosuvastatin 40 mg 1x1, Tabl. Massido a 5 mg 1x1, Tabl. Relika 4/1.25 mg 1x1, Tabl. Spirinolactone a 25 mg 1x1 and Tabl. Prazosin a 2 mg 1x1) and a recommendation for a hygienic-dietary regimen; a home blood pressure diary and regular ambulatory monitoring was given.

Discussion

Hypertension is defined as resistant if the treatment strategy fails to regulate blood pressure (for systolic <140 mm/Hg, diastolic <90 mm/Hg). Various studies report a prevalence of resistant hypertension of 5-30%. Res-HT should be distinguished from pseudo-resistant hypertension. Poor adherence to prescribed medications, white-coat hypertension, brachial artery calcification are characteristics of pseudo-resistant hypertension.
Other conditions that contribute to unsatisfactory BP level control are: increased intake of salt and alcohol, higher body weight, taking cocaine and anabolics, obstructive sleep apnea.
For diagnostic approach in a patient with resistant hypertension it is important to recognize characterristics (symptoms and signs) of patients with Res-HTA and causes of secondary resistant hypertension (Table 4).

Table 4. Resistant hypertension characteristics, secondary causes and contributing factors

Table 24 Resistant hypertension characteristics, secondary causes, and contributing factors (adapted from reference[385])

Characteristics of patients with resistant hypertension	Causes of secondary resistant hypertension	Drugs and substances that may cause raised BP
Demographics • Older age (especially >75 years) • Obesity • More common in black people • Excess dietary sodium intake • High baseline BP and chronicity of uncontrolled hypertension	**More common causes** • Primary hyperaldosteronism • Atherosclerotic renovascular disease • Sleep apnoea • CKD	**Prescribed drugs** • Oral contraceptives • Sympathomimetic agents (e.g. decongestants in proprietary cold remedies) • Non-steroidal anti-inflammatory drugs • Cyclosporin • Erythropoietin • Steroids (e.g. prednisolone and hydrocortisone) • Some cancer therapies
Concomitant disease • HMOD: LVH and/or CKD • Diabetes • Atherosclerotic vascular disease • Aortic stiffening and isolated systolic hypertension	**Uncommon causes** • Phaeochromocytoma • Fibromuscular dysplasia • Aortic coarctation • Cushing's disease • Hyperparathyroidism	**Non-prescription drugs** • Recreational drugs (e.g. cocaine, amphetamines, and anabolic steroids) • Excessive liquorice ingestion • Herbal remedies (e.g. ephedra and ma huang)

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Resistant hypertension

Recommendations	Class[a]	Level[b]
It is recommended that hypertension be defined as resistant to treatment (i.e. resistant hypertension) when: • Optimal doses (or best-tolerated doses) of an appropriate therapeutic strategy, which should include a diuretic (typically an ACE inhibitor or an ARB with a CCB and a thiazide/thiazide-type diuretic), fails to lower clinic SBP and DBP values to <140 mmHg and/or <90 mmHg, respectively; and • The inadequate control of BP has been confirmed by ABPM or HBPM; and • After exclusion of various causes of pseudo-resistant hypertension (especially poor medication adherence) and secondary hypertension.	I	C
Recommended treatment of resistant hypertension is: • Reinforcement of lifestyle measures, especially sodium restriction.[395] • Addition of low-dose spironolactone[c] to existing treatment;[310,392,394] • Or the addition of further diuretic therapy if intolerant to spironolactone, with either eplerenone,[c] amiloride,[c] a higher-dose thiazide/thiazide-like diuretic, or a loop diuretic;[d 357] • Or the addition of bisoprolol or doxazosin.[310]	I	B

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Fig. 4. Treatment of resistant hypertension
European Heart Journal (2018) 39, 3021–3104 ESC/ESH GUIDELINES doi:10.1093/eurheartj/ehy339

It is an interesting finding that β-blockers have proclivity to increase the risk of developing Res-HT, probably due to the suppression of melatonin synthesis in the pineal gland, which as a final result lead to an increased activity of the sympathetic nervous system [3-5].

Confirmation that Res-HT increases the risk of major CV events and all-cause mortality is found in S. Smith *et al.* study from 2014 [6] as well as in two other studies [1,2] in which Res-HT was associated with a poorer prognosis than nonresistant hypertension. These studies found an increased risk of adverse outcomes

and all-cause mortality as well as CV mortality in patients with Res-HT. Additionally, the Smith's study confirmed that a similar increased risk was associated with Res-HT in patients with concomitant CAD, although event rates were considerably greater in patients with CAD than in those without CAD. However, the pathophysiology of Res-HT increased CV risk is unknown and the assumption is that increased renin-angiotensin system stimulation and aldosterone production leads to increased arterial stiffness and atherosclerotic disease, i.e., increased CV risk [7]. Also, it has been observed that if a higher dose as well as a higher number of antihypertensive drugs are required (regardless of whether it leads to control of Res-HT or not) the CV risk increases [7], so the use of a larger number of antihypertensive agents may not fully mitigate the long-term risks of elevated BP [8], although this is unlikely according to other studies [6]. In agreement with these findings is VALUE study, where patients who received combined antihypertensive therapy to control non-resistant hypertension had a significantly higher risk of CV death compared to those who received only monotherapy [8]. These suggest that Res-HT is an important prognostic factor, and even more valuable than BP control.

Conclusion

Hypertension is the most common risk factor in patients with coronary vascular disease and its early diagnosis is mandatory because the population of patients with HT and CAD is growing worldwide.
Regulation of elevated BP, especially in the resistant form of hypertension, is one of the most important factors for reducing the prevalence of CAD as well as the degree of major adverse cardiovascular events in patients with existing CAD. Res-HT alone portends an increased risk of major CV events and death. Further clinical research is necessary, especially in patients with diabetes, CKD and cardiovascular disease, who as a comorbidity have Res-HT for determination of strategies for its CIE reduction and their CIE complication reductions as well.

