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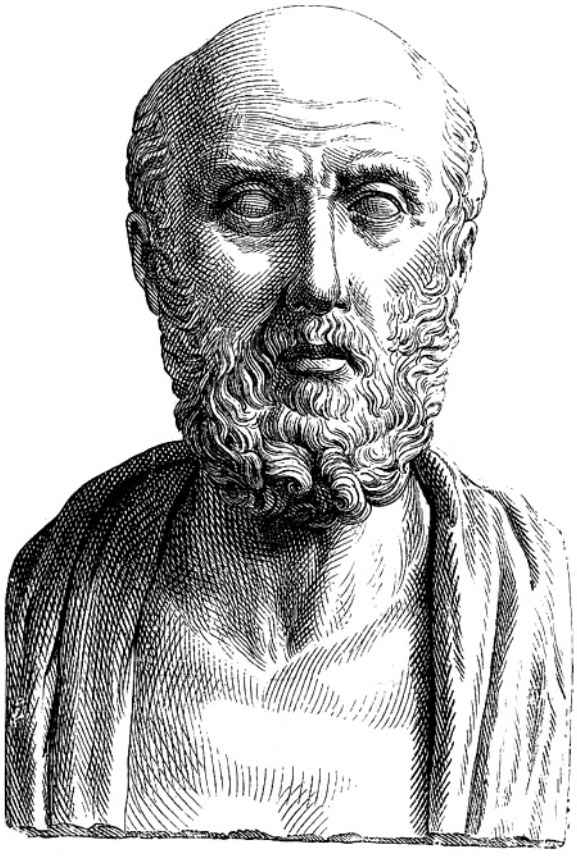

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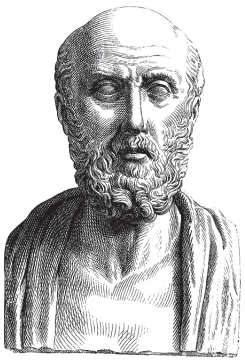

Hippocrates (born around 460 – died around 380 BC), was an ancient Greek physician who is referred to as the "Father of Modern Medicine". He revolutionized medicine in ancient Greece establishing it as a discipline distinct from other fields with which it had traditionally been associated (theurgy and philosophy), thus establishing medicine as a separate profession (see pages 1181-6).

Hipokrat (rođen oko 460. pne - umro oko 380. pne) starogrčki lekar, smatra se ocem savremene medicine. On je modernizovao medicinu u antičkoj Grčkoj odvojivši je od magije i filozofije sa kojima je, u to vreme, bila tradicionalno povezana čime je postavio temelj medicini kao posebnoj disciplini (see str. 1181-6).

Dear Authors, Editors, Peer Reviewers and Readers of the Vojnosanitetski pregled,
I thank you for your cooperation and support in the last year and wish you all the best in the coming 2017!

MERRY CHRISTMAS AND HAPPY NEW YEAR!

Cordially,
Prof. Silva Dobrić, PhD
Editor-in-Chief

Poštovani autori, urednici, recenzenti i čitaoci Vojnosanitetskog pregleda,
Uz zahvalnost na saradnji i podršci u protekloj godini,
želim vam sve najbolje u nastupajućoj 2017!

SREĆNA NOVA GODINA I BOŽIĆNI PRAZNICI!

Srdačno,
prof. dr Silva Dobrić
glavni i odgovorni urednik

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Poziv za reklamiranje u 2017. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline)*, *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je dostupan *on line* od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2017. godini su:

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E D I T O R I A L

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FMR1 gene mutations cause neurodevelopmental-degenerative disorders: Importance of fragile X testing in Serbia

Mutacije *FMR1* gena uzrokuju razvojne i degenerativne poremećaje nervnog sistema: značaj testiranja na nestabilni X hromozom u Srbiji

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Scientific advances in biomedicine have enabled translation of preclinical research breakthroughs to clinical trials during the last decades. Resources required for such effort are typically available in developed countries such as the USA. Kennedy Krieger Institute in Baltimore, Maryland is an internationally recognized institution dedicated to improving the lives of individuals with disorders of the brain, spinal cord, and musculoskeletal system (https://www.kennedykrieger.org). Clinical Trials Unit (CTU) at the Institute is one of the top-level institutions in the world that conduct the advances in translational medicine. The Unit helps advance treatment, prevention, and possible cures (https://www.kennedykrieger.org/research-training/centers-labs-cores/clinical-trials-unit). This and other similar institutions bring together world-leading experts in clinical research in order to provide state-of-the-art treatment of previously untreatable disorders for participants. One of them is fragile X syndrome (FXS), the hallmark of Fragile X-associated disorders (FXD), which is at the forefront of translational efforts to develop such targeted treatments. Specifically, FXS is the most translated among all neurodevelopmental disorders in human clinical trials. Namely, preclinical breakthroughs have generated much interest by the field to translate them into humans with FXS, and possibly autism spectrum disorder (ASD), a major public and economic health burden on society worldwide. Specifically, a recent search of www.clinicaltrials.gov, the National Institute of Health (NIH) sponsored website, and literature search revealed that 22 double-blind, placebo-controlled clinical trials [1, 2] have been registered in humans with FXS, as required by the Food and Drug Administration (FDA) Act of 2007. Gaps in translating the above successes have been identified. At present, no symptomatic or disease modifying treatments for FXS have received regulatory approval. Then the most respectable medicinal regulatory authorities in the world (e.g. FDA) greatly benefit from efforts of leading clinical and research experts in the USA to address these gaps and advance these clinical trials of humans with FXS that were conducted mostly from 2008 to 2015 [1–3]. To briefly backtrack, collaborative effort among scientific institutions in developed, and some developing countries, is key to the establishment of local specialty fragile X clinics, and even clinical and research consortiums, which is of a vital importance for the successful translation of such treatment advances. Here, we highlight the importance of the Fragile X Clinical & Research Consortium (FXCRC). The Consortium is a collaborative endeavor initiated in 2006 by the National Fragile X Foundation (NFXF) to advance clinical practice and facilitate coordinated, collaborative multi-site research on FXS, which currently consists of 28 clinics in the USA and Canada. The Consortium has formed several committees designed to address common issues with regard to best practices in evaluation and treatment, strategies for supporting and enhancing clinic work, and research priorities, such as Clinical Trials Committees. Next, Fragile X Clinical and Research Consortium Registry and Database (FORWARD) is the Center for the Disease Control (CDC) funded project now 5-year renewed through 2020 (PI: Brown, 1 U01 DD001189-01) in which the Consortium works closely with the CDC and the NFXF. The project helps establish standards of care, facilitate the conduct of multi-institutional clinical research projects, coordinate and organize research across sites, build a reliable, dynamic patient registry and assist member clinics in data collection and analysis, including effective and relevant outreach and surveillance The Institute is one of the sites for the FORWARD

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project (https://www.kennedykrieger.org/research-training/current-research-projects/clinical-research/fragile-x-clinical-and-research-cooperative (PI: Budimirovic). In addition, in 2014, Dr Budimirovic also led the Consortium's Clinical Committee effort on expert Consensus Document on ASD in FXS featured on the NFXF website [4], which was then followed by a large cohort evidence-based study that characterized ASD in FXS [5]. The Consensus Documents are supported in part by the CDC-funded grant and created and reviewed by a variety of FXCRC clinicians with many clinical backgrounds in order to offer the most up-to-date insight on a variety of subjects related to Fragile X.

An increase in life expectancy and decrease in mortality in developing countries, particularly in low, middle and upper middle-income countries, many of them in transition, has been noted during the past several decades. These positive trends are by and large because of improvements in their quality of life and diet and their health care systems, especially in disease and disorder prevention, early diagnosis, and appropriate treatments. Among other benefits, the progress delays the onset and decreases the severity of chronic diseases including neurodevelopmental [e.g., FXS, ASD, non-FXS intellectual disabilities (ID)], and neurodegenerative [i.e., fragile X-associated tremor ataxia syndrome (FX-TAS)] disorders [6–8]. For example, challenging behaviors seen in a higher proportion of children with FXS who meet criteria for ASD than those who don't can significantly impact an individual's academic and adaptive functioning, limiting their participation in the community. There are three levels of interventions aimed at decreasing the implications of these disabilities: society (healthy public policy) [9], health-care community (e.g., through education activity, training, and public campaigns) [10] and individual (e.g., counseling, screening, and availability of information about clinical preventive services) [11]. In Serbia, the incidence of FXD as the umbrella of FXS, non-FXS ID or FX-TAS is still unknown, which is a common case for these countries in transition. Consequently, socio-economic factors such as poverty and lack of adequate research support (e.g., inadequate laboratory equipment and diagnostic test) interfere with the early diagnosis and treatment of these disorders. Hence, it is often necessary to develop the collaborative programs and network between developed country and upper middle income countries such as Serbia. The effort contribute to building sustainable research capacity in these countries aimed at reaching long-term goals: prevention, early diagnosis of the FXD full mutation and adequate treatment strategies. In Serbia, such strategies don't exist but they need to include genetic, social and economic resources to prevent the above noted negative impact of FXS on brain development and function resulting in functionally interfering behavioral and other problems that negatively affect the quality of life of these individuals and their families. Together, there is a compelling need to develop new networks and an international multidisciplinary study in Serbia in order to describe the feasibility of screening for frequency of FXD among pediatric and adult populations with relevant disabiliti, and to start building their Registry, Database and Repository.

FMR1 gene mutations. There has been great progress in the fragile X field since the discovery of the Fragile X Mental Retardation 1 (*FMR1*) gene in 1991 by Drs. Ben Oostra, David Nelson, and Stephen Warren [12–14]. The gene is located on the Xq27.3 chromosome. There are two types of *FMR1* mutations that expand the number of CGG triplet repeats: normal (30–45) triplets, full-mutation (FM): > 200 triplets, and premutation (PM); 55–200 triplets [15, 16]. The impact of 45–54 CGG nucleotide repeats in the *FMR1* gene has not been studied enough [17]. The mutations of the *FMR1* gene cause both neurodevelopmental and neurodegenerative disorders under the umbrella of FXD. FXS is a global neurodevelopmental disorder caused by the FM mutation in the promotor region of the *FMR1* gene that leads to epigenetic (hypermethylation) silencing and thus, to the absence or reduction of its encoding protein: fragile X mental retardation protein (FMRP) [18]. Affecting up to 1 in 2500 boys, FXS is the leading cause of an inherited form of ID and the most known monogenetic cause of ASD that is purely behaviorally defined by Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 [American Psychiatric Association, 2013], in contrast to FXS that is a medical/genetic disorder characterized by ASD in up to 2 out of 3 boys [6, 7, 19, 20]. As such, FXS is the most studied ASD model 'disorder of synapse' due to their profound clinical and biological overlaps [4]. Specifically, FMRP, an RNA-binding protein that is heavily expressed in the brain, targets approximately 4% of the transcribed mRNAs in the brain, and as many as 842 of these transcribed mRNAs converge on the same pathway as idiopathic ASD [16, 21–25]. Therefore, the lack of a specific treatment for both FXS and ASD has propelled the need to develop core targeted therapies. Briefly, since FMRP acts as a "brake", the absence of the fragile X gene's encoded protein in FXS causes up-regulation of metabotropic glutamate receptor 5 (mGluR5), mTOR, MMP9, RAS, GSK3-beta, and PI3K signaling, and down-regulation of GABA, and cAMP [and protein kinase (PKA), CAMP response element binding protein (CREB) signaling, which leads to an imbalance in neural excitation/inhibition [7, 16, 26]. Collectively, these findings constitute 'the mGluR theory' by Bear et al. [26], providing the basis for the aforementioned clinical trials [17]. In addition, preclinical studies have shown decreased activity of insulin signaling, and that metformin can normalize at least the cAMP [and cAMP-dependent PKA and CREB] [27, 28].

Prevalence of PM is in up to 1 : 150 women and 1 : 400 men, which is about 10 times more common than FM [16]. Currently, 1.5 million individuals are affected with the PM ("carriers") in the US, and over 20 million worldwide. FX-TAS is a serious adult neurological disorder that results in cognitive, gait, and motor deficits in approximately 40–50% of carrier men and 16% of carrier women, with an average age of onset at 62 years [8]. Importantly, pathophysiological mechanisms underlying PM and FM are different and distinct. In PM, the accumulation of mRNA becomes toxic to the cell. However, in FM, the *FMR1* epigenetic silencing results in the lack of FMRP. Furthermore, PM causes different clinical phenotypes: for example, it can cause anywhere from minimal/moderate to severe cases such as fragile X-

associated primary ovarian insufficiency (FX-POI), early menopause in adult females (not the focus of this article) or FX-TAS in adult males.

FMR1 mutations diagnosis. The genetic diagnosis of FXD has much improved over time. The chromosome cytogenetic test was the only available diagnostic tool for FXD during the 1970s and 1980s. Nowadays, molecular diagnosis for FXD is possible using the most advanced method. The "*FMR1* DNA" (Southern blot fragile X testing in the text) is "standard of care" for determining the presence of *FMR1* mutations. Advanced methods assess not only the degree of CGG expansion, but also *FMR1* gene promoter methylation status (mPCR) [7]. Furthermore, the quantification of the spectrum of methylation characteristics in patients with *FMR1* expansions is available by the use of mPCR [29, 30]. Also, in order to improve the diagnosis, prognosis and treatment options for affected individuals, quantification of FMRP (qFMRP) is useful [31]. This is also of relevance for asymptomatic carriers with PM, and rare asymptomatic individuals with FM but *FMR1* is not silenced (these individuals have FMRP levels that are ~20% of normal individuals) [32, 33]. Regardless, new kits have created easy-to-use, accessible, and high performance methods for laboratories. These new kits can measure the methylation fraction for each *FMR1* allele at a higher resolution than the Southern blot. In addition, there is an opportunity to detect mosaic alleles in affected individuals. For example, scientists at Asuragen, Inc from Texas in collaboration with fragile X experts in the USA have developed Amplidex® and Xpansion® Interpreter *FMR1,* which offer a very successful suite [29, 30]. The advanced test also measures the presence and the number of AGG nucleotide triplets between CGGs (AGG interruptions), called 'speed bumps', which are important for the stability of *FMR1* PM allele. For example, a risk of PM expansion into FM in the offsprings increases 3–4 times in absence of AGGs (*vs* 2 among 60–80 CGGs) in a carrier [34]. The most frequent clinically relevant situation involves the mother with PM and her son with FM. Fragile X testing detects more than 99% of individuals with FXD, including the carriers [16]. There are three general circumstances in which fragile X testing should be considered: (i) clinical symptoms that suggest FXD, including any adult over 50 with features of FX-TAS such as cognitive, gait, and motor deficits, especially in combination with a positive family history of fragile X; (ii) a family history of FXD and intellectual or learning disabilities, or (iii) ASD and family or personal history of a fragile X carrier [18, 35, 36]. One survey revealed that almost 38% of parents of children eventually diagnosed with FXS, underwent more than 10 symptom-related visits to their health care professional before the fragile X diagnostic test was ordered [37]. From this, it is not surprising that the average age of the diagnosis of FXS is 35 to 37 months [38]. Typically, physicians do not consider it without a family history of ID or other dysmorphic features. However, these features are not present in approximately one third of individuals with FXS [39].

Fragile X testing can provide information not only for the diagnosis, but also for the treatment and prevention of FXD [16, 18]. Guidance statements from professional organizations such as the American College of Medical Genetics, and American Academy of Child & Adolescent Psychiatry, American Academy of Child Neurology emphasize the need for fragile X testing in individuals diagnosed with ASD [40]. Furthermore, the FXCRC has specific guidelines that begin with care by a physician-led team with expertise in FXD. Nevertheless, general clinical practice and available literature reveal that only one third of individuals with ASD are tested for *FMR1* mutations [41]. In countries such as Serbia, clinicians, patients and their families are by and large not familiar with the type of inheritance and phenotypes of FXD, or with the availability of precise genetic fragile X testing. Thus, there is a need to enhance the knowledge about FXD and fragile X testing. However, opportunities for medical education are limited, even at the medical school education level. Their faculties have to invest time and resources to research, and they occasionally have to organize such education programs [42]. Medical professionals often have no incentives to attend these lectures. Thus, as the first step, a well-designed educational-informative survey needs to reach a wide range of these medical health professionals. This is probably the best way to access their knowledge about FXD, and to begin an educational aspect that would disseminate this knowledge. This is also important because despite early interventions, only 9% male and 44% female patients with FXS reach a high level of independence as adults. Furthermore, while there is a need to balance between the individual needs, and the distribution of public health resources, the early diagnosis of FXS may also qualify these individuals for clinical trials. The vast majority of the aforementioned clinical trials in FXS targeted excitatory/inhibitory imbalances (14/22, 64%) [1, 2, 43, 44]. Yet, over the last few years, some of these key trials failed to meet the primary efficacy end points, including the well-powered study by Berry-Kravis et al. [45] (2016) that studied the mGluR5 antagonist mavoglurant. Nevertheless, such "negative" results in the clinical trials actually provide us with valuable lessons for designing future treatment studies in FXS, ASD, and other neurodevelopmental disorders [1, 45, 46]. For example, follow-up analyses of the arbacofen study (GABA-B agonist) showed that one of shortcomings in the design and outcome measures failed to capture areas of positive response to the newly developed therapeutics [47, 48]. Applying these 'lessons learned' a trofinetide (NNZ-2566) phase II trial conducted in adolescents and adults with FXS by the Neuren Pharmaceutical applied the Fragile X Syndrome Rating Scale which covers a wide range of behavioral symptoms, including FXS and ASD in FXS [49, 50]. It is a worthwhile effort to continue to validate this new behavior rating scale covering the core FXS behavioral phenotypes and associated symptoms. Close collaboration between the study sponsor (Neuren Pharmaceutical) and the experts in the field has been another necessary progress needed to move the field ahead faster.