Conflict of interest statement. None declared.

Reference

1. Daugherty SL, Powers JD, Magid DJ, *et al*. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012; 125: 1635-1642.
2. Kumbhani DJ, Steg PG, Cannon CP, *et al*. Resistant hypertension: a frequent and ominous finding among hypertensive patients with atherothrombosis. *Eur Heart J* 2013; 34:1204-1214.
3. Paulis L, Simko F. Blood pressure modulation and cardiovascular protection by melatonin: potential mechanisms behind. *Physiol Res* 2007; 56: 671-684.
4. Kario K. Are melatonin and its receptor agonist specific antihypertensive modulators of resistant hypertension caused by disrupted circadian rhythm? *J Am Soc Hypertens* 2011; 5: 354-358.
5. Scheer FA, Van Montfrans GA, van Someren EJ, *et al*. Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. *Hypertension* 2004; 43: 192-197.
6. Smith S, Gong Y, Handberg E, *et al*. Predictors and outcomes of resistant hypertension among patients with coronary artery disease and hypertension. *J Hypertens* 2014; 32(3): 635-643.
7. Smith SM. Epidemiology, prognosis, and treatment of resistant hypertension. *Pharmacotherapy* 2013; 33: 1071-1086,
8. Weber MA, Julius S, Kjeldsen SE, *et al*. Cardiovascular outcomes in hypertensive patients: comparing single-agent therapy with combination therapy. *J Hypertens* 2012; 30: 2213-2222.

Case report

C-REACTIVE PROTEIN IN RHEUMATOID ARTHRITIS TREATED WITH INTERLEUKIN-6 INHIBITOR

Ц-РЕАКТИВЕН ПРОТЕИН КАЈ РЕВМАТОИДЕН АРТРИТ ТРЕТИРАН СО ИНТЕРЛЕУКИН-6 ИНХИБИТОР

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Abstract

Rheumatoid arthritis (RA) is a chronic immune-mediated systemic inflammatory disease characterized by chronic synovial inflammation and hyperplasia, which cause joint erosion and damage along with systemic manifestations. Proinflammatory pathways result in localized joint and systemic inflammation with cytokines, such as interleukin-6 (IL-6), tumour necrosis factor-α (TNF-α), interleukin 1β (IL-1β), as well as downstream signalling pathways. One function of IL-6 is to drive production of the acute-phase reactant C-reactive protein (CRP) following an inflammatory event.

C-reactive protein is not only a marker of inflammation or infection, but it is also an immune regulator. C-reactive protein level is a component of several composite disease activity measures. Higher CRP levels are associated with greater RA disease activity, radiographic progression and joint destruction.

Yet, the usefulness of CRP testing as a routine measure of RA disease activity is not universal due to the substantial proportion of treated patients who experience flares in their RA but still have normal CRP levels.

There may be challenges in assessing remission with 28-joint Disease Activity Score -CRP (DAS28-CRP) when patients are treated with IL-6 inhibitors and other drugs that directly affect CRP levels because a reduction in CRP may not reflect disease activity decrease.

The case that we present is a patient with seropositive RA in whom we tried all available RA treatment modalities including IL-6 inhibitor and two other biologicals, and despite the fact that we achieved low disease activity and sometimes even remission of the underlying disease, radiographic progression and subjective complaints of the patient continued.

Keywords: C-reactive protein, disease activity, interleukin 6-inhibitor, rheumatoid arthritis

Апстракт

Ревматоидниот артритис (РА) е хронична имунопосредувана системска инфламаторна болест која се карактеризира со хронично синовијално воспаление и хиперплазија, при што се предизвикува ерозија и оштетување на зглобовите, како и системски манифестации.

Проинфламаторните настани резултираат со локализирано зглобно и системско воспаление со продукција на цитокини, како што се интерлеукин-6 (IL-6), тумор некротизирачки фактор-α (TNF-α), интерлеукин 1β (IL-1β), како и на последователни сигнални патишта. Една од улогите на IL- 6 е да го поттикне производството на реактантот на акутната фаза С-реактивен протеин (CRP) после појава на воспалителен настан.

Но CRP не е само маркер на воспаление или инфекција, туку и имунорегулатор. Вредноста на CRP е значајна компонента на неколку индекси за активност на болеста како и дел од дефиницијата за ремисија на ревматоидниот артрит.

Сепак, одредувањето на CRP како рутинска мерка за активност на RA не е универзално прифатено поради фактот што значителен дел од третираните пациенти кои доживуваат екзацербација на болеста имаат нормални нивоа на CRP. Проценката на ремисија со Индексот за активност на болеста со 28 зглобови (DAS28-CRP) кај пациентите третирани со IL-6 инхибитори и други лекови кои влијаат врз CRP е предизвикувачка. Во овие случаи ниските нивоа на CRP не корелираат со активноста на болеста кај овие пациенти.

Случајот што го презентираме е пациентка со серопозитивен RA кај која ги испробавме сите достапни модалитети на третман за ревматоиден артритис вклучувајќи го и IL-6 инхибиторот и два други биолошки лекови. Кај пациентката иако постигнавме ниска активност на болеста, а понекогаш дури и ремисија на RA, радиографската прогресија и субјективните поплаки кај пациентката продолжија.

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Клучни зборови: С-реактивен протеин, активност на болеста, 6 инхибитор, ревматоиден артрит

Introduction

Rheumatoid arthritis (RA) is a chronic immune-mediated systemic inflammatory disease characterized by chronic synovial inflammation and hyperplasia, which cause joint erosion and damage, and a range of systemic manifestations, which contribute to overall disease burden [1]. This results in functional decline, disability, and reduced quality of life in patients with rheumatoid arthritis [2]. Comorbidities are common in RA and require a holistic management approach, as multiple comorbidities are associated with poorer clinical outcomes [3].

Proinflammatory pathways result in localized joint and systemic inflammation [1] with cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), as well as downstream signalling pathways [1,4,5]. One function of IL-6 is to drive production of the acute-phase reactant C-reactive protein (CRP) following an inflammatory event [6-8].

C-reactive protein plays an important role in host defence mechanisms against infectious agents and in the inflammatory response [9,10]. C-reactive protein is not only a marker of inflammation or infection, but it is also an immune regulator [9,10]. Binding to immunoglobulin Fc gamma receptors (FcγR), it promotes the production of proinflammatory cytokines leading to an amplification loop of inflammation [11,12]. It is produced predominantly by hepatocytes in response to stimulation by IL-6 [6,7], but CRP has also been reported to be expressed by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes [10]. In healthy adults, plasma CRP concentration is usually <10 mg/L, although there is considerable inter-individual variability [9,13].

Serum CRP levels can be tested using standard or high-sensitivity (hsCRP) assays; hsCRP is used for evaluation of conditions potentially associated with inflammation in otherwise healthy individuals [14]. Additionally, multiple factors can influence baseline serum CRP levels in patients with RA. Single nucleotide polymorphisms in CRP and their haplotypes have been associated with higher or lower CRP levels [15]. Body fat, female hormone levels, dietary quality, and stress have also been shown to influence CRP levels in patients with rheumatoid arthritis [16-19].

There are two isoforms of CRP with different effects and biological properties [20]: pentameric CRP (pCRP) and monomeric CRP (mCRP).

Pentameric CRP (pCRP) is also known as native CRP synthesized in hepatocytes and secreted into the circulation; it is thought to act as an immune regulator when bound to cell membranes or liposomes; can irreversibly dissociate via a conformationally changed intermediate into monomeric CRP (mCRP) [10,21,22].

Monomeric CRP (mCRP) is a proinflammatory isoform able to activate platelets, leukocytes, and endothelial cells as well as to bind complement component 1q (C1q) to activate complement. Monomeric CRP has limited solubility compared to pCRP and is considered to be tissue bound, although transmission via microparticles and ligand complexes has been postulated [10,21,22].

Depending on its structural form, CRP interacts with a variety of leukocytes and endothelial cells, stimulating proinflammatory cytokine release, including IL-6, IL-1β, and TNF-α, upregulating adhesion molecules, increasing monocyte chemoattractant protein-1 release to recruit monocytes, inhibiting nitric oxide production, and activating platelets, thereby inducing proinflammatory and atherogenic effects [10,20,22,11,12,23].

C-reactive protein is a valuable marker and regulator of systemic inflammation in rheumatoid arthritis. CRP level is a component of several composite disease activity measures/indexes: DAS28-CRP, Simplified Disease Activity Index (SDAI), American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) definitions of remission [24-26].

Higher CRP levels are associated with:

1) Greater RA disease activity based on the core components of the 28-joint Disease Activity Score.
2) Radiographic progression and joint destruction in patients with early, moderate and severe rheumatoid arthritis [27-29]. Numerous studies in patients with early RA have shown that elevated CRP levels in patients with early, moderate and severe RA both at baseline and using time-integrated measures correlate with rapid radiological progression and joint damage within 1 year [30-34].
3) Individual aspects of disease activity, such as swollen joint count, and patient-reported measures, including functional status (Health Assessment Questionnaire score), morning stiffness, fatigue, and pain [35-39].
4) Several common comorbidities of rheumatoid arthritis.

Yet, the usefulness of CRP testing as a routine measure of RA disease activity is not universal due to the substantial proportion of treated patients who experience flares in their RA, but still have normal CRP levels suggesting that CRP levels reflect only one of the signs of disease activity and should be assessed in the context of other measures.

Rheumatoid arthritis clinical trials often specify elevated CRP (≥6 mg/L) as an eligibility criterion; patients with active RA but without elevated inflammatory markers (CRP) are commonly excluded from clinical trials [40].

As noted above, CRP is a standard component of many RA composite disease activity measures (DAS28-CRP, SDAI, ACR/EULAR remission) [24-26]. ACR

and EULAR recommend DAS28 using either CRP or erythrocyte sedimentation rate (ESR) without differentiating between them in terms of disease activity thresholds [24]. However, there is evidence that DAS28-CRP scores are consistently lower than DAS28-ESR values [41-43]. Given disease activity thresholds (high >5.1, low disease activity <3.2, and remission <2.6) were originally validated using DAS28-ESR, using the same thresholds for DAS28-CRP may underestimate residual disease activity [41,42]. Consequently, new disease activity thresholds for DAS28-CRP have been proposed: for high disease activity instead DAS28> 5.1 should be used DAS28>4.6; for low disease activity instead DAS28≥2.6 and ≤3.2 should be used DAS28<2.9, and for remission instead DAS28<2.6 should be used DAS28<2.5 [41,43]. Additionally, there may be challenges in assessing remission with DAS28-CRP when patients are treated with IL-6 inhibitors and other drugs that directly affect CRP levels because a reduction in CRP may not reflect disease activity decrease. Thus, a more stringent threshold for DAS28-CRP remission of < 1.9 has been proposed [44].

Case presentation

A female patient, 56-year-old, from Skopje, was referred to a specialist in rheumatology in March/2005 because of morning stiffness duration of about 2 hours, swelling and pain in the small joints of both hands and feet that had lasted for several months. Her blood examination showed elevated erythrocyte sedimentation rate (ESR) and CRP (ESR-35; CRP-18mg/L) and positive rheumatoid factor (RF=128 IU/ml); no anti-cyclic citrullinated peptides antibodies (anti-CCP ab.) were available. Radiographic finding of both hands interpreted by a radiologist on 21.03.2005 showed periarticular soft tissue oedema with initial subchondral cysts of the metacarpophalangeal joints (MCPs) as well as reduction of joint spaces (no radiographic image available).