In conclusion, the help from the USA in the form of collaboration is much needed for Serbia in the field of FXD, and ASD, which is currently either nonexistent or negligibly developed. Thus, building relationships with the NFXF and

the Consortium and its Clinical Trial Committee in the USA aims toward a long-term purpose of this article: to found and establish the first fragile X clinic in Serbia, and probably in the South East Europe. That also sets a stage for building a fragile X Registry, Database, and Repository in Serbia for which external international funds will be critical. In the meantime, an extensive survery has been survey has been designed as a tool for the first FXD KAP (knowledge, attitude, and practices) study in Serbia, called "Applied knowledge to early detection of genetic disorders caused by mutations of fragile X-chromosome: the most common genetic cause of ASD". The study has been approved by Ethics Committee (IRB) of School of Medicine, University of Belgrade, Serbia (No 29/IX-6; September 21, 2016; PI: Budimirovic, co-PI: Protic). The study will be conducted among physicians in primary health care in Serbia and the last grade medical students at School of Medicine, University of Belgrade, Serbia and the results will be obtained in the next few months.

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Psoriasis is the independent factor for early atherosclerosis: A prospective study of cardiometabolic risk profile

Psorijaza jeste nezavisni faktor rane ateroskleroze – prospektivna studija kardiometaboličkog rizika

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Abstract

Background/Aim. Psoriasis as multisystemic inflammatory disease is related with an increased cardiometabolic risk. The aim of the study was to analyze risk biomarkers, peripheral and renal arteries ultrasonography and echocardiography for subclinical atherosclerosis and metabolic disease in 106 subjects (66 psoriasis patients and 40 controls, 20 eczema patients and 20 healthy volunteers). **Methods.** In all exameenes following parameters were analyzed: body mass index (BMI), C-reactive protein, D-dimer, serum amyloid A (SAA), apolipoprotein (Apo) A1, ApoB, ApoB/Apo A1 index, fasting glucose, C-peptide, fasting insulinemia, homeostatic model assessment-insulin resistance (HOMA-IR), HOMA-β-cell, lipid profile, serum uric acid concentration (SUAC), 24-h proteinuria and microalbuminuria. Carotid, brachial, femoral and renal arteries ultrasonography, as well as echocardiography was also performed. **Results.** Five of 66 (7.6%) psoriasis patients had metabolic syndrome (not present in both control groups). The following variables were increased in patients with psoriasis compared to both control groups: BMI ($p = 0.012$), insulinemia ($p < 0.001$), HOMA-IR ($p = 0.003$), HOMA-β cell ($p < 0.001$), SUAC ($p = 0.006$), ApoB/ApoA1 ratio ($p = 0.006$) and microalbuminuria ($p < 0.001$). Also, increased C-peptide ($p = 0.034$), D-dimer ($p = 0.029$), triglycerides ($p = 0.044$), SAA ($p = 0.005$) and decreased ApoA1 ($p = 0.014$) were found in the psoriasis patients compared to healthy controls. HDL cholesterol was decreased in the psoriasis patients compared to the control group of eczema patients ($p = 0.004$). Common carotid (CIMT) and femoral artery intima-media thickness (FIMT) was significantly greater ($p < 0.001$) and the maximal flow speed (cm/s) in brachial artery significantly decreased ($p = 0.017$) in the patients with psoriasis in comparison to both control groups. In multivariate logistic regression analysis, after the adjustment for confounding variables, the most important predictor of CIMT and FIMT was the diagnosis of psoriasis ($p < 0.001$). **Conclusion.** Cardiometabolic risk biomarkers and ultrasonographic signs of early atherosclerosis are correlated with the diagnosis of psoriasis, and not to generalized eczema. Psoriasis was found to be an independent risk factor for subclinical atherosclerosis.

Key words:
psoriasis; arterial occlusive diseases; metabolic diseases; comorbidity; risk factor.

Apstrakt

Uvod/Cilj. Psorijaza kao multisistemska inflamatorna bolest u vezi je sa povećanim kardiometaboličkim rizikom. Cilj rada bio je da se analiziraju biomarkeri rizika, ultrasonografske odlike perifernih i renalnih arterija, kao i ehoskardiografski podaci kod 106 ispitanika (66 obolelih od psorijaze i 40 kontrolnih ispitanika 20 obolelih od ekcema i 20 zdravih dobrovoljaca). **Metode.** Kod svih ispitanika analizirani su sledeći parametri: indeks telesne mase (ITM), C-reaktivni protein, D-dimer, serumski amiloid A (SAA), apolipoprotein (Apo) A1, ApoB, ApoB/Apo A1 odnos, jutarnja glikemija, bazalna insulinemija, C-peptid, *homeostatic model assessment-insulin resistance* (HOMA-IR), HOMA-β-ćelija, serumska mokraćna kiselina (SMK), 24-h proteinurija i mikroalbuminurija; učinjeni su ultrasonografija karotidne, brahijalne, femoralne i renalnih arterija, kao i ehokardiografija. **Rezultati.** Pet od 66 (7,6%) bolesnika sa psorijazom ispunjavalo je kriterijume za metabolički sindrom (nije registrovan u kontrolnim grupama). Sledeće varijable bile su povećane kod obolelih od psorijaze u poređenju sa obe kontrolne grupe: ITM ($p = 0,012$), insulinemija ($p < 0,001$), HOMA-IR ($p = 0,003$), HOMA-β ćelija ($p < 0,001$), SMK ($p = 0,006$), ApoB/ApoA1 odnos ($p = 0,006$) i mikroalbuminurija ($p < 0,001$). Takođe, povećane koncentracije C-

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peptida ($p = 0,034$), D-dimera ($p = 0,029$), triglicerida ($p = 0,044$), SAA ($p = 0,005$) kao i snižena koncentracija ApoA1 ($p = 0,014$) nađeni su kod obolelih od psorijaze u poređenju sa zdravim kontrolnim ispitanicima. HDL holesterol bio je snižen kod obolelih od psorijaze u poređenju sa kontrolnom grupom obolelih od ekcema ($p = 0,004$). Debljina intime-medije karotidne i femoralne arterije bila je značajno veća ($p < 0,001$), a maksimalna brzina protoka (cm/s) u brahijalnoj arteriji bila je značajno manja ($p = 0,017$) kod obolelih od psorijaze negó kod ispitanika obe kontrolne grupe. Multivarijantna regresiona analiza pokazala je da ga posle prilagođavanja za pridružene varijable, najznačajniji prediktor za debljinu intime-medije karotidne i femoralne arterije sama psorijaza ($p < 0,001$). **Zaključak.** Kardiometabolički biomarkeri rizika i ultrasonografski znaci rane ateroskleroze u vezi su sa postojećom psorijazom, a ne sa generalizovanim ekcemom. Nađeno je da je psorijaza nezavisan faktor rizika od supkliničke ateroskleroze.

Ključne reči:
psorijaza; arterije, okluzione bolesti; metaboličke bolesti; komorbiditet; faktori rizika.

Introduction

Psoriasis is multisystemic inflammatory disease mainly affecting skin and joints, but also associated with significant cardiovascular and metabolic states and comorbidities, on the so-called "psoriatic march" [1]: insulin resistance, atherosclerosis, myocardial infarction, obesity and metabolic syndrome [1–4]. Three to 4 years of reduction in life expectancy was noted in patients with severe form of disease [5], and decrease in longevity may be as much as 20 years in patients whose psoriasis begins before 25 years of age [6].

A pathogenetic link between psoriasis and metabolic syndrome is chronic Th1 and Th17 lymphocyte-mediated inflammation, that leads to epidermal hyperplasia in psoriatic lesions, production of proinflammatory cytokines such as TNF-α and IL-6 and expression of inflammatory markers on endothelial cells [7], leading to development of insulin resistance, obesity, type 2 diabetes and atherosclerosis.

The majority of data on this topic come from large epidemiological studies, and studies that explored separately laboratory biomarkers of cardiovascular risk, or ultrasonographic signs of subclinical atherosclerosis, but there are few studies exploring their correlation [8–11]. Also, there is a paucity of data on these findings in patients with generalized eczema, except a study which finds higher risk of ischemic stroke in patients with atopic dermatitis [12].

In this study, we analized cardiovascular and metabolic biomarkers, ultrasonographic signs of subclinical atherosclerosis at peripheral arteries and echocardiographic findings in the patients with chronic plaque psoriasis, compared with the controls.

Methods

Study population

This study enrolled 66 patients with chronic plaque psoriasis. The age and sex matched control group comprised of 20 patients with generalized eczema and 20 healthy volunteers. Inclusion criteria for patients with psoriasis were the clinical diagnosis of chronic plaque psoriasis for at least 6 months, age range 18–60 years and the absence of the earlier or present diagnosis of cardiovascular, renal and metabolic diseases, or any other systemic disease. Exclusion criteria was receiving any systemic therapy such as cyclosporine, methotrexate, acitretine, biologic therapy and phototherapy. The control group of eczema patients comprised generalized nummular eczema, contact dermatitis and atopic dermatitis, involving ≥ 30% of skin, without earlier or present comorbidities and systemic therapy. The following data were collected after signing informed consent: age, gender, weight, height, waist circumference, blood pressure, smoking habit, the age of psoriasis onset, severity of psoriasis (PASI score), the percentage of skin involvement (BSA), presence of psoriatic arthropathy (according to standard criteria) and frequency of physical activity. Body mass index (BMI) was determined by the formula: weight (kg)/height^2 (m); waist circumference was measured by the standard procedure. Blood pressure was recorded as the average of two measurements after subjects had been sitting for 5 minutes. Metabolic syndrome was verified using the criteria (3 or more) of the National Cholesterol Education Programm's Adult Treatment Panel III (NCEP's ATP III) [13]. Blood samples were taken after subjects had fasted overnight, at least 8 hours. The following study parameters were analyzed: erythrocyte sedimentation rate (ESR), fibrinogen, C-reactive protein (CRP), D-dimer, serum amyloid A (SAA), apolipoprotein (Apo) A1, Apo B, Apo B/ApoA1 ratio, serum uric acid concentration (SUAC), fasting insulin, C-peptide, creatinine clearance (by Cockroft-Gault formula (mL/min)), 24-h-proteinuria and microalbuminuria. The homeostasis model assessments (HOMA IR and HOMA β-cell), based on plasma levels of fasting glucose and insulin, were computed using international formulas [14].

Renal color Doppler duplex ultrasonography (Toshiba Powervision 6000 ultrasound imaging system with frequency 3.7 MHz convex transducer) was used to evaluate renal arterial resistive index [15] (< 0.7 was considered normal).

The ultrasonographic study was performed to measure the intima–media thickness of the common carotid artery distal to the carotid bifurcation in the posterior wall (CIMT) and femoral artery (FIMT), resistivity and pulsatility indices (RI, PI) as well as the maximal flow speed (Vmax) of femoral and brachial arteries, by high-resolution ultrasound B-mode TOSHIBA AplioMX with a 5–10 MHz broadband linear array transducer.

The echocardiographic study included variables such as aortic diameter, left atrium and right ventricle diameters, thickness of septal and posterior walls, peak of early diastolic (E wave) and late diastolic (A wave) flow velocity, E/A ratio, the presence of mitral and tricuspidal regurgitation, ejec-

tion fraction (EF), systolic pressure in the right ventricle, end-systolic and end-diastolic diameter (ESD, EDD), as well as EDD/ESD ratio. Diastolic dysfunction (impaired diastolic relaxation) was based on the reductions in transmitral ratios of early to late ventricular filling-by the presence of impaired relation pattern if the E/A ratio was < 1.1. Echocardiography was performed by using GE medical systems Vivid 7 Proultrasound imaging system, with the patient in the left lateral position.

Statistical analysis

Numeric data were presented as the mean ± standard deviation (SD) or median with the interquartile range (IQR), depending on the normality of data distribution. Categorical variables are displayed in the form of absolute numbers with percentages. To compare continuous variables Student's *t*-test was used for independent samples or Mann Whitney test, depending on the normality of distribution, which was checked by Kolmogorov-Smirnov test. χ^2-test was used for comparison of frequencies for categorical variables. Predictors of subclinical atherosclerosis were identified by univariate and multivariate logistic regression analysis. The results were considered statistically significant if the probability of the null hypothesis was less than 5% ($p < 0.05$). All statistical calculations were made using a commercial software package SPSS 21.0.

Results

There were 66 patients with chronic plaque psoriasis (among them 4 subjects with psoriatic arthritis), mean age 36.77 ± 11.56 years, range 18–60 years. There were 23 (34.85%) patients with PASI < 10 and 43 patients (65.15%) with PASI ≥ 10. The median PASI score was 13.45 [(13.2), range 1.9–50, and median BSA was 21% (33). The median age of psoriasis onset was 23.97 ± 10.05 years, range 9–60 years. Also, the control groups comprised of 20 patients with eczema and 20 healthy volunteers. Descriptive features of all the subjects are shown in Table 1.

Personal history and clinical data

BMI was significantly higher in the psoriasis patients comparing to both control groups (26.86 *vs* 23.71 *vs* 24.41 kg/m^2; $p = 0.012$). Overweight (defined as BMI > 25 kg/m^2) was noticed in 43.9% of the patients with psoriasis and in 15% of the patients in each control group ($p = 0.009$). Waist was significantly wider in the patients with psoriasis compared to the control groups (93.09 ± 14.05 *vs* 81.25 ± 11.14 *vs* 82.8 ± 10.739 cm; $p < 0.001$). There were no significant differences regarding systolic and diastolic blood pressure and the prevalence of arterial hypertension (Table 1).

Metabolic syndrome criteria in the patients with psoriasis

There were no cases of metabolic syndrome (MS) in both control groups. Among the patients with psoriasis there were 5 patients with MS (7.6%), predominantly in males older than 40 years (M : F ratio 4 : 1), with later onset (28.8 ± 5.01 years) and a longer duration of psoriasis (14.8 ± 7.69 years).

Cardiovascular and metabolic risk markers

Fasting insulinemia was increased in the psoriasis patients compared to both control groups ($p < 0.001$), as well as the HOMA-IR ($p = 0.003$), HOMA-β cell ($p < 0.001$) and SUAC ($p = 0.006$); C peptide was increased in the psoriasis patients compared to the healthy control group ($p = 0.034$), as well as D-dimer ($p = 0.029$). Decreased Apo A1 concentration was found in the psoriasis patients compared to the healthy control group ($p = 0.014$). ApoB/ApoA1 ratio was higher in the psoriasis patients compared to both control groups ($p = 0.006$). Triglycerides were increased in the psoriasis patients compared to the healthy control group ($p = 0.044$), al-

Table 1

Descriptive characteristics of the patients with psoriasis and matched controls: demographics and clinical findings

Characteristics of the patients	Psoriasis (n = 66)	Eczema (n = 20)	Healthy (n = 20)	*p*
	x̄ ± SD	x̄ ± SD	x̄ ± SD	
Gender (male), n (%)	45 (68)	10 (50)	10 (50)	0.176
Age (years)	36.77 ± 11.56	37.55 ± 10.85	37.7 ± 6.43	0.923
Age at disease onset (years)	23.97 ± 10.05	28.35 ± 13.37		0.119
Duration of disease† (years)	10 (18)	10 (11)		0.935
PASI score ≥ 10, n (%)	43 (65)			
BSA† med (IQR)	21 (33)			
Current smoker, n (%)	25 (38)	9 (45)	3 (15)	0.098
Systolic BP (mmHg)	123.79 ± 10.26	119 ± 6.6	121 ± 5.28	0.088
Diastolic BP (mmHg)	77.88 ± 6.44	76.75 ± 5.68	78.5 ± 4	0.634
Waist (cm)	93.09 ± 14.05 [e,h]	81.25 ± 11.14	82.8 ± 10.73	< 0.001
BMI (kg/m^2)	26.868 ± 5.22 [e,h]	23.715 ± 3.32	24.41 ± 3.7	0.012
Exercise (not at all), n (%)	42 (64)	16 (80)	11 (55)	0.163
Overweight, n (%)	29 (44) [e,d]	3 (15)	3 (15)	0.009

PASI – psoriasis area and severity index; BSA – body surface area; BMI – body mass index.
†data are presented as median (interquartile range) [med(IQR)]; [e]significant difference from eczema; [h]significant difference from healthy controls.

so HDL cholesterol was decreased in the psoriasis patients compared to the control group of eczema patients ($p = 0.004$). SAA was increased in the patients with psoriasis compared to the healthy control group ($p = 0.005$), but also in the patients with eczema compared to the healthy control group ($p = 0.005$). 24-h proteinuria was increased in the patients with eczema compared to the healthy controls ($p = 0.042$) and microalbuminuria was increased in the psoriasis patients compared to both control groups ($p < 0.001$), but not outside the normal range in any of the patient. There was a trend of increased creatinine clearance in patients with psoriasis compared to both control groups ($p = 0.053$). Data about cardiovascular and metabolic risk markers are shown in Table 2.

Carotid, brachial and femoral ultrasonography

Considering the carotid, brachial and femoral arteries the psoriasis patients had a greater CIMT and FIMT than both control groups ($p < 0.001$). Also, a decreased maximal flow speed (cm/s) in the brachial artery in the patients with psoriasis compared to both control groups was found ($p = 0.017$). No differences between the groups were found in resistivity and pulsatility indices of the brachial and femoral arteries and maximal flow speed of the femoral artery. Data are shown in Table 3.