On physical examination, there was arthritis of the left wrist, bilateral arthritis of the proximal interphalangeal joints 2,3 (PIP2,3) of the hands and bilateral arthritis of PIP 4,5 of the feet, and arthritis of the metatarsophalangeal joint 3 (MTP 3) of the left foot.

Using the ACR criteria for RA, we established the diagnosis of seropositive RA and we started the treatment. The initial therapy was with glucocorticoids (GC) 5 mg Pronizone equivalent/daily, Chloroquine 250 mg daily, Nonsteroidal anti-inflammatory drug (NSAID) in full recommended daily dose and Proton Pump Inhibitor Drug (PPI). It was impossible to achieve remission with this treatment, so we changed this modality after one year and started with a new disease-modifying antirheumatic drug (DMARD) Methotrexate as a monotherapy at a dose of max. 25 mg/weekly. The remaining high disease activity forced us to add a second DMARD-Sulfasalazine (SSZ) 2 gr/daily, and after several months the third DMARD-Hydroxichloroquine (HCQ) 400 mg/daily. Even with the triple basic therapy we could not achieve a good control over the disease activity, so we suspended Methotrexate and changed SSZ with Leflunomide 20 mg/daily and continued with HCQ 400 mg/daily. We were not able to achieve remission following DAS-28 values (See Appendix1).

Appendix 1.
RA-Disease activity: treatment with conventional DMARDs and Rituximab

	Therapy	**ESR**	**CRP**	**DAS28**
03/2006-01/2010	Amp. MTX 15 mg-25 mg i.m/weekly+ GC5-10 mg Pronizone equivalent daily +NSAID + PPI + Folic Acid			
05/2008		23	**7.3**	
02/2010-09/2012	Amp. MTX 15 mg i.m/weekly + Tbl.SSZ 2 gr/daily, CQ 500 mg/daily, GC7.5 mg Pronizone equivalent daily + NSAID + PPI + Folic Acid			
09/2012		27	**9.6**	
10/2012-11/2013	Tbl. Leflunomide 20 mg/daily, CQ 500 mg/daily, GC 5 mg Pronizone equivalent daily + NSAID + PPI			
11/2013		29	**8.3**	**4.03**
12/2013-11/2017	Amp. Rituximab 500 mg i.v. (5 cycles every 6 months) Tbl. Leflunomide 20 mg 1x1, GC 5 mg Pronizone equivalent daily + NSAID + PPI			
09/2015		31	**7.9**	**4.06**
11/2017		18	**10.2**	**4.01**
12/2017-10/2018	Tbl. Leflunomide 20 mg 1x1, GC 5 mg Pronizone equivalent daily + NSAID + PPI			
10/2018		15	**12.7**	**6.39**

ESR - Erythrocyte sedimentation rate, **CRP** - C-reactive protein, **DAS28** - Disease activity score with 28 joints, **MTX** - Methotrexate**; GC** - Glucocorticoids, **NSAID** - Nonsteroidal Anti-inflammatory Drug, **PPI** - Proton Pump Inhibitor Drug (PPI), **CQ** - Chloroquine, **SSZ** - Sulfasalazine, **DMARDs** - Disease modifying Anti-rheumatic drugs

Appendix 3:
Radiographic progression: hands during treatment with conventional DMARDs and Rituximab

Peryarticular osteoporosis; Right radiocarpal joint: pseudocysts, reactive sclerosis and partial ankylosis. Distal part of the ulna remodeled on both sides, with subcortical erosion on the left side. Carpus: partial ankylosis on both sides, partial ankylosis of the carpo-metacarpal joints.The MCP-1 joint of the both hands and MCP- 3 of the right hand with pseudocysts.

Progresion of the ankylosis on the radiocarpal joint on the right side ,initial ankylosis on the radiocarpal joint on the left side.

Generalizated osteoporosis of the sceleton. Progression of radiocarpal and carpal joint ankylosis with reactive sclerosis, remodeling of os lunatum, subluxation of MCP joints on both hands more pronounced on MCP-5.

This time we had the possibility to use the first biological agent available in North Macedonia-Rituximab in combination with Leflunomide 20 mg/ daily. After five cycles of Rituximab, the disease activity remained high. The DAS28-CRP showed that our patient experienced flare. The radiological progression during treatment with conventional DMARDs and Rituximab was obvious, and hence we excluded Rituximab due to ineffectiveness. Detailed chronological treatment and disease activity in our patient in a period beginning from 2006 to 2018 is shown in Appendix 1.

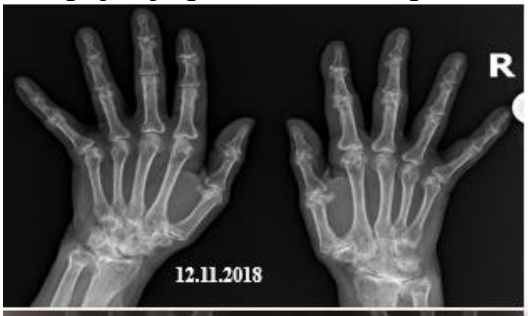
We added IL-6 inhibitor-Tocilizumab as a new biological agent in November, 2018. The treatment with IL-6-inhibitor has been given continuously for four years at a dose of 165 mg subcutaneously once a week in combination with tbl. Leflunomide of 20 mg/daily. The CRP values were monitored periodically, and we always got low CRP values that did not correlate with the patient's symptoms. Disease activity score - CRP value when starting anti-IL-6 was 2.69 (March, 2022) but the patient still had active arthritis.

Appendix 2:
RA-disease activity: treatment with anti –IL6 biological and Leflunomide

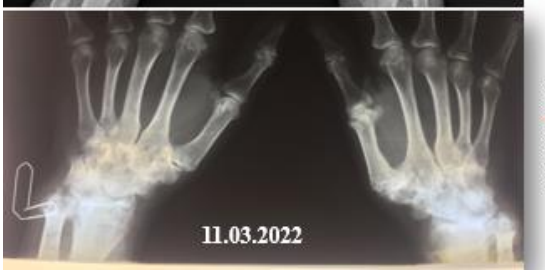
	DAS-28	CDAI	SDAI	HAQ-100	ESR	**CRP**
30.11.2018	**6.39**	45.00	46.20	38.70	15	**12.7**
08.01.2019	**3.21**	22.00	22.10	43.75	2	**1**
16.04.2019	**2.74**	4.00	4.10	46.25	2	**1**
16.07.2019	**2.53**	1.00	1.00	31.25	4	**0.5**
08.11.2019	**2.87**	4.00	4.10	36.25	6	**1**
21.02.2020	**2.63**	9.00	9.10	16.25	4	**1**
07.09.2020	**2.76**	10.00	10.10	40.00	4	**1**
10.06.2021	**2.61**	9.00	9.00	38.75	6	**0.6**
11.03.2022	**2.69**	32.00	33.20	38.25	7	**6**

DAS28 - Disease Activity Score with 28 joints, **CDAI** - Clinical Disease Activity Index, **SDAI** - Simplified Disease Activity Index, **HAQ100** - Health Assessment Questionnaire, **ESR** - Erythrocyte sedimentation rate, **CRP** - C-reactive protein

Appendix 4:
Radiographic progression: hands during treatment with IL-6 inhibitor + Leflunomide



Generalisated steoporosis of the skeleton. Progression of radiocarpal and carpal joint ankylosis with reactive sclerosis, subluxation of MCP joints on both hands more pronounced on MCP-5. Distal part of the ulna remodeled on both sides. Carpus: partial ankylosis on both sides, partial ankylosis of the carpo-metacarpal joints. Remodeling of os lunatum.



Osteoporosis of the skeleton shown on both hands, with marked ankylosis of MCP joints. Subluxation and ulnar deviation of several MCP joints with pseudocysts and subcortical erosions. Marked carpal joint ankylosis with reactive sclerosis. Progresive ankylosis on the radiocarpal joint on both sides.

Having a disproportion between the patient's complaints and the low DAS28-CRP, and considering the fact that IL-6 inhibitor directly influences CRP values and thus we could not have a clear picture of the activity of the disease, we decided to introduce a new biological agent, TNF-α inhibitor-Infliximab. Three months later, we assessed the disease activity using the same DAS28-CRP. This time its value was 2.35 and there was also a correlation of DAS28-CRP values with the objective and subjective symptoms of the patient.

Discussion

We have had a rare opportunity to treat a RA patient not only with several conventional treatment modalities but also with three biological agents: anti-CD20 agent, anti-IL-6 agent and TNF-α inhibitor. We used several disease activity scores. For the purpose of this case report, we focused on DAS28-CRP and anti-IL-6 agent treatment in our patient. We periodically performed DAS28-CRP to assess RA-activity. DAS28-CRP values indicated a low disease activity, but those measures were uncorrelated with the actual disease activity in our patient, in whom we constantly had multifocal active symmetrical arthritis, stiffness and radiological progression (see Appendix 2 and Appendix 4).
Low levels of CRP while assessing remission with DAS28-CRP in our patient treated with IL-6 inhibitor did not reflect disease activity decrease. We agree with the authors R.M. Fleischmann, *et al* [41,43] that a more stringent threshold for DAS28-CRP should be used: for high disease activity instead DAS28>5.1 should be used DAS28>4.6; for low disease activity instead DAS28≥2.6 and ≤3.2 should be used DAS28< 2.9, and for remission instead DAS28<2.6 should be used DAS28<2.5 and for patients treated with IL6-inhibitor agent DAS28-CRP remission of<1.9 [44].
We have witnessed radiographic progression and joint destruction in our patient (See Appendix 3 and Appendix 4). Our findings are in accordance with numerous studies in patients with RA treated with anti-IL-6 agent which have shown that low CRP levels at baseline and using time-integrated measures do not correlate with radiological progression and joint damage.

Conclusion

1. During ongoing treatment with IL-6 inhibitor, our patient with active RA did not have an elevated CRP.
2. Low level of CRP did not reflect a disease activity decrease in our patient treated with IL-6 inhibitor agent.
3. We documented radiographic progression and bone destruction in spite of the low levels of C-reactive protein.
4. Assessing remission with DAS28-CRP in our patient treated with IL-6 inhibitor (that directly affected levels of CRP), was difficult and inaccurate.
5. Following DAS based on 28 joints thresholds (for high disease activity >5.1, low disease activity <3.2, and remission <2.6), we underestimated residual disease activity in our patient.
6. It is wise to introduce new disease activity thresholds for DAS28-CRP in patients treated with anti-IL-6 agent as follows: for high disease activity >4.6, for low disease activity <2.9, and <1.9 for remission, as has been proposed before.

Conflict of interest statement. None declared.