Echocardiographic analysis

Evaluation revealed a significantly increased aortal diameter in the psoriasis patients compared to both control groups ($p = 0.009$), and increased systolic pressure in the right ventricle ($p = 0.035$), septal wall thickness ($p = 0.034$) and posterior wall thickness ($p = 0.019$) in comparison to the healthy individuals. No difference was found between the groups for the left atrium diameter, E and A-waves, E/A ratio, end-systolic and end-diastolic diameters, EDD/ESD ra-

Table 2

Cardiovascular and metabolic risk markers

Biomarkers	Psoriasis (n = 66)	Eczema (n = 20)	Healthy (n = 20)	*p*
	x̄ ± SD	x̄ ± SD	x̄ ± SD	
ESR† (mm/h)	14 (15)	13.5 (11)	11 (6)	0.456
Fibrinogen (mg/dL)	3.58 ± 1.15	3.48 ± 1.08	3.65 ± 0.67	0.308
C reactive protein† (mg/L)	3.47 (2.85)	3.47 (0)	3.47 (0)	0.581
D-dimer† (meg/mL)	0.31 (0.30)[h]	0.23 (0.26)	0.17 (0.12)	0.029
Serum amyloid A† (mg/L)	4.39 (5.2)[h]	4.65 (3.9)[h]	3.09 (1.3)	0.005
Insulin† (pmol/L$^-$)	12 (11.8)[e,h]	8.2 (7.5)	7.5 (4.9)	< 0.001
Serum C-peptide† (ng/mL)	1.79 (1.27)[h]	1.20 (0.88)	1.14 (0.63)	0.034
Blood glucose (mg/dL)	5.10 ± 1.05	5.33 ± 0.61	5.21 ± 0.84	0.634
HOMA-IR†	2.66 (2.87)[e,h]	2.15 (1.84)	1.71 (1.37)	0.003
HOMA- β-cell†	161.07 (183.93)[e,h]	90.21 (82.43)	86.68 (61.15)	< 0.001
Uric acid (mg/dL)	322.95 ± 73.74[e,h]	285.05 ± 81.28	266.55 ± 63.04	0.006
Apo A1 (mg/dL)	1.30 ± 0.26[h]	1.44 ± 0.21	1.53 ± 0.50	0.014
Apo B (mg/dL)	0.93 ± 0.25	0.84 ± 0.16	0.85 ± 0.20	0.217
ApoB/ApoA1 ratio	0.73 ± 0.21[e,h]	0.60 ± 0.14	0.60 ± 0.20	0.006
Triglycerides† (mg/dL)	1.35 (0.81)[h]	1.12 (0.64)	0.96 (0.80)	0.044
Cholesterol (mmol/L)	5.14 ± 1.34	5.12 ± 1.01	4.96 ± 0.99	0.852
HDL† (mmol/L)	1.33 (0.32)[e]	1.57 (0.39)	1.35 (0.23)	0.004
LDL (mmol/L)	3.29 ± 1.15	3.08 ± 0.85	2.94 ± 0.91	0.380
Creatinine clearance* (mL/min)	130.29 ± 29.25	115.21 ± 32.44	116.06 ± 28.49	0.053
Proteinuria† (mg/day)	0.1 (0.06)	0.11 (0.99)[h]	0.07 (0.06)	0.042
Microalbuminuria† (mg/day)	11 (1.5)[e,h]	10 (0)	10 (0)	< 0.001

ESR – erythrocyte sedimentation rate; HDL – high-density lipoprotein; LDL – low-density lipoprotein.
†data are presented as median (interquartile range) [med(IQR)]; [e]significant difference from eczema; [h]significant difference from healthy controls; HOMA – homeostatic model assessment; IR – insulin resistance.

Table 3

Ultrasonographic findings of carotid and femoral arteries' intima-media thickness (IMT), resistivity (RI) and pulsatility indices (PI) and maximal flow speed of femoral and brachial arteries

Ultrasonographic features	Psoriasis (n = 66)	Eczema (n = 20)	Healthy (n = 20)	*p*
	x̄ ± SD	x̄ ± SD	x̄ ± SD	
Carotid IMT† (mm)	1.09 (0.2)[e,h]	0.7 (0.6)	0.65 (0.2)	< 0.001
Femoral IMT† (mm)	1.1 (0.16)[e,h]	0.7 (0.4)	0.7 (0.1)	< 0.001
Brachial RI	0.99 ± 0.10	1.02 ± 0.09	1.01 ± 0.08	0.246
Brachial PI	5.91v2.67	6.24 ± 2.21	5.31 ± 1.64	0.458
Femoral RI†	1.01 (0.05)	1.015 (0.03)	1.02 (0.02)	0.815
Femoral PI	7.66 ± 2.98	6.69 ± 1.61	6.52 ± 2.25	0.157
Brachial Vmax (cm/s)	115.76 ± 39.71[e,h]	148.6 ± 70.27	146.33 ± 65.22	0.017
Femoral Vmax (cm/s)	112.75 ± 33.63	114.04 ± 34.	123.98 ± 27.61	0.411

Vmax – maximal flow speed; †data are presented as median (interquartile range) [med(IQR)]; [e]significant difference from eczema; [h]significant difference from healthy controls.

tio and ejection fraction (EF). Tricuspid and mitral regurgitation were more frequently found in the patients with psoriasis compared to the healthy control group ($p < 0.05$). Echocardiographic data are shown in Table 4.

Renal ultrasonography

Renal arterial resistive indices were found normal in all the groups, with no difference between the groups (right kidney: $p = 0.830$; left kidney: $p = 0.203$).

Metabolic and cardiovascular biomarkers in the eczema patients

Considering the generalized eczema patients, there were no significant differences between the groups in cardiovascular and metabolic biomarkers, and ultrasonographic signs of subclinical atherosclerosis, except for the SAA ($p = 0.005$) and 24-h proteinuria ($p = 0.042$) that were increased in comparison to those in the healthy controls (Table 2).

Multivariate analysis of cardiovascular and metabolic biomarkers

In multivariate logistic regression analysis, after the adjustment for confounding variables (Table 5), the most important predictor of CIMT and FIMT was the diagnosis of psoriasis: CIMT relative risk (RR) = 11.886 (95% confidence interval (CI): 3.267–43.237, $p < 0.001$) and FIMT RR = 15.955 (95% CI: 4.326–58.846, $p < 0.001$). These results point out that psoriasis can be independent factor for early (subclinical) atherosclerosis.

Discussion

Understanding psoriasis genetics and immunopathogenesis moved focus from common dermatosis to multisystemic inflammatory disease [16]. Psoriasis patients have an increased risk of having cardiovascular disease and metabolic syndrome. Also, there is a higher prevalence of cardiovascular risk contributors such as overweight-obesity,

Table 4

Echocardiographic findings

Echocardiographic features	Psoriasis (n = 66)	Eczema (n = 20)	Healthy (n = 20)	*p*
	x̄ ± SD	x̄ ± SD	x̄ ± SD	
Aortal diameter (cm)	3.08 ± 0.36 [e,h]	2.90 ± 0.30	2.84 ± 0.30	0.009
Septal wall thickness† (cm)	1 (0.13)[h]	1 (0.1)	0.9 (0.2)	0.034
Posterior wall thickness† (cm)	1 (0.1)[h]	0.975 (0.1)	0.9 (0.2)	0.019
Left atrium diameter† (cm)	3.6 (0.5)	3.4 (0.6)	3.45 (0.5)	0.077
Right ventricle diameter (cm)	2.17 ± 0.37	1.99 ± 0.26	2.09 ± 0.42	0.144
E wave (cm)	0.87 ± 0.13	0.85 ± 0.163	0.94 ± 0.11	0.106
A wave (cm)	0.68 ± 0.12	0.65 ± 0.13	0.69 ± 0.16	0.656
E/A ratio (cm)	1.31 ± 0.29	1.36 ± 0.41	1.34 ± 0.33	0.820
Mitral regurgitation, n (%)	16 (24%)[h]	6 (30%)	0 (0%)	0.034
Tricuspidal regurgitation, n (%)	32 (48%)[h]	9 (45%)	3 (15%)	0.027
Right ventricle systolic pressure†	29 (3.25)[h]	28 (4.75)	28 (2.75)	0.035
End-systolic diameter (cm)	3.01 ± 0.39	3 ± 0.30	2.94 ± 0.30	0.690
End-diastolic diameter (cm)	4.98 ± 0.46	4.81 ± 0.38	4.85 ± 0.36	0.227
EDD/ESD	1.66 ± 0.11	1.6 ± 0.07	1.65 ± 0.10	0.090
EF (%)	67.0 ± 4.2	66.5 ± 2.8	67.4 ± 3.3	0.770

EDD/ESD – end-diastolic diameter/end-systolic diameter; EF – ejection fraction.
†data are presented as median (interquartile range) [med(IQR)]; [e]significant difference from eczema; [h]significant difference from controls.

Table 5

Psoriasis as predictor of intima-media thickness of the common carotid artery (CIMT) > 0.8 mm and femoral intima-media thickness (FIMT) > 0.8 mm in multivariate logistic regression model, after adjustment for confounding variables

Variable	p_{CIMT}	p_{FIMT}
Psoriasis	< 0.001	< 0.001
Body mass index (kg/m^2)	0.920	0.533
Basal insulin (mmol/L)	0.497	0.807
Serum uric acid (mg/dL)	0.693	0.152
Triglyceride (mg/dL)	0.352	0.233
ApoB/ApoA1 index	0.752	0.514
Serum C peptide (ng/mL)	0.928	0.711
D dimer (mcg/mL)	0.080	0.519
Creatinine clearance (mg/L)	0.085	0.492
HOMA IR	0.523	0.731
HOMA β cell	0.244	0.386

HOMA – homeostatic model assessment; IR – insulin resistance.

smoking habit, physical inactivity, emotional stress, dyslipidemia and hyperhomocysteinemia [17]. Psoriasis is regarded as an independent risk factor for the increased cardiovascular morbidity in general and myocardial infarction in particular and patients are affected by systemic inflammation even if they do not have any major cardiovascular risk factors [18, 19].

The concomitant occurrence of decreased high-density lipoprotein, hypertriglyceridemia, impaired glucose regulation, abdominal obesity and hypertension constitutes MS, which lead to chronic systemic inflammation [20–26]. In many studies it was shown that individual pathophysiological components of MS are enriched in patients with psoriasis [27–31]. The prevalence of MS in our patients with psoriasis was 7.6% with a higher prevalence in the males older than 40 years with longer duration of psoriasis (14.8 years) and older age at psoriasis onset (28.8 years), while no MS cases were registered in the control groups.

Intra-abdominal obesity and its surrogate measure BMI are directly linked to the MS. Increased BMI and waist circumferences are positively and strongly correlated with increased risk for coronary heart disease, with or without metabolic syndrome [32]. Intra-abdominal fat is an endocrine organ which secretes adipocytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-6 and plasminogen activator inhibitor type 1 (PAI-1), promoting inflammation and affecting glucose metabolism and vascular endothelial biology [33]. Positively correlated with increased BMI, overproduction of TNF-α and IL-6 contributes to insulin resistance and development of type 2 diabetes mellitus [34]. Obesity, as proinflammatory state, may potentiate inflammation in psoriasis driven by adipocytokines TNF-α and IL-6, which leads to impaired glucose regulation, dyslipidemia, endothelial dysfunction and hypertension [33]. In our study, significantly higher BMI was found in the psoriasis patients comparing to both control groups. Overweight was noticed in 43.9% of the patients with psoriasis and in 15% of the patients in each control group. Waist circumference was significantly wider in the patients with psoriasis compared to control groups.

Impaired glucose regulation with insulin resistance as a consequence of chronic inflammation, favors diabetes mellitus and atherosclerosis, and there is an impact of obesity on insulin resistance, which supports the concept of synergistic effects of chronic inflammation [35, 36]. It was found that in the patients with psoriasis serum C-peptide and insulin levels were significantly increased, in correlation with BMI. Increase in the mean serum C-peptide and insulin levels was constant and independent from clinical stage of the disease [37]. In our study, the patients with psoriasis had significantly higher levels of fasting insulin, as well as HOMA index of insulin resistance and the HOMA β-cell index of insulin secretion. Serum C-peptide was also increased in the psoriasis patients compared to the healthy controls. These results can strongly point out to the impairment of glucose metabolism in this group of patients.

Regarding serum lipid abnormalities in patients with psoriasis, in most of the studies a statistically significant elevated level of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol and/or triglycerides (TG) and a decrease in high density lipoprotein (HDL) was found compared to healthy controls [38–44]. Decreased ApoA1 lipoprotein, which is in correlation with decreasing HDL cholesterol, and increased serum ApoB lipoprotein is a marker of higher risk for development of atherosclerosis [45, 46]. In our study, increased triglycerides were found in the psoriasis patients compared to healthy controls; a higher ApoB/ApoA1 atherogenicity index, and a decreased HDL concentration were found, compared to the control group of eczema patients. These findings further confirm atherogenic potential in our patients with psoriasis.

Regarding proteins of acute phase inflammation, sensitive C-reactive protein (CRP) and serum amyloid A (SAA) concentrations are elevated in inflammatory diseases and they are widely accepted as predictors of risk for the development of cardiovascular diseases [47]. Increased CRP in patients with psoriasis suggests that systemic inflammation provides a background conducive for the development of cardiovascular diseases and other comorbidities [48–50]. Also, a positive relationship between CRP and BMI in the psoriasis patients was found [51]. We did not found statistically significant differences among the groups regarding CRP, but SAA was found to be increased in the patients with psoriasis compared to the healthy control group, but also in the patients with eczema compared to the healthy control group.

In previous studies, fibrinogen and D-dimer, showed elevated levels both in the group of psoriatic patients with cardiovascular disease (CVD) and cardiovascular risk factors, in comparison with the psoriatic patients without CVD and risk factors [52]. In our study, no significant difference in fibrinogen levels was found, but D-dimer was increased in psoriasis patients compared to the healthy controls, with possible prothrombotic effects.

Renal disease was not found to be correlated with chronic plaque psoriasis in several studies, but in one recent study it has been found that moderate to severe psoriasis is associated with an increased risk for moderate to advanced chronic renal disease independent of traditional risk factors, with increased relative risk in younger patients [53–55]. An increased prevalence of pathologic albuminuria and its positive correlation with psoriasis severity, which may suggest subclinical glomerular dysfunction, was found in a study of Dervisoglu et al. [56]. In our study, microalbuminuria was found to be more pronounced in the psoriasis patients compared to both control groups, but no pathologic proteinuria/albuminuria was found in any group. There was a trend of increased creatinine clearance in the patients with psoriasis compared to both control groups.

El-Mongy et al. [11] explored possible subclinical atherosclerosis in 80 psoriasis patients without cardiovascular risk factors, and found the increased CIMT in patients with psoriasis, positively correlated with patients' age and severity of psoriasis. In our study the psoriasis patients had a greater CIMT than both control groups, as well as FIMT.

In multivariate analysis, the most important predictor of CIMT and FIMT is psoriasis itself after the adjustment for confounding variables. A decreased maximal flow speed in the brachial artery in the patients with psoriasis was found compared to both control groups. Since endothelial changes

in the brachial artery are in correlation with similar changes in coronary arteries [57], these findings points out to the need for monitoring of patients with psoriasis for possible subsequent manifestations of coronary heart disease.

Psoriasis patient had more prevalent valvular regurgitation, abnormal diastolic relaxation, left ventricular hypertrophy, left ventricular diastolic dysfunction, left ventricular wall motion abnormalities, mitral valve and tricuspid valve prolapse in few studies, but in others these results were not confirmed [11, 58, 59]. Our echocardiographic findings demonstrated that the patients with psoriasis had greater aortal diameter compared with the control groups, also greater septal and posterior wall thickness compared to healthy controls; more frequent tricuspid and mitral regurgitation and increased systolic pressure in the right ventricle were found in the group of patients with psoriasis. Our results show that early atherosclerotic echocardiography predictors were found in the patients with the diagnosis of psoriasis.

Considering the generalized eczema patients, there was no correlation between this diagnosis and cardiovascular and metabolic biomarkers, and signs of subclinical atherosclerosis. Increased SAA points out to the inflammation that is a hallmark of eczema, and increased 24-h proteinuria in comparison to healthy subjects could be explained by s small sample size, but certainly demands further exploration.

Conclusion

Early atherosclerosis ultrasound predictors (CIMT and FIMT) are found to be correlated with the diagnosis of psoriasis itself, after adjustment for all confounding factors, while a decreased flow speed in the brachial artery points out to the risk for future possible coronary disease. Identification of patients with early atherosclerosis ultrasonographic predictors and increased inflammatory and metabolic risk biomarkers could lead to preventive and therapeutic interventions.

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Prevalence of dental caries in hospitalized patients with schizophrenia

Prevalencija karijesa kod bolesnika hospitalizovanih zbog shizofrenije

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Abstract

Background/Aim. It is considered that over 450 million people worldwide suffer from some form of mental disorder. Previous studies in other countries have shown that schizophrenia is among the most frequent. Oral health is significant for general health and should not be separated from mental health. Studies in other countries have shown an increased incidence of carious and extracted teeth, and less incidence of filled teeth in this group of psychiatric patients. The aim of this study was to establish condition of the existing teeth, to determine the prevalence of caries and to consider possible risk factors that contribute to the current oral health status of hospitalized patients with schizophrenia. **Methods.** The study comprised 190 patients with schizophrenia, hospitalized at the Clinic for Psychiatric Disorders "Dr. Laza Lazarević" in Belgrade, and 190 mentally healthy patients at the Clinic for Periodontology and Oral Medicine, Faculty of Dental Medicine in Belgrade. The decayed, missing, filled (DMF) index, sociodemographic and economic characteristics were registered in both groups, as well as characteristics of the primary disease of hospitalized patients with schizophrenia. **Results.** The value of DMF index (representing the sum of carious, extracted and filled teeth), in the hospitalized patients with schizophrenia was 18.57 ± 7.07 and 12.47 ± 5.64 in the healthy group ($p = 0.000$). The structure of the DMF index in the study group showed that caries and extracted teeth dominated with 88.1%; in the control group, filled teeth dominated with 55.6%, which was a statistically significant difference for all the three observed variables. **Conclusion.** Hospitalized patients with schizophrenia had twice as many caries and extracted teeth, and five time less filled teeth than healthy people. The patient's age and taking antiparkinsonics were established as predictors of the increased DMF index in hospitalized patients with schizophrenia.

Key words:
schizophrenia; hospitals, psychiatric; dental caries; prevalence; dmf index; oral hygiene.