References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011; 365: 2205-2219.
2. Bombardier C, *et al.* The relationship between joint damage and functional disability in rheumatoid arthritis: a systematic review. *Ann Rheum Dis* 2012; 71: 836-844.
3. Ranganath VK, *et al*. Comorbidities are associated with poorer outcomes in community patients with rheumatoid arthritis. *Rheumatology* 2013; 52: 1809-1817.
4. Firestein GS, McInnes IB. Immunopathogenesis of rheumatoid arthritis. *Immunity* 2017; 46: 183-196.
5. Ridgley LA, Anderson AE, Pratt AG. What are the dominant cytokines in early rheumatoid arthritis? *Curr Opin Rheumatol* 2018; 30: 207-214.
6. Castell JV, *et al.*Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989; 242: 237-239.
7. Choy E, Rose-John S. Interleukin-6 as a multifunctional regulator: inflammation, immune response, and fibrosis. *J Scleroderma RelatDisord* 2017; 2: S1-S5.
8. Jones SA, *et al*. Interleukin 6: the biology behind the therapy. *Consid Med* 2018; 2: 2-6.
9. Ansar W, Ghosh S. C-reactive protein and the biology of disease. *Immunol Res* 2013; 56: 131-142.
10. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol* 2018; 9: 754.
11. Newling M, *et al.* C-Reactive protein promotes inflammation through FcγR-Induced glycolytic reprogramming of human macrophages. *J Immunol* 2019; 203: 225-235.
12. Salazar J, *et al.* C-reactive protein: an in-depth look into structure, function, and regulation. *IntSch Res Notices* 2014; (2014), Article 653045.
13. Pepys MB, Hirschfield GM. C-reactive protein: a critical update, *J Clin Invest* 2003; 111: 1805-1812.
14. FDA. Review Criteria for Assessment of C Reactive Protein (CRP), High Sensitivity C-Reactive Protein (hsCRP) and Cardiac C-Reactive Protein (cCRP) Assays - Guidance for Industry and FDA Staff, <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/review-criteria-assessment-c-reactive-protein-crp-high-sensitivity-c-reactive-protein-hscrp-and>(2005).
15. Ammitzboll CG, *et al.* CRP genotype and haplotype associations with serum C-reactive protein level and DAS28 in untreated early rheumatoid arthritis patients. *Arthritis Res Ther* 2014; 16: 475.
16. Bärebring L, Winkvist A, Gjertsson I, Lindqvist HM. Poor dietary quality is associated with increased inflammation in Swedish patients with rheumatoid arthritis. *Nutrients* 2018; 10; 1535.
17. Giles JT , *et al.* Association of body fat with C-reactive protein in rheumatoid arthritis. *Arthritis Rheum* 2008; 58: 2632-2641.
18. Kass AS, Lea TE, Torjesen PA, *et al*. The association of luteinizing hormone and follicle-stimulating hormone with cytokines and markers of disease activity in rheumatoid arthritis: a case-control study. *Scand J Rheumatol* 2010; 39: 109-117.
19. Veldhuijzen van Zanten JJ, Ring C, Carroll D, Kitas GD. Increased C reactive protein in response to acute stress in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 1299-1304.
20. Eisenhardt SU, Thiele JR, Bannasch H, *et al*. C-reactive protein: how conformational changes influence inflammatory properties. *Cell Cycle* 2009; 8: 3885-3892.
21. Thiele JR, *et al.* Targeting C-reactive protein in inflammatory disease by preventing conformational changes. *Mediators Inflamm* 2015 (2015), Article 372432.
22. McFadyen JD, *et al.* Dissociation of C-reactive protein localizes and amplifies inflammation: evidence for a direct biological role of C-reactive protein and its conformational changes. *Front Immunol* 2018; 9: 1351.
23. Urman A, Taklalsingh N, Sorrento C, McFarlane IM. Inflammation beyond the joints: rheumatoid arthritis and cardiovascular disease. *Scifed J Cardiol* 2018; https://www.researchgate.net/publication/329525180_Inflammation_beyond_the_Joints_Rheumatoid_Arthritis_and_Cardiovascular_Disease.
24. England BR, *et al.* 2019 Update of the American College of Rheumatology recommended rheumatoid arthritis disease activity measures. *Arthitis Care Res* 2019; 71: 1540-1555.
25. Felson DT, *et al.* American College of Rheumatology/ European League against Rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. *Ann Rheum Dis* 2011; 70: 404-413.
26. Wells G, *et al.* Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis* 2009; 68: 954-960.
27. Jansen LM, van der Horst-Bruinsma IE, van Schaardenburg D, *et al*. Predictors of radiographic joint damage in patients with early rheumatoid arthritis. *Ann Rheum Dis* 2001; 60: 924-927.
28. Bay-Jensen AC, *et al.* Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis. *BMC Rheumatol* 2019; 3: 3.
29. Yeh JC, *et al*. Non-hepatic alkaline phosphatase, hs-CRP and progression of vertebral fracture in patients with rheumatoid arthritis: a population-based longitudinal study. *J Clin Med* 2018; 7: 439.
30. Curtis JR, *et al.* Predicting risk for radiographic damage in rheumatoid arthritis: comparative analysis of the multi-biomarker disease activity score and conventional measures of disease activity in multiple studies. *Curr Med Res Opin* 2019; 1-11.
31. Emery P, Gabay C, Kraan M, Gomez-Reino J. Evidence-based review of biologic markers as indicators of disease progression and remission in rheumatoid arthritis. *RheumatolInt* 2007; 27: 793-806.
32. Bagdi R, Aswani P, Singh VK, & Verma MK. C-reactive protein as a disease activity marker in rheumatoid arthritis. *International Journal of Health Sciences* 2022; 6(S2): 10587²10593. https://doi.org/10.53730/ ijhs.v6nS2.7717.
33. Navarro-Compán V, *et al.* Relationship between disease activity indices and their individual components and radiographic progression in RA: a systematic literature review. *Rheumatology* 2015; 54: 994-1007.
34. Vanier A, *et al.* An updated matrix to predict rapid radiographic progression of early rheumatoid arthritis patients: pooled analyses from several databases. *Rheumatology* 2019; kez542.
35. Dessein PH, Joffe BI, Stanwix AE. High sensitivity C-reactive protein as a disease activity marker in rheumatoid arthritis. *J Rheumatol* 2004; 31: 1095-1097.
36. Jansen LM, van Schaardenburg D, van Der Horst-Bruinsma IE, *et al*. Predictors of functional status in patients with early rheumatoid arthritis. *Ann Rheum Dis* 2000; 59: 223-226.
37. Keenan RT, Swearingen CJ, Yazici Y. Erythrocyte sedimentation rate and C-reactive protein levels are poorly

correlated with clinical measures of disease activity in rheumatoid arthritis, systemic lupus erythematosus and osteoarthritis patients. *ClinExpRheumatol* 2008; 26: 814-819.

38. Madsen SG, Danneskiold-Samsøe B, Stockmarr A, Bartels EM. Correlations between fatigue and disease duration, disease activity, and pain in patients with rheumatoid arthritis: a systematic review. *Scand J Rheumatol* 2016; 45: 255-261.
39. Sarzi-Puttini P, *et al.* Correlation of the score for subjective pain with physical disability, clinical and radiographic scores in recent onset rheumatoid arthritis. *BMC MusculoskeletDisord* 2002; 3: 18.
40. Janet E Pope, Ernest H Choy. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. *Semin Arthritis Rheum* 2021; 51(1): 219-229.
41. Fleischmann RM, *et al.*DAS28-CRP and DAS28-ESR cut-offs for high disease activity in rheumatoid arthritis are not interchangeable. *RMD Open* 2017; 3: Article e000382.
42. Hamann PDH, *et al.* Gender stratified adjustment of the DAS28-CRP improves inter-score agreement with the DAS28-ESR in rheumatoid arthritis.*Rheumatology* 2019; 58: 831-835.
43. Kuriya B, *et al.* Thresholds for the 28-joint disease activity score (DAS28) using C-reactive protein are lower compared to DAS28 using erythrocyte sedimentation rate in early rheumatoid arthritis. *ClinExpRheumatol* 2017; 35: 799-803.
44. Schoels M, Alasti F, Smolen JS, Aletaha D. Evaluation of newly proposed remission cut-points for disease activity score in 28 joints (DAS28) in rheumatoid arthritis patients upon IL-6 pathway inhibition. *Arthritis Res Ther* 2017; 19: 155.

Case report

PULMONARY EMBOLISM AS A COMPLICATION AFTER ARTHROSCOPIC INTERVENTION OF THE KNEE

ПУЛМОНАЛНА ЕМБОЛИЈА КАКО КОМПЛИКАЦИЈА ПОСЛЕ АРТРОСКОПСКА ИНТЕРВЕНЦИЈА НА КОЛЕНО

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Abstract

Arthroscopy is a minimally invasive surgical procedure on the joints that is used to examine and, if necessary, proceed to the treatment of possible damage to the joint with the help of an endoscope that is inserted through a small incision. The advantage over traditional open surgery is that the joint does not have to be completely opened. For knee arthroscopy, only two small incisions are made, one for the arthroscope and one for the surgical instruments used in the knee socket. This reduces recovery time and increases the success rate due to less trauma to the surrounding tissue. Due to the faster recovery and fewer postoperative scars, it has become a more acceptable method compared to the classic surgical treatment.
Venous thromboembolism (VTE) is clinically presented as DVT or PE and is globally the third most common acute cardiovascular syndrome after myocardial infarction and stroke. In epidemiological studies, annual incidence rates for PE range from 39.115 per 100.000 population, for DVT incidence rates range from 53.162 per 100,000 population [1,2]. It is known that the risk of deep vein thrombosis resulting in pulmonary embolism, also known as venous thromboembolism (VTE), is increased by operations requiring general anesthesia lasting longer than 30 min. However, even minor surgeries such as knee arthroplasties are known to significantly increase the risk of VTE [3]. Despite this, the overall incidence of VTE is extremely low and is estimated to be <0.1% [4].

Keywords: ?

Апстракт

Во приказов презентираме 50 годишна пациентка која се јави на ортопедски преглед во нашата болница, три недели после артроскопска операција на колено. Се жали на болка во градите и во рбетен столб, но поради податокот дека исплукува крвава содржина, индициран е интернистички преглед. Пациентката на преглед е тахикардична, тахипноична, со периферна цијаноза и со сатурација sPO2=83%. Според стратификацијата на Женева скалата, пациентката е со висока клиничка веројатност за пулмонална емболија и индицирана е КТ ангиографија со контраст на бели дробови. На направената ангиографија се следат дефекти во полнењето на главното стебло на левата пулмонална артерија, како и консолидација во инфериорниот лобус.

Клучни зборови: ?

Case report

We present a case of a patient who came to our hospital for an orthopedic examination, but due to chest pain and hemoptysis, an internist was called for consultation. The 50-year-old patient gave us information about chest pain, rapid heartbeat, and for the last few days, a cough with spitting up blood. The problems started two weeks ago, one week after a knee arthroscopy. From pharmacological therapy, she took analgesics and anticoagulant therapy (tbl. Rivaroxaban a 10 mg 1x1) which she stopped 5 days after the operation. Physical examination revealed tachycardia up to 128/min, TA=85/65 mmHg, peripheral cyanosis with sPO2=83% and weakened breathing on the left side with moist rales up to the middle parts of the lung. A risk stratification was performed according to the Geneva score, which revealed that the patient presented with a high clinical probability of pulmonary embolism. A contrast-enhanced CT angiography of the lungs was immediately performed, which showed filling defects of the main trunk of the left pulmonary artery, as well as consolidation in the inferior lobe.

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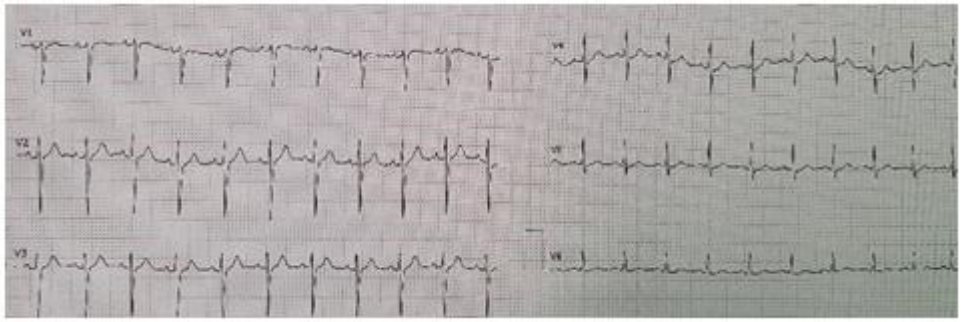