Apstrakt

Uvod/Cilj. Smatra se da preko 450 miliona ljudi širom sveta pati od nekog oblika mentalnog poremećaja, a istraživanja sprovedena u drugim zemljama pokazala su da je shizofrenija među najzastupljenijima. Oralno zdravlje zauzima značajno mesto u celokupnom zdravlju čoveka i ne treba ga razdvajati od mentalnog zdravlja. Istraživanja sprovedena u drugim zemljama pokazala su povećanu zastupljenost karijesnih i izvađenih zuba, a manje plombiranih zuba kod ove grupe psihijatrijskih bolesnika. Cilj studije bio je da se istraži stanje prisutnih zuba, odredi prevalencija karijesa i ispitaju mogući faktori rizika koji doprinose postojećem stanju oralnog zdravlja kod bolesnika hospitalizovanih zbog shizofrenije. **Metode.** U istraživanju je učestvovalo 190 bolesnika sa shizofrenijom, hospitalizovanih na Klinici za psihijatrijske bolesti „Dr Laza Lazarević" u Beogradu i 190 mentalno zdravih ispitanika, pacijenata Klinike za parodontologiju i oralnu medicinu Stomatološkog fakulteta u Beogradu. Ispitanicima obe grupe registrovane su vrednosti KEP indeksa, sociodemografske i ekonomske karakteristike, a bolesnicima hospitalizovanim zbog shizofrenije i karakteristike primarne bolesti. **Rezultati.** Vrednost KEP indeksa kod bolesnika hospitalizovanih zbog shizofrenije iznosila je 18,57 ± 7,07, a kod zdravih osoba 12,47 ± 5,64 ($p = 0,000$). U strukturi indeksa koji pokazuje zbir brojeva karioznih, ekstrahovanih i plombiranih zuba (KEP) kod bolesnika sa shizofrenijom, dominirali su karijesni i ekstrahovani zubi – 88,1%, a u kontrolnoj grupi plombirani zubi – 55,6%, što je bila statistički značajna razlika za sve tri posmatrane varijable. **Zaključak.** Bolesnici hospitalizovani zbog shizofrenije imali su dvostruko više karijesnih i izvađenih zuba, a pet puta manje plombiranih zuba od zdravih osoba. Kao prediktori povećanog KEP indeksa kod bolesnika hospitalizovanih zbog shizofrenije ustanovljeni su starost ispitanika i korišćenje antiparkinsonika.

Ključne reči:
shizofrenija; bolnice, psihijatrijske; zub, karijes; prevalenca; dmf indeks; higijena, oralna.

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Introduction

Mental health is an integral part of general health [1]. It is considered that over 450 million people worldwide suffer from some form of mental disorder [2]. Previous studies in other countries have shown that schizophrenia is among the most frequent [3–7].

Schizophrenia is a chronic mental disorder characterized with remissions and exacerbations, which leads to social and occupational disability of patients. It is a burden for the patient's familly and for the community, making it a disease of major public health importance [8]. Epidemiological features of schizophrenia are many due to the specific symptoms, behavioral imbalance and chronic course [9]. The disease occurs in 1% of the general population and it is one of the ten leading causes of disability in the population between 15 and 44 years of age [8].

Oral health is significant for general health and should not be separated from mental health [10]. The disease which has a large impact on oral health of general population is caries [11]. Previous studies in other countries have shown an increased occurrence of caries in psychiatric patients compared to mentally healthy people. Psychiatric patients have more caries and extracted teeth, and less filled teeth [12–14]. It is reasonable to expect that the prevalence of dental caries in hospitalized patients with schizophrenia should be higher than in healthy individuals for several reasons: psychiatric disease leads to a weakening habits in oral hygiene, some antipsychotic drugs cause adverse effects in the oral cavity, such as dry mouth, the access of these patients to the dentist is, as a rule, limited, for several reasons[15, 16].

In Serbia no research has been conducted related to oral health of this vulnerable group of psychiatric patients, although this disease in Serbia is present in 1% of the population, and these patients occupy 50% of hospital beds in psychiatric institutions in Serbia [17]. Therefore, the aim of this study was to establish condition of present teeth, to determine the prevalence of dental caries and consider possible risk factors that have contributed to the current oral health status of hospitalized patients with schizophrenia.

Methods

The study was conducted as an observational cross-sectional study. It had received approval of the Ethics Committee of the Clinic for Psychiatric Disorders "Dr Laza Lazarević" in Belgrade and the Faculty of Dental Medicine, University of Belgrade. The research was conducted in accordance with the Declaration of Helsinki. The participation of all participants was voluntary. Each participant was informed, through a special brochure, of the type of the research, data collection procedure, and other aspects of the study, and written consent was obtained from all subjects or their legal representatives to use personal data for research purposes.

Two groups of participants were formed: the study group comprised 190 randomly selected patients with schizophrenia, hospitalized at the Clinic for Psychiatric Disorders "Dr Laza Lazarević" in Belgrade. The sample size was determined in regard to the prevalence of schizophrenia in general population in the Republic of Serbia [8], with 95% confidence level. Therefore, the calculated sample size was 190. The inclusion criteria for the study were that the patient was hospitalized, older than 18 years and diagnosed with schizophrenia (according to the 10th Revision of the International Classification of Diseases) two years prior to the study. The exclusion criteria were the primary diagnosis of another mental disorder, hospitalized patients diagnosed with schizophrenia in the period shorter than two years from the time of the survey, the simultaneous presence of severe somatic illnesses or severe disability, and inability to communicate or the refusal to cooperate. The control group comprised 190 randomly chosen mentally healthy people who were being treated at the Clinic of Periodontology and Oral Medicine, Faculty of Dental Medicine, University of Belgrade. They were matched to the study group by number, gender and roughly by age. The exclusion criteria were the diagnosis of any psychiatric or somatic illness and the use of drugs that can cause oral changes (antibiotics, antifungals, blood pressure medications, corticosteroids, diabetes medications, etc.) [18].

All the participants were subjected to targeted dental examinations in accordance with the criteria recommended by the World Health Organization [19]. Dental check-ups were carried out by the dentist (first author) in the dental office at the Clinic for Psychiatric Disorders "Dr Laza Lazarević" in Belgrade, and the Clinic for Periodontology and Oral Medicine, Faculty of Dental Medicine. The examinations were perfomed in the daylight, using flat dental mirrors and sharp probes. Dental check-ups were carried out with the aim of measuring parameters for oral health evaluation and assessment of the decayed, missing, filled (DMF) index [20], which is used for oral health assesment. Clearly visible lesions with cavities on tooth surfaces were registered as caries; teeth with only a change in transparency, but with intact surface and without cavitation were registered as being healthy.

A questionnaire was designed for the study with the aim to record socioeconomic and demographic characteristics of the participants. It also recorded health data from medical records related to a mental disorder (the diagnosis, duration of the disease, previous hospitalizations and current medications).

The primary data obtained entered the SPSS 17.0 and were analyzed by descriptive statistical parameters, methods for testing hypotheses and regression models. The descriptive statistical methods were represented by the measures of central tendency (mean and median), a measure of variability (standard deviation and variation interval) and were expressed in percentages. The methods for testing the difference in numerical data (age, DMF index) were represented by the *t*-test of the independent groups. If there were no grounds for application of the parametric statistical methods, Mann-Whitney or Kruskal-Wallis test were applied. For testing data of different cathegories (gender, education level, employment status, marital status and residence), Pearson's χ^2 test was used.

Results

The study group comprised 190 hospitalized patients with schizophrenia, 95 males and 95 females, the mean age being: mean ± SD = 43.59 ± 11.95; med (min-max) = 43

(19–67) years. Most participants (32.1%) were in the age group over 50 years of age. In the control group there were 190 participants, 95 males and 95 females, the mean age being; mean ± SD = 43.20 ± 11.89; med (min-max) = 45.5 (19–72) years; the most participants (30%) were between 41 and 50 years of age. These data indicate the comparability of the groups concerning their age structure (Student's *t*-test for independent samples; $p = 0.747$).

Table 1

Characteristics of patients with schizophrenia

Characteristics	Number (%) of patients
Duration of disease (years)	
[($\bar{x}$ ± SD; med (min-max)]	14.69 ± 9.608; 14 (2–45)
≤ 10	67 (35.1)
11–20	82 (43.3)
21–30	30 (16.5)
≥ 31	11 (5.1)
Hospitalizations *per* patient (number)	
[($\bar{x}$ ± SD; Med (min-max)]	8. 52 ± 5.712; 7 (1–30)
≤ 10	129 (67.9)
11–20	55 (28.9)
≥ 21	6 (3.2)
Antipsychotic drugs *per* patient (number)	
[($\bar{x}$ ± SD; Med (min-max)]	1.64 ± 0.657; 2 (1–3)
1	87 (45.8)
2	84 (44.2)
3	19 (10.0)
Antipsychotic drugs	
haloperidole	52 (27.4)
clozapine	34 (17.9)
chlorpromazine	34 (17.9)
olanzapine	34 (17.9)
Other medications	
antiepileptics	135 (71.1)
hypnotics and sedatives	63 (33.2)
anxiolytics	160 (84.2)
antidepressants	15 (7.9)
antiparkinsonics	110 (57.9)

Most of the participants of the study group had the diagnosis of residual schizophrenia (42.6%) and paranoid schizophrenia (37.9%). The disease lasted over 14 years on the average (from 2 to 45 years), and the average number of hospitalizations was 8.52 ± 5.71 (from 1 to 30). The patients were treated with an average of 1.64 ± 0.66 antipsychotics (1 to 3 drugs). Most patients had been treated with one or two antipsychotics (45.8% and 44.2%, respectively). Apart from antipsychotic drugs, the patients commonly received haloperidole (alone or in combination with other antipsychotics), and they were receiving other drugs, too (Table 1).

Socioeconomic and demographic characteristics of the respondents are shown in Table 2. Statistically significant differences were observed between the level of education, employment status, marital status and ownership of the residence. Educational structure of hospitalized patients with schizophrenia was lower than that in the control group. Also, the percentage of employees among hospitalized people with schizophrenia was significantly lower than that in healthy individuals. Hospitalized people with schizophrenia were in the highest percentage unmarried (68.9%) in contrast to the control group (45.3%). Only 33.7% participants of the study group had residence in their ownership, as opposed to mentally healthy individuals (52.6%).

The patients of study group had significantly more carious and extracted teeth, and five times less filled teeth than patients of the control group. The mean value of the DMF index in the study group also was significaly higher than in the control group (Table 3).

The mean values of the DMF index in the subgroups showed a statistically significant difference (Table 4) in comparison to the control group. The highest value of the DMF index in the studied group had the patients older than 50 years of age, those who suffered from the disease for a

Table 2

Socioeconomic and demographic characteristics of the participants

Characteristics	Participants		*p*-values*
	Study group, n (%)	Control group, n (%)	
Educational level			
without school / primary school	40 (21.1)	6 (3.2)	
secondary school	109 (57.3)	93 (48.9)	
high school	16 (8.4)	33 (17.4)	
faculty	25 (13.2)	58 (13.2)	0.000
Employment			
unemployed / occasionally employed	117 (61.6)	99 (52.1)	
employed	10 (5.2)	73 (38.4)	
disability pension	37 (19.5)	3 (1.6)	
age / surviver pension	26 (13.7)	15 (7.9)	0.000
Marital status			
married	21 (11.1)	67 (35.3)	
divorced	30 (15.8)	31 (16.2)	
unmarried	131 (68.9)	86 (45.3)	
widow	8 (4.2)	6 (3.2)	0.000
Residance			
own property	64 (33.7)	100 (52.6)	
parents property	101 (53.2)	40 (21.1)	
rent	9 (4.7)	49 (25.8)	
other	16 (8.4)	1 (0.5)	0.000

***Pearson's χ^2 test.**

Study group – patients with schizophrenia; Control group – mentally healthy people treated at the Clinic of Periodontology and Oral Medicine.

Table 3

Distribution of carious, extracted, filled teeth and the value of the decayed, missing, filled (DMF) index

Variables	Study group		Control group		*p*-values*
	$\bar{x}$ ± SD; med (min–max)	% of patients	$\bar{x}$ ± SD; med (min–max)	% of patients	
Carious teeth	7.59 ± 5.563; 7 (0–31)	40.9	2.15 ± 2.609; 6.5 (0–13)	17	0.000
Extracted teeth	8.77 ± 7.928; 7 (0–28)	47.2	3.27 ± 3.791; 9.5 (0–19)	27.3	0.000
Filled teeth	2.21 ± 2.852; 1 (0–14)	11.9	6.97 ± 4.060; 9.5 (0–19)	55.6	0.000
DMF index	18.57 ± 7.068; 19 (3–32)	100	12.47 ± 5.644; 14.5 (1–28)	100	0.000

*** Mann-Whitney test.**

Study group – patients with schizophrenia; Control group – mentally healthy people treated at the Clinic of Periodontology and Oral Medicine.

Table 4

The mean values of the decayed, missing, filled (DMF) index among patients with shizophrenia

Characteristics	The mean value of DMF index [($\bar{x}$ ± SD; med (min-max)]	*p*-values
Gender:		
men	18.76 ± 7.114; 18 (4–32)	
womens	18.39 ± 7.055; 19 (3–32)	0.787*
Age (years):		
≤ 30	14.85 ± 7.262; 14.5 (3–32)	
31–40	15.80 ± 6.535; 16 (3–30)	
41–50	19.52 ± 6.238; 20 (5–32)	
≥ 51	21.92 ± 6.288; 22 (4–32)	0.000†
Duration of disease (years):		
≤ 10	16.67 ± 6.673; 16 (3–29)	
11–20	18. 38 ± 7. 368; 18 (3–32)	
21–30	22.27 ± 5. 447; 22.5 (12–32)	
≥ 31	21.55 ± 6. 962; 22 (4–29)	0.001†
Hospitalization (number)		
≤ 10	17.64 ± 7.110; 18 (3–32)	
11–20	20.29 ± 6.235; 19 (5–32)	
≥ 21	23.00 ± 9. 798; 27.5 (4–30)	0.013†
Antipsychotic drugs, number *per* patient		
1	18.41 ± 7.592; 19 (3–32)	
2	18.73 ± 6. 908; 18.5 (3–30)	
3	18.63 ± 5.377; 18 (9–30)	0.980†
Other medications		
antiepileptics		
yes	18.55 ± 6. 771; 18 (3–32)	
no	18.59 ± 7. 210; 19 (3–32)	0.884*
hypnotics and sedatives		
yes	17.97 ± 7. 542; 18 (3–32)	
no	19.79 ± 5. 867; 19 (5–32)	
anxiolytics		
yes	17.80 ± 7. 658; 17. 5 (4–29)	
no	18.72 ± 6. 968; 19 (3–32)	0.172*
antidepressants		
yes	18.65 ± 7. 111; 19 (3–32)	
no	17.67 ± 6. 715; 16 (8–28)	0.513*
antiparkinsonics		
yes	17.28 ± 7. 412; 17.5 (3–32)	
no	19.52 ± 6. 684; 20 (4–32)	0.049*
Education level		
without school / primary school	20.23 ± 7. 794; 21.5 (3–32)	
secondary school	18.48 ± 6. 890; 18 (3–32)	
high school	17.88 ± 6. 541; 18.5 (5–28)	
faculty	16.80 ± 6. 795; 16 (4–29)	0.226†
Employment		
unemployed / occasionally employed	17.90 ± 7.475; 18 (3–32)	
employed	18.10 ± 6.574; 15.5 (9–28)	
disability pension	18.46 ± 6.145; 18 (5–30)	
age / surviver pension	21.96 ± 5.862; 23.5 (9–29)	0.074†
Marital status:		
married	21.57 ± 5.904; 22 (8–29)	
divorces	17.80 ± 6. 562; 18.5 (4–28)	
unmarried	18.07 ± 7. 105; 18 (3–32)	
widow	21.88 ± 9. 311; 26 (3–28)	0.054†
Residance:		
own property	19.66 ± 7.087; 20.5 (3–30)	
parents property	17.58 ± 7.102; 18 (3–32)	
rent	16.22 ± 7.102; 16 (5–28)	
other	21.81 ± 5.406; 20.5 (15–32)	0.054†

***Mann-Whitney test; †Kruskal-Wallis test.**

long time, and patients who were more often hospitalized. Depending on the drugs used, a statistically significant difference in values of the DMF index was observed between those patients who were taking and those who were not taking antiparkinsonic drugs. The highest values of the DMF index in the study group had patients who were taking antiparkinsonic drugs (Table 4).

The impact of sociodemographic and economic characteristics, as well as the characteristics of the underlying disease, on the value of the DMF index among the study group patients was examined by linear regression model. Univariate regression analysis emphasized age of the participants, total duration of illness, number of hospitalizations and use of antiparkinsonic. However, statistically significant factors separated in the univariate linear model were included into the multivariate regression model, showing a statistical significance only for the age of the participants and application of antiparkinsonic drugs (Table 5).

health education of patients. The mean DMF index of hospitalized patients with schizophrenia in this study was 18.57 ± 7.07, and carious and extracted teeth dominated in its structure. Age, total duration of the disease and total number of hospitalizations significantly affected the DMF index value. Used drugs and sociodemographic and economic characteristics of participants did not influence the results significantly, which suggests that the underlying disease affects oral health indirectly, reducing patients' motivation for preservation of oral health.

The mean value of the number of carious teeth in hospitalized patients with schizophrenia, in this study, was 7.59 ± 5.56, which is almost twice as much as in the research performed by Arnaiz et al. [12], and four times more than in the study of Adeniyl et al. [21]. Naturally, the higher number of carious teeth in people with schizophrenia, noticed in this study, may be explained by hyposalivation associated with antipsychotics that have antimuscarinic effect [22]. Hyposalivation was even

Table 5

The value of the decayed, missing, filled (DMF) index, examined by linear regression models, among patients with shisophrenia

Characteristics	Univariate linear regression analysis		Multivariate linear regression analysis	
	#B (95%CI)	*p*-values	#B (95% CI)	*p*-values
Age of participants	0.212	0.000	0.210	0.000*
Gender of participants	-0.846	0.412	/	/
Educational level	-0.966	0.085	/	/
Employment	0.556	0.052	/	/
Marital status	-0.963	0.183	/	/
Residence	0.394	0.523	/	/
Duration of disease	0.133	0.018	-0.710	0.330
Number of hospitalizations	0.276	0.003*	0.139	0.231
Number of antipsychotic drugs	0.281	0.717	/	/
Antiepileptics	0.470	0.682	/	/
Anxiolytics	1.999	0.170	/	/
Hypnotics and sedatives	1.703	0.120	/	/
Antidepressants	-2.008	0.310	/	/
Antiparkinsonics	2.382	0.022*	2.242	0.021*

***statistical significance; #Unistandardized Coefficient B; CI – confidence interval.**

Discussion

The research enabled assessment of dental status of hospitalized patients with schizophrenia, determining the prevalence of dental caries and potential risk factors that might contribute to this state of the oral health. When interpreting the values obtained for the DMF index, in addition to the importance of its absolute value, the value of each parameter of its structure (carious, extracted and filled teeth) is also essential. When the value of the DMF index is large indicating domination of filled teeth, it points to the fact that the overall oral health of the patient was previously bad, but that dental health service is well organized and the patients' motivation exists. However, if the value of DMF index is large indicating domination of carious teeth (including extracted teeth as well), it shows a patient's lack of awareness concerning the need of periodic visits to the dentist, difficult availability of dental health service or poor preventive health programs and more pronounced because many patients were treated with conventional antipsychotics (first generation antipsychotics), such as haloperidole. Results of Tani et al. [23] showed that the majority of their patients used risperidone and olanzapine, a newer generation of antipsychotics. Hyposalivation consequently leads to a buildup of dental plaque on marginal gingiva, which is a major etiologic factor for the occurrence of caries [11]. However, in addition to hyposalivation, antipsychotics lead to extrapyramidal syndrome, which is reflected in the involuntary motor functions [8], which further prevent patients to maintain regular and adequate oral hygiene encouraging accumulation of dental plaque. The results of this study show a relatively little value of filled teeth in the DMF index (2.21 ± 2.85), indicating the lack of motivation for rehabilitation of carious teeth and weakening habits in maintaining oral hygiene as well [15, 16].

The mean age of the hospitalized participants was 43.59 ± 11.96, which is similar to other studies [6, 12, 21]. The disease, on the average lasted 14.69 ± 9.61 years, which is lower than

previously reported [23]. The patients were treated with the average number of 1.64 ± 0.66 antipsychotics (1 to 3 drugs), which is also similar to the reported results [12, 23]. The parameter that is tracked in the research of other authors was the duration of the last hospitalization. This study showed a large number of hospitalizations per patient (8.52 ± 5.71; 1–30), which points to the fact that the patients were hospitalized for a proportionally long period of time.

This survey also found that most of the patients had completed secondary school (57.4%), which is consistent with the results obtained by the study of Adeniyl et al. [21]. The majority of participants were unemployed, unmarried and lived with their parents. Similar results were obtained by Chu et al. [22], where 76.9% of participants were unmarried, and 78.3% of lower sociodemographic and economic status.

This study showed that the age of participants and the application of antiparkinsonics could influence the DMF index in hospitalized patients with schizophrenia. However, it seems that high value of the DMF index can be expected in older subjects and in subjects treated with antiparkinsonics. Similarly, previous studies showed an association between age and increased incidence of dental caries and higher values of DMF index. When it comes to the application of antiparkinsonic drugs, a survey conducted in Bosnia, among females suffering from schizophrenia, showed similar results [24]. Women who, in addition to antipsychotic drugs, were receiving antiparkinsonic drug biperiden which is of anticholinenergic type had higher values of the DMF index than women treated with antipsychotic drugs only (antiparkinsonics were used to suppress the effects of parkinsonism, tremor and akinesia, consequently caused by antipsychotic drugs).

Summarizing the results, this study showed a high prevalence of caries in hospitalized patients with schizophrenia and high value of the DMF index, considerably higher compared to healthy population, but also in comparison with mentally ill persons in the world [6, 13, 21, 22]. The population of schizophrenic persons is certainly of lower socioeconomic status. The characteristics of the underlying disease did not significantly affect the high value of the DMF index. As the predictors for the increased DMF index, the age of participants and the application of antiparkinsonic drugs with anticholinenergic effects could be stressed.

There are two possible limitations of this study: firstly, all the patients of the study group were hospitalized at the Clinic for Psychiatric Disorders which has a separate dental office enabling dental care within patients' reach. Therefore, the results could be even worse in other psychiatric hospitals. Secondly, patients of the control group used to regularly visit the Clinic for Periodontology and Oral Medicine, and the results concerning their DMF index were possibly slightly better than in general population.

It is important to stress that our own experience in treating hospitalized patients with schizophrenia suggests that this population of the psychiatric patients is not especially difficult for establishing communication necessary to complete dental treatment. During their hospitalization, they showed apparent interest for taking care of their oral health and desire to repair their teeth. Even more, most of them seek recommendations for continuation of dental treatments after termination of hospitalization. However, in our society still exist prejudices among dental personnel concernig motivation for improving oral and general health of mentally ill persons, which should be overcomed.

Conclusion

The results of this study indicate the need for continuous research of oral health of psychiatric patients in general in order to determine the current state of their oral health and determine modes of its improvement, with the emphasisis on primary systems of health care, and implementation of optimal measures for its improvement.

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Community-acquired urinary tract infections: causative agents and their resistance to antimicrobial drugs

Vanbolničke infekcije urinarnog trakta: uzročnici i njihova rezistencija na antimikrobne lekove

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Abstract

Background/Aim. Urinary tract infections (UTIs) are among the most common infections in outpatients. The aim of this study was to define the causative agents of urinary tract infections and their resistance to antimicrobial drugs in the urban area of central Serbia, as well as to evaluate eventual differences associated with age and gender of the patients. **Methods.** This retrospective study analysed data taken from routine, consecutively collected urine cultures of outpatients with symptomatic UTIs, collected from the Department of Microbiology, Institute of Public Health in Kragujevac, Serbia, from January 2009 to December 2013. **Results.** There were 71,905 urine cultures, and 24,713 (34.37%) of them were positive for bacterial pathogens. The most common pathogen was *Escherichia coli* (*E. coli*) (56.56%), followed by Klebsiella spp. (16.20%), *Proteus* spp. (14.68%), *Enterococcus* spp. (5.29%) and *Pseudomonas aeruginosa* (3.74%). *E. coli* and *Enterococcus* spp. isolation rates were lower in males ≥ 60 years old (23.71% and 4.87%, respectively), while *Klebsiella* spp. was more prevalent in this group (32.06%). The most common causative agents isolated from 15–29 years old male patients were *Enterococcus* spp. and *Pseudomonas aeruginosa* (13.28% each). Among women, the isolation rate of *E.coli* was high in all age groups (around 70%). *Proteus spp.* was frequently isolated from females ≤ 14 years old (13.27%), while *Klebsiella* spp. was the most frequent in the oldest age female group (10.99%). **Conclusion.** Choice of antibiotics for treatment of UTIs should be governed not only by the local resistance patterns, but also by gender and age of patients.

Key words:
urinary tract infections; urine; bacteria; drug resistance, microbial; outpatients; serbia; age factors; sex factors.

Apstrakt

Uvod/Cilj. Infekcije urinarnog trakta jedne su od najčešće prisutnih infekcija u vanbolničkoj praksi. Cilj ovog istraživanja bio je da se identifikuju uzročnici infekcija urinarnog trakta i stepen njihove rezistencije na antimikrobne lekove u urbanom području centralne Srbije, kao i njihova povezanost sa starošću i polom bolesnika. **Metode.** Studija je bila sprovedena kao retrospektivna analiza podataka prikupljenih tokom rutinskog rada na obradi urinokultura vanbolničkih pacijenata sa simptomatskom infekcijom urinarnog trakta u periodu od januara 2009. do decembra 2013. godine. **Rezultati.** Ukupno je bilo analizirano 71 905 kultura, od kojih je 24 713 (34,37%) bilo pozitivno na prisustvo bakterijskih patogena. Najčešće izolovani uzročnik urinarnih infekcija bila je *Escherichia coli* (*E. coli)* (56,56%), zatim vrste *Klebsiella* (16,20%), *Proteus* (14,68%), *Enterococcus* (5,29%) odnosno *Pseudomonas aeruginosa* (3,74%). *E. coli* i *Enterococcus* izolati bili su manje zastupljeni kod muškaraca starosti ≥ 60 godina (23,71%, odnosno 4,87%), dok su uzročnici *Klebsiella* vrsta preovladavali u toj starosnoj grupi (32,06%). Najčešće izolovani uzročnici kod osoba muškog pola starosti 15–29 godina bili su pripadnici vrsta *Enterococcus* i *Pseudomonas aeruginosa* (13,28% svaki). Među ženama, učestalost izolacije *E. coli* bila je visoka u svim starosnim grupama (oko 70%). *Proteus* vrste često su bile izolovane kod pripadnica ženskog pola starosti do 14 godina (13,27%), dok je *Klebsiella* bila najčešće zastupljena u najstarijoj grupi žena (10,99%). **Zaključak.** Izbor antibiotske terapije za urinarne infekcije treba da bude baziran na lokalnim obrascima rezistencije i usklađen sa polom i životnim dobom bolesnika.

Ključne reči:
urinarni trakt, infekcije; mokraća; bakterije; lekovi, rezistencija bakterija; bolesnici, vanbolničko lečenje; srbija; životno doba, faktor; pol, faktor.

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Introduction

Urinary tract infections (UTIs) are among the most common infections in outpatients. They are associated with a significant morbidity and mortality in general population and impose substantial financial burden to the society. About 150 million people worldwide are affected by UTIs every year, spending about 6 billion US dollars [1]. According to the 2007 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTIs are responsible for nearly 7 million office visits and 100,000 hospitalizations [2]. In Serbia, over 350,000 people is diagnosed with acute cystitis in primary care annually, and UTIs are the fourth leading cause of visits to general practitioners [3].

Earlier studies indicated that 50–80% of uncomplicated UTIs are solely due to *Escherichia coli (E. coli)*, while the remaining cases are caused by other *Enterobacteriaceae* (*Proteus, Klebsiella, Enterobacter*) together with *Pseudomonas* spp and gram-positive bacteria such as *Enterococci, Streptococci* and *Staphylococci* [4, 5]. The host risk factors as well as the virulence of a pathogen determine clinical course of UTI. Well-known risk factors for UTIs are female gender (especially pregnancy), diabetes mellitus, spinal cord injuries, multiple sclerosis, anatomic abnormalities of the urinary tract, incontinence, urinary bladder catheterization and advanced age [6, 7].

According to the guidelines of the European Association of Urology (EAU), treatment of UTIs includes fosfomycin trometamol, pivmecillinam or nitrofurantoin as the first-line therapy; alternative therapy includes fluoroquinolones, cefpodoxime proxetil, and combination of sulfamethoxazole and trimethoprim, if the local resistance of *E. coli* to the latter is less than 20% [8]. However, local and regional adjustments of these recommendations are necessary, since there are significant local differences in frequency of urinary pathogens, emergence of new agents or susceptibility to antimicrobial drugs [9]. Two recent studies from Serbia [10] and Bosnia and Herzegovina [11] support these recommendations, since isolated gram-negative causative agents of UTIs were highly resistant to beta-lactam antibiotics (> 25%), especially to ampicillin, amoxicillin and cephalosporins.

The aim of this study was to define the causative agents of UTIs and their resistance to antimicrobial drugs in outpatients in the urban area of central Serbia, as well as to evaluate eventual differences associated with age and gender of the patients.

Methods

This retrospective study included data taken from routine, consecutively collected urine cultures of outpatients with symptomatic UTIs, collected from the Department of Microbiology, Institute of Public Health in Kragujevac, Serbia, from January 2009 to December 2013. For each outpatient, the following data were extracted: the date of the sample obtaining, age, gender, urine culture results, identification of the bacterial strain responsible for an UTI and results of the corresponding antimicrobial susceptibility test (AST).

The Department of Microbiology has internal quality control procedures and participates in the external program for quality assurance by The United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology and by Institute of Public Health of Belgrade, Serbia. The Institute of Public Health in Kragujevac is the competent UTI diagnostic center for 6 municipalities of the Šumadija region with 240,000 inhabitants.

The study was approved by the Ethics Committee of the Clinical Centre, Kragujevac, Serbia.

Before giving the urine sample, the outpatients received instructions for avoiding contamination with antimicrobials and for appropriate sampling technique, as a part of the routine procedure. The urine sample was collected early in the course of the disease, by midstream clean-catch technique after usual daily hygiene of genital area. The initial and the end portion of the micturition stream were discarded and the middle part was collected directly into a sterile recipient. In children up to two years of age urine samples were collected by collection bags taped to the skin surrounding the urethral orificium. Urine samples were transported to the laboratory and analyzed within the two hours after collection. When this procedure was not possible, urine samples were stored at 4°C and processed within the 24 hours after collection.

Identification of microorganisms was made by plating on chromogen coagulase positive *staphylococci* (CPS) agar (BioMerieux, France) and by incubation for 18–24 h at 35 ± 2^0C.

The exclusion criteria were contamination (growth of two or more bacterial species) and negative samples [bacterial growth lower than 10^3 colony-forming units (CFU)/mL of urine]. The inclusion criterion was monomorphic bacterial growth higher than 10^5 CFU/mL of the culture. All isolates were subjected to antimicrobial susceptibility testing AST.

The AST was made by the disk-diffusion method on Mueller-Hinton Agar (Biomerieux, France) and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [12] by measuring the diameter of the zones of inhibition. The following antibiotics were analyzed: penicillin (10 μg/mL), ampicillin (25 μg/mL), cephalexin (30 μg/mL), cefaclor (30 μg/mL), cefotaxime (30 μg/mL), ceftriaxone (30 μg/mL), meropenem (10 μg/mL), tetracycline (30 μg/mL), gentamicin (10 μg/mL), amikacin (30 μg/mL), ofloxacin (5 μg/mL), ciprofloxacin (5 μg/mL), trimethoprim-sulfamethoxazole (2.5 μg/mL) and nitroxolin (20 μg/mL).

Primary analysis of collected data was made by descriptive statistics. The difference between females and males in the frequency of positive samples to each of the agents was analyzed by $\chi 2$-test. Statistical hypotheses were considered true if probability of null-hypothesis was less than 0.05. All calculations were performed by the statistical software SPSS (SPSS Inc, ver.18, Chicago, IL).

Results

During the study period, there were 71,905 urine cultures, and 24,713 (34.37%) of them were positive for bacterial pathogens. Generally, the most common pathogen was *E. coli* (56.56%), followed by *Klebsiella* spp (16.20%),

Proteus spp (14.68%), *Enterococcus* spp (5.29%) *and Pseudomonas aeruginosa* (3.74%), all accounting for over 95% of total isolates (Table 1). Gram-negative agents consisted 93.28% of urinary pathogens.

The isolates were obtained from 24,713 patients, 1 to 94 years of age (median 58.1 years). Nearly 70% of all isolates were from women [female to male ratio (F/M) was 2.21 (17,015/7,698)] (Table 2). The isolates frequency according to the age distribution of the patients is presented in Table 3. Female to male ratio was the highest in 15–29 years age group (F/M = 13.0) and the lowest in the oldest one (F/M = 1.5). There were significant gender differences in the isolation rates for four of the top five causative agents (the difference was not significant only for *Enterococcus* spp.): *E. coli* was isolated more frequently in females (11,953/17,015; 70.25%), whereas *Klebsiella* spp. (2,305/7698; 29.94%), *Proteus* spp. (1,970/7698; 25.59%) and *Pseudomonas aeruginosa* (646/7,698; 8.39%) were more common in men (Table 2).

All five the most prevalent bacterial isolates differed in regard to the isolation rate between the age groups (Table 3). *E. coli* was less prevalent in the oldest subjects (7,676/14,816; 51.81%) and more prevalent in the age groups 15–29 (1,268/1,797; 70.56%) and 30–59 years (3,798/6,071; 62.56%).

The data stratification according to both gender and age showed significant differences in regard to frequency of isolation between females and males throughout all age groups (Table 3). Furthermore, *E. coli* and *Enterococcus* spp were less frequently isolated in males ≥ 60 years old (1,407/5,935; 23.71% and 289/5,935; 4.87%, respectively), while *Klebsiella* spp was more prevalent in this group (1,903/5,935; 32.06%). The most common causative agents isolated from 15–29 years old patients were *Enterococcus* spp. and *Pseudomonas aeruginosa* (17/128; 13.28% each). Interestingly, *Proteus* spp. and *E. coli* were the most prevalent (170/477; 35.64 % each) isolates in young males ≤ 14 years old.

Among women, the isolation rate of *E. coli* was high in all age groups (around 70%). *Proteus* spp. was frequently isolated from females ≤ 14 years old (206/1,552; 13.27%), while *Klebsiella spp.* was the most frequent in the oldest age group (976/8,881; 10.99%) (Table 3).

The pattern of resistance to antibiotics of main isolated uropathogens is shown in Table 4. Isolates of *E. coli* showed moderate degree of resistance to trimethoprim-sulfamethoxazole (40.1%), while resistance to fluoroquinolones was lower (32.6% ofloxacin and ciprofloxacin 26.1%), as well as the resistance to aminoglycosides (23.0% for gentamicin, and 6.1% for amikacin). Percentage of the isolates resistant to the 1st and 2nd generation of cephalosporins was the same, 32.1%, while that to the 3rd generation was 10.7%.

Isolated uropathogen *Klebsiella* spp. showed high degree of resistance to fluoroquinolones (64.4–66.6%), trimethoprim-sulfamethoxazole (69.1%) and cephalosporins of the 1st, 2nd and 3rd generation (57.7–72.5%).