Fig. 1. ECG on admission

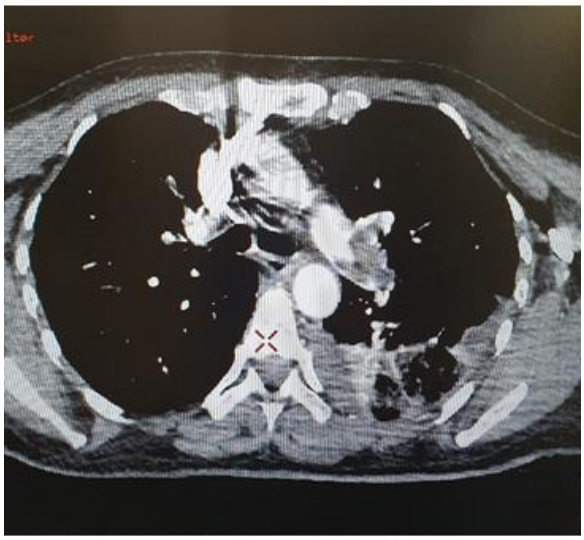

Fig. 2. CT angiography on admission

The patient was immediately hospitalized in the intensive care unit. She was placed on oxygen support. Due to hemodynamic instability and despite the fact that several days had passed since the onset of symptoms, it was decided to start thrombolytic therapy. Parenteral antibiotic therapy was also given, due to the presence of consolidation in the inferior lobe. The next day, an echocardiography was performed, which did not show any significant right heart overload. In two days, symptoms improved, sPO2=92%, heart rate was 94/min. After the initial thrombolytic therapy, the patient was placed on oral NOAC therapy (apixaban 10 mg 2x1 for 7 days, then apixaban 5 mg 2x1). The patient was discharged from the hospital after 10 days of hospitalization, with normal vital and laboratory parameters.

Discussion

Conventional therapy with vitamin K antagonists or low-molecular-weight heparin after orthopedic interventions for the prevention of venous thromboembolism has been shown not to be superior to therapy with direct oral anticoagulants. Evidence from four RECORD studies shows that NOACs are superior to enoxaparin after major orthopedic interventions. In the overall primary outcome for any deep vein thrombosis, non-fatal pulmonary embolism, and death from all these causes, the risk was reduced by 70-79% after hip surgery and 31-49% after knee surgery. Rivaroxaban is also non-inferior to low molecular weight heparin in terms of safety against major bleeding. Apart from prevention, the RECORD3 and RECORD4 studies showed that the optimal duration of therapy was 10-14 days, in order to achieve a satisfactory level of prevention of events related to venous thromboembolism [5].

Conclusion

Despite the excellent anticoagulant effect of NOAC in the prevention of venous thromboembolism after orthopedic interventions, it is extremely important to apply the appropriate dose and duration of therapy when a drug from the NOAC group is the drug of choice. Otherwise, with a lower dose than intended, or too short treatment time, not only the risk of bleeding is not reduced, but the patient is exposed to an increased risk of fatal or non-fatal venous thromboembolism.

Conflict of interest statement. None declared.

References

1. Wendelboe AM, Raskob GE. Global burden of thrombosis: epidemiologic aspects. *Circ Res* 2016; 118: 1340-1347.
2. Keller K, Hobohm L, Ebner M, *et al*. Trends in thrombolytic treatment and outcomes of acute pulmonary embolism in Germany. *Eur Heart J* 2020; 41: 522-529.
3. Adrichem RA, Nelissen RG, Schipper IB, *et al*. Risk of venous thrombosis after arthroscopy of the knee: results from a large population-based case-control study. *Thromb Haemost* 2015; 13: 1441-1448.
4. Hetsroni I, Lyman S, Do H, *et al*. Symptomatic pulmonary embolism after outpatient arthroscopic procedures of the knee: the incidence and risk factors in 418, 323 arthroscopies. *J Bone Joint Surg Br* 2011; 93: 47-51.
5. Turpie AG, Lassen MR, Davidson BL, *et al*. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial, *Lancet* 2009; 373(9676): 1673-1680.

УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед" (ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.

Списанието ги има следниве рубрики и категории на трудови:

1. **Изворни трудови**
2. **Соопштувања за клинички и лабораториски искуства**
3. **Прикази на случаи**
4. **Од практика за практика**
5. **Едукативни статии**
6. **Вариае (**писма од редакцијата, општествена хроника**,** прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката „Во сеќавање„ и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриките 2-5 имаат белези на стручни трудови.
Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.
Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.
Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

1. ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Тимес Нењ Роман големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.
Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

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Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспондеција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со МеСХ (Медицал Сибјецт Хеадингс) листата на Индеџ Медицус.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните СИ единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

3. ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).
Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

4. ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. Ш3-6Ќ).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Индекс Медицус):
а) ***статија во списание*** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор.*) Неглиа ЈП Меадоњс АТ, Робисон ЛЛ *ет ал*. Секонд неопласмс афтер ацуте лsмпхобластиц леукемиа ин цхилдхоод. Н Енгл Ј Мед 1991; 325:1330-6.
б) заеднички автор
ГИВИО (Интердисциплинарs гроуп фор цанцер царе евалуатион). Редуцинг диагностиц делаs ин бреаст цанцер. Поссибле тхерапеутиц имплицатионс. *Цанцер* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Бреаст сцреенинг: нењ евиденце. (*Едиториалл Ланцет* 1984; и :1217-8).
г) ***поглавје во книга или монографија***
Њеинстеин Л, Сњартз МН. Патхогениц пропертиес оф инвадинг мицроорганисмс. Во: Содеман ЊА Јр, Содеман ЊА, Ед. Патхогениц пхscиологs: мецханисмс оф дисеасе. Пхиладелпхиа; Њ Б Саундерс, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

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Детални информации можете да добиете на телефонот на Друштвото 02 3 162 557.

Известување за рецензентите за ММП

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