Table 1

Distribution of bacterial isolates from urine samples

Microorganism	n	%
All gram-negative	23,056	93.28
Escherichia coli	13,977	56.56
Klebsiella spp.	4,004	16.20
Proteus spp.	3,629	14.68
Pseudomonas aeruginosa	924	3.74
Acinetobacter spp.	270	1.09
Pseudomonas spp.	203	0.82
Providencia spp.	49	0.19
All Gram-positive	1,657	6.72
Enterococcus spp.	1,307	5.29
Streptococcus beta-haemolyticus group B	272	1.10
Coagulase-negative staphylococci	47	0.19
Staphylococcus aureus	27	0.12
Staphylococcus saprophiticus	4	0.02
Total	24,713	100.0

Table 2

Distribution of the most common bacterial isolate from urine samples by gender of the patients

Microorganism	Isolates, n (%)			*p*-values
	All (n = 24,713)	Males (n = 7,698)	Females (n = 17,015)	
Escherichia coli	13,977 (56.56)	2,024 (26.29)	11,953 (70.25)	< 0.001
Klebsiella spp.	4,004 (16.20)	2,305 (29.94)	1,699 (9.99)	< 0.001
Proteus spp.	3,629 (14.68)	1,970 (25.59)	1,659 (9.75)	< 0.001
Enterococcus spp.	1,307 (5.29)	411 (5.34)	896 (5.27)	0.708
Pseudomonas aeruginosa	924 (3.74)	646 (8.39)	278 (1.63)	< 0.001
All other Gram-negative	522 (2.11)	295 (3.83)	227 (1.33)	< 0.001
All other Gram- positive	350 (1.42)	47 (0.61)	303 (1.78)	< 0.001

Table 3

Distribution of the five most common bacterial isolates from urine samples by gender and age groups of the patients

Microorganism	≤ 14 years	15–29 years	30–59 years	≥ 60 years	*p*-values
Total, n					
males	477	128	1,158	5,935	
females	1,552	1,669	4,913	8,881	
all	2,029	1,797	6,071	14,816	
Escherichia coli, n (%)					
males	170 (35.64)	37 (28.91)	410 (35.41)	1,407 (23.71)	0.002[a]
females	1,065 (68.62)	1,231 (73.76)	3,388 (68.96)	6,269 (70.59)	
all	1,235 (60.87)	1,268 (70.56)	3,798 (62.56)	7,676 (51.81)	< 0.001[b]
Klebsiella spp, n (%)					
males	99 (20.75)	21 (16.21)	282 (24.35)	1,903 (32.06)	< 0.001
females	98 (6.31)	100 (5.99)	525 (10.69)	976 (10.99)	
all	197 (9.71)	121 (6.73)	807 (13.29)	2,879 (19.42)	< 0.001
Proteus spp, n (%)					
males	170 (35.64)	29 (22.66)	215 (18.57)	1,556 (26.22)	< 0.001
females	206 (13.27)	133 (7.97)	460 (9.36)	860 (9.68)	
all	376 (18.53)	162 (9.02)	675 (11.12)	2,416 (16.31)	< 0.001
Enterococcus spp, n (%)					
males	28 (5.87)	17 (13.28)	77 (6.65)	289 (4.87)	< 0.001
females	89 (5.73)	129 (7.73)	276 (5.61)	402 (4.53)	
all	117 (5.77)	146 (8.12)	353 (5.81)	691 (4.66)	< 0.001
Pseudomonas aeruginosa, n (%)					
males	6 (1.26)	17 (13.28)	105 (9.07)	518 (8.73)	< 0.001
females	57 (3.67)	6 (0.36)	74 (1.51)	141 (1.59)	
all	63 (3.1)	23 (1.28)	179 (2.95)	659 (4.45)	< 0.001

a – analysis of distribution of isolat rates among age groups by gender of patients or b – in all patients.

Table 4

Resistance pattern (%) of the most common bacterial isolates from urine samples

Microorganism	Antibiotic													
	PEN	AMP	CFL	CFC	CET	CTR	MER	TR	GEN	AMC	OFX	CIP	SXT	NTX
Escherichia coli	-	57.8	32.1	32.1	10.7	10.7	9.8	-	23.0	6.1	32.6	26.1	40.1	2.7
Klebsiella spp	-		72.5	72.5	57.7	58.0	11.2	-	59.8	30.2	66.6	64.4	69.1	3.6
Proteus spp	-	79.5	72.8	70.2	50.4	49.3	10.4	-	63.6	44.5	63.9	60.2	74.7	2.1
Pseudomonas aeruginosa	-	98.8	98.9	98.8	78.1	78.0	29.2	-	81.5	47.5	82.6	78.3	98.1	-
Acinetobacter spp	-	85.8	77.8	77.5	57.7	58.6	18.2	-	57.7	25.1	69.4	63.7	44.5	-
Pseudomonas spp	-	87.2	76.3	77.2	51.2	45.1	23.9	-	55.8	36.4	56.1	60.4	81.9	-
Enterococcus spp	7.0	7.5	-	-	-	-	-	84.4	69.9	-	-	43.1	9.1	-
Streptococus beta-haemolyticus group B	5.1	4.1	11.9	15.4	11.1	6.3	-	49.3	25.0	50.0	-	16.5	37.4	-

PEN – penicillin, AMP – ampicillin, CFL – cephalexin, CFC – cefaclor, CET – cefotaxime, CTR – ceftriaxone, MER – meropenem, TR – tetracycline, GEN – gentamicin, AMC – amikacin, OFX – ofloxacin, CIP – ciprofloxacin, SXT– trimethoprim-sulfamethoxazole, NTX – nitroxolin; -: not tested.

Proteus spp. isolates were highly resistant to trimethoprim-sulfamethoxazole (74.7%), ampicillin (79.5%) and the fluoroquinolones (63.9% ofloxacin and 60.2% ciprofloxacin). The other gram-negative bacteria also showed high degree of resistance to tested antimicrobial drugs.

The most commonly isolated uropathogen from the gram-positive group, *Enterococcus* spp. showed a low grade of resistance to ampicillin (7.5%), and trimethoprim-sulfamethoxazole (9.1%), but a high grade of resistance to tetracyclines (84.4%).

Discussion

Knowledge of the local or regional etiology of UTIs and antimicrobial resistance can be very useful as a guide for empirical therapy, because the frequency of pathogens and their features vary according to time and geographical area. As these infections are very common, adequate treatment have an important role in regard to the patients' health, development of antibiotic resistance and health care costs [1]. A large number of bacterial isolates included in this study (obtained from routine urine analyses), allowed stratification

of data according to gender and age, and evaluation of association of these variables and UTI etiology, as well as determination of susceptibility of uropathogens to commonly prescribed antimicrobial drugs.

In our study, over 90% of all isolates were gram-negative pathogens. As it was expected, *E. coli* was the most frequent isolate (56.56%). It was also the most frequent uropathogen associated with the community-acquired UTIs (being implicated in more than a half of all the UTIs) in other studies [5, 13]. *E. coli* generally belongs to normal flora of human colon and therefore may easily colonize the urinary tract. The other gram-negative pathogens found in this study were *Klebsiella* spp. *Proteus* spp. and although they were isolated in small percentages, they play substantial role in UTIs due to their pathogenicity and high resistance to antibiotics [14, 15].

In our study the obtained isolation rate of gram-positive bacteria was relatively low (6.72%) and among them, *Enterococcus* spp was responsible to 5.28% of UTIs. The other studies show similar results, confirming that these bacteria have minor role in UTIs [16]. However, true frequency is still unknown, since the studies published about the topic differ in design, sample size, inclusion and exclusion criteria and presentation style.

Women are more likely to experience UTIs than men. Nearly 70% of all isolates in our study were obtained from women. This could be explained by anatomical differences: the urethra is shorter and closer to the anal orifice in women than in men. Furthermore, women are more likely to get an infection after sexual activity or when using a diaphragm for birth control. Pregnancy and menopause also increase risk from UTIs [6].

In our study, significant difference was also found in frequency of certain uropathogens in relation to gender: *E. coli* was isolated more frequently in females, whereas *Klebsiella* spp., *Proteus* spp. and *Pseudomonas aeruginosa* were more common in men, which is consistent with the results of other authors [4]. Previous studies have indicated that some uropathogens, especially *Pseudomonas aeruginosa*, were strongly associated with particular host characteristics, including male gender, recent antibiotic therapy, prior urinary tract procedures and neurogenic bladder [17].

In our study significant differences in etiology of UTIs among different age groups were observed, too. Besides, frequencies of urinary pathogens were different across both age- and gender-stratified groups. *E. coli*, for example, was less prevalent in the oldest males (23.71%), but highly frequent in female patients from all age groups (approximately 70%). *Klebsiella* spp. was the most common in the oldest age group in both men and women (32.06% and 10.99%, respectively), and *Proteus* spp. frequency was highest in younger age groups of both males and females (35.64% and 13.27%, respectively). Age of the patients was linked to etiology of UTIs in several recent publications [18–20]: the study similar to our with the data stratification according to both age and gender showed lower *E. coli* isolation rate in both males ≥ 60 years old (52.2%) [*Escherichia faecalis* and *Pseudomonas aeruginosa* were frequent in this group (11.6% and 7.8%, resp.), and in those ≤ 14 years old (51.3%) (*Proteus mirabilis* was highly prevalent in this group: 21.2%]. On the other hand, Linhares et al. [21] in a ten-year study did not find differences in uropathogen isolation rates among age groups of patients with an UTI. However, when the age groups were stratified according to gender, the isolation rate increased with the age.

Proteus mirabilis is the most frequent uropathogen in boys [22], which should be borne in mind when prescribing antimicrobial drugs to boys. On the other hand, the results of our study indicate that *Proteus* spp. is an important urinary pathogen in young females, in spite of its low frequency in the preadolescent female genital tract flora [23].

The misuse of antibiotic drugs in medicine has led to an alarming increase of the microbial resistance [24] and the consequent spread of antibiotics-resistant strains is a serious public health problem. Approximately 15% of all community-prescribed antibiotics in the USA [25] and some European countries [26] are dispensed for UTIs. Prudent use of available antibiotics is the only option to delay the development of resistance [27] and the urological community has a responsibility to contribute to these efforts. Therefore, it is necessary to follow the guidelines of EAU in treatment of UTIs. Also, it must be noted that the recommended antibiotic for the first-line therapy pivmecillinam is not registered in Serbia and that the fosfomycin and nitrofurantoin are not frequently used, so it was not possible to draw some conclusions about their effectiveness in treatment of UTIs.

In our study, 40.1% of isolates of *E. coli* were resistant to trimethoprim-sulfamethoxazole, while the percentage of resistance to fluoroquinolones was lower (32.6% ofloxacin and ciprofloxacin 26.1%), but still relatively high and in line with other European countries [28, 29]. This is probably due to extensive utilization of these antibiotics in treatment of community-acquired UTIs over the past decade in this region. Although values may vary among reports, resistance rate of recently community-isolated of *E. coli* to trimethoprim-sulfamethoxazole in Europe tends to be higher than 30% [30, 31].

According to the international Antimicrobial Resistance Epidemiological Survey on Cystitis (ARESC) conducted from 2003 to 2006, *E. coli* showed a high resistance to sulfonamides (29.4%) and to fluoroquinolone ciprofloxacin (8.1%) in nine European countries and in Brazil [32], thus limiting use of these antibiotics in empirical therapy. It is necessary that entire community makes significant effort to maintain sensitivity of urinary pathogens to antibiotics which could be given for treatment of UTIs. What was encouraging from this study is relatively low level of resistance to second-line antibiotics for UTIs, aminoglycosides (gentamicin and amikacin, 23.0% and 6.1%, respectively) and third generation cephalosporins (cefotaxime and ceftriaxone, 10.7% both). However, some of these drugs do not exist in the oral form and are more expensive for the treatment of UTIs.

The isolates of *Klebsiella* spp. in our study showed a high degree of resistance to fluoroquinolones (64.4–66.6%), trimethoprim-sulfamethoxazole (69.1%) and second- and third-generation cephalosporins (57.7–72.5%), which is 2–3

times higher than in other recent European studies [21]. This result is worrisome due to a high proportion of UTIs caused by *Klebsiella* spp. in our community (16.2%).

The resistance rate to uropathogens *Proteus* spp. isolated in our study was generally high for all tested antibiotics. The resistance rate of this isolate to trimethoprim-sulfamethoxazole is 74.7%, 79.5% to ampicillin, 63.9% to ofloxacin and 60.2% to ciprofloxacin, which is much higher than the rates shown in other similar studies [20].

Conclusion

The results of our study are a useful tool for doctors who should prescribe antibiotics to patients with UTIs, as well as for regional health authorities who intend to formulate recommendations for rational antibiotic use and define standard treatment guidelines. When prescribing drugs for UTIs, Serbian physicians should be aware of a high resistance rate of urinary pathogens not only to semi-synthetic penicillins and cephalosporins (> 30%), but also to fluoroquinolones (> 25%) and trimethoprim-sulfamethoxazole (> 40%), and choose among the antibiotics with still low resistance rates. Choice of antibiotics for treatment of UTIs should be governed not only by the local resistance patterns, but also by gender and age of patients.

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Stigmatization and discrimination of patients with chronic hepatitis C

Stigmatizacija i diskriminacija obolelih od hroničnog hepatitisa C

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Abstract

Background/Aim. Chronic hepatitis C (CHC) is often associated with injectable drug users and human immunodeficiency virus coinfection for which there is stigmatization in society. The aim of this study was to identify the presence of stigma and discrimination of patients with CHC, as well as the influence of sociodemographic factors on the occurrence of stigmatization. **Methods.** A cross-sectional study was performed. Patients with CHC and conducted antiviral therapy completed an anonymous structured questionnaire consisting of sociodemographic questions and Hepatitis C stigma scale. **Results.** Out of 154 patients 61.7% were male and 72.1% from the city; 59.7% have completed secondary school; 61.7% were employed before the disease while 31.8% after the disease; 45.5% were unsatisfactory with financial situation; 54.5% were married; 37.7% lived with a spouse and children; 86.4% in their own house/apartment; 5.2% of the patients were abandoned by their partners, while 35.7% consumed drugs. A statistical significance of the stigma score was found in those who lived in the city ($p = 0.018$), unmarried ($p = 0.005$), abandoned by the partners after the diagnosis of CHC ($p < 0.001$), drug users ($p = 0.002$) and those living with parents ($p = 0.034$). Univariate regression analysis singled out as significant: residence ($p = 0.018$), living with their parents ($p = 0.046$), abandonment by a partner ($p < 0.001$) and drug use ($p = 0.002$). A multivariate regression model of independent variables singled out abandonment by partners (Beta = 5.158, $p = 0.007$). Men disagree significantly with the two elements inside stigma [not the same as the others ($p = 0.035$)] and hurt by the reaction of others ($p = 0.047$)). **Conclusion.** The presence of stigma in patients with CHC was proven. The results indicate the need to strengthen anti-stigma programs that will reduce their psychological and social problems and reduce stigmatization in society.

Key words:
hepatitis c; hepatitis, chronic; social stigma; socioeconomic factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Hronični hepatitis C (HHC) često se povezuje sa korisnicima droga koji ih injektiraju i sa koinfekcijom virusom humane imunodeficijencije kod kojih postoji stigmatizacija u društvu. Cilj ove studije bio je da se identifikuje prisustvo stigmatizacije i diskriminacije obolelih od HHC, i ispita uticaj sociodemografskih faktora na pojavu stigmatizacije. **Metode.** Ova studija preseka obuhvatila je ispitanike sa HHC i sprovedenom antivirusnom terapijom koji su anonimno popunjavali strukturisani upitnik sastavljen od sociodemografskih pitanja i hepatitis C stigma skale. **Rezultati.** Od ukupno 154 ispitanika, 61,7% bilo je muškog pola, 72,1% iz grada, 59,7% sa srednjom školom, 61,7% radilo je pre bolesti, a posle 31,8%, 45,5% je bio nezadovoljavajućeg materijalnog stanja, 54,5% oženjeno/udato, u zajednici sa supružnikom i decom 37,7%, u sopstvenoj kući/stanu 86,4%. Partner je napustio 5,2% obolelih, dok je drogu konzumiralo 35,7% ispitanika. Statistička značajnost stigma skora nađena je kod bolesnika koji su živeli u gradu ($p = 0,018$), neoženjenih/neudatih ($p = 0,005$), ostavljenih od strane partnera posle dijagnostikovanja HHC ($p < 0,001$), korisnika droga ($p = 0,002$) i onih koji su živeli sa roditeljima ($p = 0,034$). Univarijantnom regresionom analizom izdvojeni su, kao statistički značajni: prebivalište ($p = 0,018$), život sa roditeljima ($p = 0,046$), napuštanje od strane partnera ($p < 0,001$) i upotreba droge ($p = 0,002$). Multivarijantnim regresionim modelom nezavisnih varijabli, kao značajno izdvojeno je napuštanje od strane partnera (Beta = 5,158, $p = 0,007$). Muškarci se značajno nisu slagali sa dva elementa unutrašnje stigme (nije isti kao drugi – $p = 0,035$ i povređeni reakcijom drugih – $p = 0,047$)). **Zaključak.** Utvrđeno je prisustvo stigmatizacije kod obolelih od HHC. Rezultati ukazuju na potrebu jačanja antistigma programa koji će umanjiti psihičke i socijalne probleme kod obolelih od HHC i sniziti njihovu stigmatizaciju u društvu.

Ključne reči:
hepatitis c; hepatitis, hronični; socijalna stigma; socioekonomski faktori; ankete i upitnici.

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Introduction

Stigmatization of patients is often associated with a fear of chronic diseases: the fear of the disease, the fear that they will infect others and the fear of death. Stigmatization is attributing traits that significantly discredit a person in the eyes of others. In the beginning, it indicated stamping unfit person. Now it is commonly used in the context of social alienation from the people with discrediting condition or disease, which further helps reduce the chances of life by limiting access to employment, education, housing, income, health care, etc. [1]. The influence of stigma leads to different social interactions [2]. According to data from the literature, the most stigmatized are those suffering from mental illness, AIDS, chronic hepatitis C (CHC), tuberculosis and others [3–8]. The reaction is not only to the symptoms of the disease but also to the opinion that the society has about the disease. People avoid the infected ones because of the ignorance of the ways of transmission, especially blood transmitted infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV), partly because of the fears of contagion, or their negative attitudes and socially accepted view that only people with high-risk behavior usually get sick with these diseases, so the society has little understanding and marginalizes them. As a result, patients avoid social gatherings, isolate themselves, lose self-esteem and fall into depression [9–11]. Sometimes the stigma and discrimination of patients happens in the health institution, usually in the hospital, general practice and dental practice [12]. There is a rejection of the provision of health services, stigma when providing services or violation of confidentiality.

The aim of this study was to identify the presence of stigma and discrimination of patients with CHC and explore the impact of sociodemographic factors on the occurrence of stigmatization.

Methods

This-cross sectional study was performed in the period from January 1 to March 31, 2015. Consecutive, ambulatory and hospitalized patients (154 patients of both sexes) of the Clinic for Infectious Diseases, Clinical Centre Niš, Serbia, diagnosed with CHC were interviewed and included in the study. All the patients were informed about the research objectives in details. All data were collected anonymously by voluntary filling in the questionnaire by the patients in the presence of the interviewer. The approval of the Ethics Committee of the Clinical Centre Niš (No. 338/41 of 13 January 2015) and the Public Health Institute Niš (No. 07-4693 from December 26, 2014) was obtained.

The criterion for inclusion in the study was the diagnosis of CHC by the infectologist and treatment of patients of both sexes aged over 18 years by antiviral therapy. All criteria for the diagnosis and treatment are implemented by the infectologist.

The criteria by which people were not included: aged under 18, people who at the time of testing or previously were serving a prison sentence, psychiatric patients and patients with dementia, pregnant women, hemodialysis patients with a contraindication to receiving ribavirin, ignorance of Serbian language and not accepting participation in the study.

By using a structured questionnaire designed for this study following parameters were examined: sociodemographic characteristics (age, sex, place of residence, education level, employment status, financial status, marital status, number of household members), the time elapsed from the time of diagnosis to the initiation therapy and characteristics of behavior (use of psychoactive substances). To determine the level of stigmatization HCV stigma scale was used. The self-administered questionnaires were used.

The hepatitis C stigma scale is a questionnaire that is derived from the original HIV stigma scale with 40 questions [13]. A short form with 10 questions was adapted by Wright et al. [14]. The edited version contains minimum two questions from the following subscales: personalized stigma (consequences of knowing the status of the patient), concerns about the disclosure of the diseases, negative self-image: shame, guilt, not as good as others) and 4 concern over public attitudes. The scale has been adapted for patients with other health disorders (lung cancer) and population groups, as well. The scale was shortened for 1 question (factor disclosure in Berger scale), because it reduces the scale validity and reliability [15, 16]. HCV stigma scale with 9 questions enables to see the connection with depression and mental health. Tests have shown that the maximum scores of stigmatization are associated with depression and mental health. Answers are ranked from 1 to 4 (with modalities: I strongly disagree – 1, I disagree – 2, I agree – 3, I strongly agree – 4), with the total score that ranges from 9 to 36. Maximum score is the greatest stigmatization.

Statistical analysis

From the basic descriptive statistical parameters, the following standard statistical methods for qualitative and quantitative assessment of the results were used: absolute numbers, relative numbers (%), arithmetic mean ($\bar{x}$) and standard deviation (SD). The normality of the distribution of individual values was investigated with Kolomogorov Smirnov test. Comparing the arithmetic mean of the two sample was performed by *t*-test while, in cases of improper distribution of data, nonparametric Mann-Whitney *U* test was used. For the purpose of comparing the value of the test characteristics among several samples ANOVA was used (i.e. the Kruskal-Wallis test in cases of irregular distribution), where for the *post hoc* analysis, i.e. for the analysis of the values between two sample, the Bonferroni test was used. To test the statistical significance of the difference of absolute frequencies between samples, χ^2 test was used. For the correlation analysis Pearson's linear correlation coefficient was used. In order to determine predictors stigma score multivariate regression analysis was used. Statistical hypothesis was tested at the level of significance for the risk of $\alpha = 0.05$, i.e. the difference between the samples was considered significant if $p < 0.05$.

Results

Table 1 shows the descriptive characteristics of the patients. There were more male subjects, 95 (61.7%); the average age of the patients was 46.81 ± 13.86 (range 22–75 years); the city as residence had 111 (72.1%) patients. The highest percentage of subjects had secondary education (59.7%), followed by completed college and university 27 (17.5%), basic 25 (16.2%), while there was the smallest number of those without school 10 (6.5%). A higher percentage of patients at the time of morbidity was employed (61.7%), while 31.8% were currently working. Only one (0.65%) health worker was registered among respondents with the diagnosis of CHC. More than half (51.3%) of the subjects believed themselves satisfactory, 45.5% poor, while 3.2% were in excellent financial situation. The largest number of the patients were married, 84 (54.5%), and living with a spouse and children, 58 (37.7%). Also, most of them were living in their own house / flat, 133 (86.4%).

Table 1

Baseline descriptive characteristics of the patients with the diagnosis of hepatitis C infection

Characteristics	Patients, n (%)
Gender	
male	95 (61.7)
female	59 (38.3)
Residence	
city	111 (72.1)
village	43 (27.9)
Level of education	
incomplete primary	10 (6.5)
primary	25 (16.2)
secondary	92 (59.7)
college and university	27 (17.5)
Employment status at the time of morbidity	
unemployed	59 (38.3)
employed	95 (61.7)
Current employment status	
unemployed	105 (68.2)
employed	49 (31.8)
Financial state	
poor	70 (45.5)
satisfactory	79 (51.3)
excellent	5 (3.2)
Marital status	
not married	42 (27.3)
married	84 (54.5)
cohabitation	4 (2.6)
divorced	16 (10.4)
a widower / widow	8 (5.2)
Abandonment by the partners after the diagnosis of hepatitis C	
no	146 (94.8)
yes	8 (5.2)
Life in your house / flat	
no	21 (13.6)
yes	133 (86.4)
Family status	
lives alone	13 (8.4)
lives with a spouse	28 (18.2)
lives with a spouse and children	58 (37.7)
lives with children	12 (7.8)
lives with his parents	43 (27.9)
The use of psychoactive substances before morbidity	
no	99 (64.3)
yes	55 (35.7)
The use of heroin before morbidity	48 (31.17)
Current use of psychoactive substances	
no	149 (96.8)
yes	5 (3.2)
On cessation therapy	6 (3.9)

A total of 8 (5.2%) patients were left by their partners after learning they had hepatitis C. Psychoactive substances were consumed by 55 (35.7%) patients before learning they had the disease, mostly heroin (31.17%). Currently, 6 (3.9%) patients were on cessation therapy.

The average age of the patients at the time of the morbidity was 39.92 ± 14.67 years, with the average age at the time of initiation of symptomatic treatment 37.13 ± 13.38 years, and at the time of starting therapy with interferon and ribavirin 43.36 ± 13.59 years (Table 2).

In this study, only in two (1.3%) male patients the highest stigma scores (34 or all 36) were found, while 6 (3.90%) patients had the lowest score – 9, i.e. these patients did not have any form of stigmatization.

Table 3 shows that the patients who lived in the city (p = 0.018) had higher values of stigma score. There was a

Table 2

Age structure of the patients depending on the period of disease

Period of the disease	Age (years) $\bar{x}$ ± SD; Med (min–max)
At the time of the study	46.81 ± 13.86; 44.5 (22–75)
At the time of commencement symptomatic therapy	37.13 ± 13.38; 34,00 (13–66)
At the time of commencement therapy with interferon and ribavirin	43.36 ± 13.59; 41.00 (14–73)

$\bar{x}$ – mean value; SD – standard deviation; Med – median.

Table 3

Values of stigma score according to certain sociodemographic factors

Variables	Stigma score ($\bar{x}$ ± SD)	t/F*	p
Gender			
male	18.44 ± 5.27	0.874	0.774
female	19.19 ± 4.91		
Residence			
city	19.33 ± 5.15	2.389	0.018
village	17.16 ± 4.81		
Educational attainment			
no education	19.00 ± 5.89		
primary	16.68 ± 4.98	2.284*	0.081
secondary	19.49 ± 5.16		
college and university	17.93 ± 4.51		
Current employment status			
unemployed	19.01 ± 5.25	0.998	0.887
employed	18.12 ± 4.87		
Financial state			
poor	19.13 ± 5.39	0.713	0.492
satisfactory	18.51 ± 4.99		
excellent	16.60 ± 3.51		
Marital status			
not married [a]	20.74 ± 5.73		
married	17.39 ± 4.75	3.654*	0.007
cohabitation	18.00 ± 2.58		
divorced	20.00 ± 4.71		
a widower / widow	20.00 ± 3.85		
Leaving from a partner			
no	18.38 ± 4.92	3.692	< 0.001
yes	25.00 ± 5.18		
Family status			
lives alone	20.23 ± 1.55		
lives with a spouse [b]	17.43 ± 4.81	3.239*	0.014
lives with a spouse and children	17.45 ± 4.71		
lives with children	20.17 ± 4.04		
lives with parents	20.44 ± 5.46		
The use of psychoactive substances before morbidity			
no	17.79 ± 4.61	3.132	0.002
yes	20.42 ± 5.63		

***ANOVA; [a] (single *vs* married, p = 0.005); [b] (spouse and children *vs* parents, p = 0.034).**

statistically significant difference in the stigma score in relation to marital status (p = 0.007). It was observed that the unmarried subjects had a greater stigma score than the married subjects (20.74 ± 5.73 *vs* 17.39 ± 4.75, p = 0.007).

The subjects abandoned by the partners after learning about the disease had a significantly higher stigma score than those who were not abandoned (25.00 ± 5.18 *vs* 18.38 ± 4.92, $p < 0.001$).

In relation to who subjects lived with, a statistically significant difference was observed (p = 0.14). Further analysis found that people who lived with their parents had significantly higher scores compared to those living with a spouse and children (20.44 ± 5.46 *vs* 17.45 ± 4.71, p = 0.034).

The subjects who used drugs prior to the diagnosis of hepatitis C had a significantly higher stigma score compared to those who did not consumed drugs (20.42 ± 5.63 *vs* 17.79 ± 4.61, p = 0.002).

Univariate regression analysis was conducted to evaluate the effect of some independent factors to the values of stigma score. As statistically significant were singled out: residence (p = 0.018), the person the subjects lived with (p = 0.046), abandonment by a partner ($p < 0.001$) and the use of psychoactive substances before the diagnosis of hepatitis C (p = 0.002) (Table 4).

The method of standard multiple regression analysis was performed to examine the place of residence, the person the subjects lived with, abandonment by the partners, drug use before morbidity and marital status on the value of the stigma score. The tested model explains 12.80% of the variance of stigma score (adjusted R2 = 0.098, F = 4.326, p = 0.001). Only abandonment by partners was allocated as an independent risk factor (Beta = 5.158, p = 0.007) (Table 5).

To the question (Table 6) whether they feel not the same as others because of hepatitis C, a statistically significant difference was found in the responses between men and women. A significantly more men disagreed with this statement (χ^2 = 4.4288, p = 0.035).

No statisticaly significant differences were found in the responses to gender questions: whether they felt dirty, whether they had the feeling of being a bad person, whether they believed people with hepatitis C repulsive, whether they stopped to socialize with some people because of their reactions, whether they had lost friends after the announcement of hepatitis C, whether they worried about what others would say about their disease. When asked whether they were hurt by the reaction of others to their hepatitis C, the statistically significant differences in the responses in relation to sex was found. Significantly more men disagreed with this statement (χ^2 = 3.391, p = 0.047).

Discussion

This study examined the presence of stigma and discrimination of patients with CHC, as well as the influence of some sociodemographic factors on the presence of stigma. Stigmatization and some sociodemographic factors, such as marital status, abandonment by partners after finding that the subject got infected by hepatitis C, drug users and living with parents, were found as significantly influential. The patients suffered from distortion of mental health [17], lowering of self-esteem, fear of disclosing a positive HCV status, even to health care workers and, consequently, less access to medical care. It seems that the stigma of HCV hurts more than the knowledge that they are suffering from hepatitis C.

Suarez [18] believes that research too often excludes structural inequalities in the evaluation of stigmatization and that minorities, already stigmatized, less acknowledge stigmatization due to HCV infection.

Table 4

Univariate regression analysis of certain sociodemographic factors according to stigma score

Variables	Beta	95% CI	*p*
Gender	0.071	-0.939– 2.427	0.384
Residence	-0.190	-3.966– -0.376	0.018
Educational	0.040	-0.805–1.341	0.622
Financial state	-0.088	-2.284-0.656	0.276
Marital status	-0.019	-0.848–0.669	0.816
Family status	0.161	0.011–1.278	0.046
Abandonment by partners	6.616	3.076–10.157	< 0.001
The use of psychoactive substances before morbidity	2.630	0.971–4.289	0.002

CI – confidence interval.

Table 5

Multivariate regression model of independent variables according to stigma score

Variables	Beta	95% CI	*p*
Residence	-0.109	-3.076–0.579	0.179
Family status	0.061	-0.409–0.902	0.459
Abandonment by partners	5.158	1.411–8.905	0.007
The use of psychoactive substances before morbidity	0.137	-0.336–3.265	0.110
Marital status	0.059	-0.464–1.021	0.460

CI – confidence interval.

Table 6

The presence of stigmatization by gender

Type of stigmatization	Disagree n (%)	Agree n (%)	χ^2	*p*
It is not the same as others				
male	63 (66.3)	32 (33.7)		
female	29 (49.2)	30 (50.8)		
total	92 (59.7)	62 (40.3)	4.4288	0.035*
Feels dirty				
male	69 (72.6)	26 (27.4)		
female	35 (59.3)	24 (40.7)		
total	104 (67.5)	50 (32.5)	2.9214	0.08
Feels that he/she is a bad person				
male	84 (88.4)	11 (11.6)		
female	53 (89.8)	6 (10.2)		
total	137 (89)	17 (11)	0.0731	0.786
People with hepatitis C are repulsive				
male	55 (57.9)	40 (42.1)		
female	33 (55.9)	26 (44.1)		
total	88 (57.1)	66 (42.9)	0.0569	0.8115
People with hepatitis C are rejected				
male	50 (52.6)	45 (47.4)		
female	28 (47.5)	31 (52.5)		
total	78 (50.6)	76 (49.4)	0.3873	0.533
Hurt by the reaction of others				
male	68 (71.6)	27 (28.4)		
female	33 (55.9)	26 (44.1)		
total	101 (65.6)	53 (34.4)	3.9221	0.047*
Stopped hanging out with some people because of their reactions				
male	83 (87.4)	12 (12.6)		
female	52 (88.1)	7 (11.9)		
total	135 (87.7)	19 (12.3)	0.0197	0.8884
Lost friends				
male	82 (86.3)	13 (13.7)		
female	56 (94.9)	3 (5.1)		
total	138 (89.6)	16 (10.4)	2.04	0.1079
Worried that others tell about their illness				
male	68 (71.6)	27 (28.4)		
female	42 (71.2)	17 (28.8)		
total	110 (71.4)	44 (28.6)	0.0027	0.958

General mental pain is associated with the total score of stigma, while specific symptoms are associated with certain types of stigma. Depression and anxiety are positively correlated with more personal effects of stigma in contrast to concerns about public attitudes or disclosure. Fear of rejection and negative self-image are essential for mental health. Social support shows a similar pattern [19]. Upon the recommendation of Mikocka-Walus et al. [20] all patients suffering from hepatitis C should be tested for depression because in their study 34% of patients with hepatitis C had depression [20].

It is often thought that suffering from HCV infection are to be blamed themselves for their risky behavior so a negative attitude toward them follows, even by health care workers, which indicates the lack of information) and the level of knowledge of health workers about HCV infection. The study shows that 41% of HCV patients had some kind of difficulties in communicating with their doctor. The patients reported twice more often the difficulties with subspecialists compared with general practitioners. The authors [21] believe that this may be the consequence of the coexistence of emotional and social problems. In the Australian study, 65% of patients with HCV said they had experienced stigmatization in the health institution. Due to the disease, 35% to more than 85% of HCV-infected were stigmatized [22].

In their research Moore et al. [23], showed the fact that 84.6% of patients with hepatitis C experienced stigmatization. Older than 65 years and Hispanics were stigmatized. The most common stigmatizers were health care workers (at 54.5%), and the patients experienced stigmatization in the family environment, at work, in health care institutions and society [24].

HCV stigmatization and discrimination are often equaled to the one of HIV infection. Cabrera's data support the links between greater stigma and increased depressive syndrome and impaired quality of life of people living with

HIV / HCV. In fact, people living with HIV are at increased risk of coinfection with HCV: in Canada the percentage is around 25% and in North America in injecting drug users even 50% –90% [15].

Between the sexes there was no statistically significant difference in the overall stigma score. According to the type of stigmatization in two, "is not like the other" and "hurt by the reaction of others", it was registered that a significantly large number of men answered that they did not agree with this view. The explanation may be that some of the subjects did not experience lowering of self-esteem due to their education and knowledge of the disease transmission, but eventually the effects of stigma could be changed [25]. Studies show that women are generally more stigmatized and have a higher prevalence of diagnosis of posttraumatic stress disorder (45.16%) than men (20.58%) [22, 26].

In Egypt, the current response of over 60% of patients with HCV is that they feel sadness and concern and over 40% feel dirty and dangerous for others. This is more expressed in women. Later less than 30% felt like this. Over 35% reported disturbances in relationships with family. Because of the nervousness, 32.6% alienated from the family; due to the fear 24.8%. Women had a higher degree of alienation from the partners in marriage. More than 35% reported problems in relationships with friends (due to fatigue 25.4% of women and due to nervousness 20.4% of men isolated themselves). The loss of friends, due to fear that they will transmit the disease to them, more women (12.4%) suffer than men (5.7%). A higher number of men (52.3%) reported employment problems, and job loss (40.2%). As more as 70% of patients have financial problems; 13% of patients are stigmatized, of whom 71% are male. The main cause of stigmatization is a false image of the transmission mode (operation and sharing of food). Because of sexual orientation, 12.2% of men and 15% of women are stigmatized [27].

Significantly higher values of stigma score have been found in subjects who live in the city (72.1% of subjects) compared to those living in the countryside. In contrast, another study shows that the fear of discovering HCV status and violation of patient confidentiality may even be stronger in rural areas and all this is because of the fear of being isolated from the community [28].

Family plays a decisive role in health and disease of its members. It solves with its own resources about 75% of the total health needs. Many failures and poor results are due to superficiality and errors in communication, the lack of empathic attitude and the lack of partnership [29]. Marital condition significantly affects stigmatization. This study found that the unmarried subjects (27.3%) have a higher degree of stigmatization compared to married ones. A study on the health status, health needs and utilization of health services of population in Serbia in 2000 finds that in the population of Serbia single ones are more satisfied with the quality of their life than the married ones. Other studies show that married people are happier than others [30]. This indicates that stigmatization is more tolerant in those who have the support of their families and are socially active.

The subjects left by the partners due to their disease had significantly higher level of stigmatization than not abandoned. Multiple regression analysis found that this was the only predictor of the occurrence of stigmatization. To the question that people with hepatitis C are rejected due to the disease, 49.4% of the subjects agreed with that, more women agreed. For fear of detection and rejection, a large number of patients decide to keep silent about their disease. In their study Blasiole et al. [31] find that 45% of patients with hepatitis C lose at least one relationship, due to their illness, 17% of patients have the deterioration of social contacts, which caused less sexual intercourse, and 16% had deterioration of relations with family members. A total of 12% of the subjects lose at least one friend, 8% state multiple losses that led to social isolation and 7% isolate themselves from the family and friends. A quarter of the patients report that their family members are concerned about their own health.

People who live with their parents have a significantly higher stigma score than those who live with a spouse and children. People who develop a positive identity as members belonging to the family will develop a positive perception and experience of themselves because they got social support from their family and will not feel stigmatized nor will they stigmatize themselves [29]. Many patients in the studies by Manos et al. [32] declare that they deliberately isolate themselves from friends and activities, sometimes to avoid explaining or talking about their situation. Their illness and/or treatment 24.5% conceale from their friends, but they have the support of their family members in 79.5%, while 14.8% have no support of their spouse.

Before their disease had been diagnosed 35.7% of the subjects consumed drugs (31.17% heroin), and after that 3.2% consumed marijuana. The subjects who used drugs prior to the diagnosis of hepatitis C virus were more stigmatized than those who did not consume drugs. The World Health Organization estimates that in Europe, more than 19 million people are infected with hepatitis C virus and that 67% of infected are injectable drug users [33]. The central and special feature of the stigma of HCV in the Western world is its connection with injecting drug users [34]. In Poland, a high prevalence of HCV (42.7%) and HIV (3.2%) infections among intravenous drug users, who inject drugs less than 2 years, and the users under the age of 25 years old (49.5% and 8.8% HCV HIV), indicates the permanent transfer of both infections in this population. There was a strong association of low socioeconomic status and deprivation of liberty with a higher prevalence of blood-borne diseases [35]. A study of veterans, mostly from the Vietnam War, has found a high prevalence of psychiatric disorders and drug use in outpatients with CHC when they are going through the identification of the therapy. People with this comorbidity are contraindicated for treatment with pegylated interferon [36]. As in our region there was the war, posttraumatic stress and its consequences are not rare, so treatment of these patients requires multidisciplinary access.

In this study, only one health worker was registered (0.65% of respondents) with the diagnosis of CHC while he was a student, so it can not be taken as an occupational hazard. In the study conducted in Serbia and Montenegro, it was found that 6.1% of health care workers (HCWs) are with hepatitis C [37]. Occupational exposure of HCWs and medical students to percutaneous injuries represents a significant source of bloodborne diseases infection

(40% of cases of hepatitis B and C, and 2.5% of HIV cases among HCWs) [38]. Vaccination is an important measure of prevention against hepatitis B, but not against HCV infection [39]. In Egypt, there is a high rate of HCV infection among HCWs due to percutaneous injuries while surgical and dental interventions and perinatal care are responsible for the transmission of HCV in the population [40]. Not all cases of accidental injuries are reported [41] probably because of irregularities, speed and recklessness at work. Studies mainly investigate stigmatization and discrimination of patients by HCWs while professionally ill health workers are rarely the subjects of the investigation [42]. This points to the possibility that the characteristics of the patients under their care, who have already been subjected to the social stigma, are also projected on them [43].

Conclusion

Stigmatization is significantly present in people who are not married, live in the city, who were left by the partner due to hepatitis C, in patients who used drugs before the diagnosis of the disease and in those who live with their parents. Multivariant analysis allocated, as a significant predictor, abandonment by the partners after the diagnosis. Statistical significance was found in two elements of internal stigmatization. It is necessary to intensify activities on educating patients and people living with them, as well as service providers in various sectors, on risk factors, modes of transmission and protection measures and thus remove the obstacles which prevent patients suffering chronic HCV to seek help.

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Agreement between admission and discharge diagnoses: analysis by the groups of International Classification of Diseases, 10th revision

Slaganja uputne i otpusne dijagnoze: analiza po grupama Međunarodne klasifikacije bolesti, X revizija

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Abstract

Background/Aim. Admission diagnosis represents the diagnosis of an illness, injury or condition due to which a patient is referred to hospital to be admitted. Discharge diagnosis represents the main reason of illness or condition due to which a patient is admitted. The aim of this study was to analyze the agreement between admission diagnostic groups and discharge diagnostic groups of patients in the Clinical Center Kragujevac in the period from January 1, 2006 to December 31, 2013 on the basis of the hospitalization report. **Methods.** From the basic set of reports, 5% of random samples were singled out and they contained 20,422 reports. Out of the given number of reports, 18,173 hospitalization reports were complete and then further analyzed in the paper. Admission diagnostic groups given by the primary care doctor were compared with discharge diagnostic groups filled out by the practicing physician in the hospital ward from which a patient was discharged. The agreement of these two diagnostic groups was an indication of the high-quality performance of the primary care doctor. Agreement analysis was conducted using Cohen's Kappa statistics. **Results.** Agreement analysis showed that the values of the Kappa coefficient for the five leading admission diagnostic groups were in the range of $\kappa = 0.61$ to $\kappa = 0.94$. The values of the Kappa coefficient for the five most common discharge diagnostic groups were in the range of $\kappa = 0.55$ to $\kappa = 0.81$. **Conclusion.** Hospitalization report is a reliable individual report on inpatient care, so it could be used in determining the degree of agreement between admission diagnostic groups and discharge diagnostic groups.

Key words:
patient admission; patient discharge; diagnosis; international classification of diseases.

Apstrakt

Uvod/Cilj. Uputna dijagnoza ukazuje na oboljenje, povredu ili stanje zbog kojeg je bolesnik upućen na prijem u bolnicu. Otpusna dijagoza pokazuje glavni uzrok bolesti ili stanja zbog kojeg je bolesnik primljen u bolnicu. Cilj ovog rada bio je analiza slaganja uputne dijagnoze i osnovnog uzroka hospitalizacije bolesnika u Kliničkom centru Kragujevac u periodu od 1. 1. 2006 do 31. 12. 2013. godine na osnovu izveštaja o hospitalizaciji. **Metode.** Iz osnovnog skupa izdvojen je slučajni uzorak koji je sadržao 20 422 izveštaja (5%). Od datog broja 18 173 izveštaja o hospitalizaciji bilo je potpuno i oni su u daljem radu analizirani. Poređena je uputna dijagnoza koju propisuje lekar u primarnoj zdravstvenoj zaštiti sa osnovnim uzrokom hospitalizacije koji popunjava ordinirajući lekar odeljenja sa kojeg se bolesnik otpušta. Slaganje dveju dijagnoza predstavlja indikator kvaliteta rada lekara u primarnoj zdravstvenoj zaštiti. Analiza slaganja urađena je pomoću kapa statistike. **Rezultati.** Analiza slaganja pokazala je da se vrednosti kapa koeficijenta za pet vodećih uputnih dijagnoza kreću u rasponu od $\kappa = 0,61$ do $\kappa = 0,94$. Vrednosti kapa koeficijenta za pet najčešćih osnovnih uzroka hospitalizacije bile su u rasopnu od $\kappa = 0,55$ do $\kappa = 0,81$. **Zaključak.** Izveštaj o hospitalizaciji je pouzdani individualni izveštaj o stacionarnom lečenju i može se koristiti u određivanju stepena slaganja uputne dijagnoze i osnovnog uzroka hospitalizacije.

Ključne reči:
bolesnik, prijem; bolesnik, otpust; dijagnoza; međunarodna klasifikacija bolesti.

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Introduction

Admission diagnosis represents the diagnosis of an illness, injury or condition due to which a patient is referred to hospital to be admitted. Discharge diagnosis represents the main reason of illness or condition due to which a patient is admitted. They are established after the period of treatment and diagnostic procedures which are recorded in the medical documentation. Discharge diagnoses are filled out by the practicing physician in the hospital ward from which a patient is discharged and they can, if they want, confirm the diagnosis given at the time of admission by the primary health care doctor.

The reliability of diagnoses indicates a high-quality work of the primary care physician. Analysis of sensitivity, positive predictive value and accuracy of the hospitalization report in which discharge diagnostic group is referred to as a health disorder for which it is necessary to keep a register (for example, a stroke register) which is considered a "gold standard", shows that there is an agreement and that hospital discharge data are reliable and that it can be used [1]. In contrast to this, and the fact that these data are available to researchers in the Institute of Public Health in Serbia and hospital management, this kind of research is not often conducted in Serbia. The reason could be that the use of large databases for assessment of people's health condition, evaluation of the performance and planning further activities are not just a routine. Namely, despite the fact that data are reliable [2] and that they exist for years [3], there are problems such as: discrepancy between defining and coding of diagnoses and practical procedures, underestimation of comorbidity [4], as well as a partial coverage of health institutions [5].

Although the quality of the available data varies [6] every doctor-researcher must possess a skill for perceiving and understanding the variability of the data [7].

The aim of the paper was to analyze the agreement between admission diagnostic groups and discharge diagnostic groups on the basis of the hospitalization report.

Methods

This retrospective cohort study included as a basic set all the hospitalization reports of the patients admitted in the Clinical Centre in Kragujevac in the period from January 1, 2006 to December 31, 2013. The data were taken from the database of the Biostatistics and Medical Informatics Center in the Institute of Public Health in Kragujevac as a referent institution which Clinical Centre in Kragujevac provides with hospitalization reports.

The basic set contained more than 400,000 reports. It would be unrealistic to analyze such a large set of reports, so a representative subset of 5% of simple and random samples was made, without repetition which contained 20,422 hospitalization reports. Out of the given number of reports, 18,173 hospitalization reports were complete and they were further analyzed in the paper. By ensuring that all the reports had the same probability of being chosen, many sampling errors, biased sampling and other mistakes unrelated to sampling have been avoided, and given conclusions are reliable and valid and they can be generalized to the whole set of reports.

Admission diagnoses and discharge diagnoses are recorded in the form of 4-digit numbers. At the beginning of the analysis, diagnoses were grouped according to the International Classification of Diseases, 10th revision (ICD10) into 21 groups. One report could contain only: one admission diagnoses, one discharge diagnoses and two comorbidities.

The diagnostic group agreement was measured in two ways. Firstly, we compared the agreement of admission diagnostic groups with the main causes of illness and then the agreement of main causes of illnesses with admission diagnostic groups. The agreement was defined within ICD10 disease groups. In order to avoid robustness of the system, a new variable was formed that monitored the diagnostic group accordance. The advantage of diagnostic group comparison according to the ICD groups is better clarity, but the disadvantage is the lack of preciseness. Discharge diagnostic groups are used as a "gold standard" in the comparison.

The analysis of the diagnostic group agreement according to ICD10 groups was conducted using Cohen's Kappa statistic and 95% of confidence interval. In case of perfect matching, the value of the Kappa coefficient is 1. If the value of the Kappa coefficient is close to 0, that means that matching is coincidental and if it is less than 0, the probability of matching is even less than coincidental. Multiple testing was done firstly, by testing the whole sample, then only by reports which contained an additional illness beside a primary one and finally by testing those samples with no comorbidities.

Mann-Whitney U-test and χ^2-test was used to test the importance of hospital length of stay, the age and gender of patients.

Statistical significance was defined by the value of $p \leq 0.05$. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 19.0.

Results

From the total of 20,422 hospitalization reports, 2,184 reports (10.7%) lacked admission diagnoses, 65 reports (0.3%) did not have the discharge diagnostic group.

By analyzing only the complete reports (those which had both admission and discharge diagnostic groups), a total of 18,173, we noticed that in 22% of cases there was a disagreement between ICD10 admission diagnostic groups and discharge diagnostic groups. In those reports, we noticed a significantly longer hospital length of stay, the patients were older and often of male gender with a larger number of comorbidities. The value of the Kappa coefficient for the whole model in the specified period of time was 0.76 (0.75–0.77) (Table 1).

Beside the primary illness, comorbidities were found in 24.1% of reports (4372), either one (in 2,670 reports) or two comorbidities (in 1,702 repots). The most common comorbidities were diseases of the circulatory system (more than 20%). The value of the Kappa coefficient in the reports containing comorbidities (one or two) was 0.67 (0.64–0.69),

Table 1

Sample characteristics

Variable		Agreement	Disagreement	*p*	Kappa	95% CI
LOS (days), $\bar{x} \pm SD$		7.2 ± 8.8	7.5 ± 7.8	< 0.01		
Age (years), $\bar{x} \pm SD$		47.4 ± 22.2	54.0 ± 21.5	< 0.01		
Gender, n (%)				< 0.01		
female	9,685 (53.3)	7,927 (81.8)	1,758 (18.2)			
male	8,488 (46.7)	6,257 (73.7)	2,231 (26.3)		0.76	0.75–0.77

LOS – length of stay; $\bar{x}$ – mean; SD – standard deviation; CI – confidence intervals.

while the value of the same coefficient in the reports which did not have comorbidities was 0.78 (0.77–0.79).

The most common admission diagnostic groups were: neoplasm, pregnancy, childbirth and puerperium, diseases of the circulatory system, diseases of the digestive system and diseases of the respiratory system with the Kappa coefficient ranging from 0.59 (diseases of the respiratory system) to 0.94 (neoplasm) (Table 2).

The analysis of certain ICD10 subgroups showed that the value of the Kappa coefficient range from $\kappa = 0.33$ for hypertensive diseases to $\kappa = 0.90$ for hernia (Table 3).

The 5 most common ICD10 discharge diagnostic groups matched five, previously mentioned, most common ICD10 admission diagnostic groups but the Kappa coefficient value was different, ranging from 0.38 (circulatory system diseases) to 0.81 (neoplasm) (Table 4).

The order of occurrence of ICD10 admission and discharge diagnostic groups in relation to the total number of ICD10 admission and discharge diagnostic groups is shown in the Table 5. It can be seen that the biggest change happens with diseases of the genitourinary system. Namely, disease of the genitourinary tract as an admission diagnostic group occupied the 3rd place and as a discharge diagnostic group it occupied the 9th place with Kappa coefficient value of 0.21 (Table 2). As it can be seen in Table 6, a large number of admission diagnostic groups for ICD10 XIV (Diseases of the genitourinary system) matched the discharge diagnostic group ICD XXI (Factors influencing health status and contact with health services). Analysis of reports in which the admission diagnostic group was chronic renal insufficiency (N18) showed that in 99.3% of reports factors influencing health status and contact with health services were listed as the discharge diagnostic group.

The change from the 5th to the 3rd place can be seen in the diseases of the circulatory system while the Kappa coefficient value was 0.61 (Table 2). Table 6 shows that the most common discharge diagnostic groups after diseases of the circulatory system were diseases of the nervous system, symptoms, signs and abnormal clinical and lab results which were not classified in the 2nd place and endocrine, nutritional and metabolic diseases. Mental and behavioral disorders fell from the 10th place to the 12th. The Kappa coefficient value was 0.79 (Table 2) and as the most common discharge diagnostic group apart from mental disorders, there were symptoms, signs and abnormal clinical and lab results which were not classified in the 2nd place (Table 6). With other diagnostic groups, the variations were insignificant changing the order by one place.

Table 2

Admission diagnostic groups of International Classification of Diseases, 10th revision (ICD 10)
Kappa statistics and 95% CI

Admission diagnoses (ICD 10)	n	Agreement n (%)	Disagreement n (%)	Kappa	95% CI
Certain infectious and parasitic diseases	671	639 (95.2)	32 (4.8)	0.84	0.81–0.87
Neoplasm	3,426	3,351 (97.8)	75 (2.2)	0.94	0.93–0.95
Diseases of the blood and blood-forming organs	318	294 (92.5)	24 (7.5)	0.88	0.84–0.92
Endocrine, nutritional and metabolic diseases	454	366 (80.6)	88 (19.4)	0.75	0.71–0.79
Mental and behavioral disorders	469	425 (90.6)	44 (9.4)	0.79	0.75–0.83
Diseases of the nervous system	981	748 (76.2)	233 (23.8)	0.67	0.64–0.70
Diseases of the eye and adnexa	409	387 (94.6)	22 (5.4)	0.93	0.85–0.99
Diseases of the ear and mastoid process	59	45 (76.3)	14 (23.7)	0.68	0.56–0.80
Diseases of the circulatory system	1,403	1,144 (81.5)	259 (18.5)	0.61	0.58–0.64
Diseases of the respiratory system	1,030	876 (85)	154 (15)	0.59	0.56–0.62
Diseases of the digestive system	1,192	1,092 (91.6)	100 (8.4)	0.77	0.75–0.79
Diseases of the skin and subcutaneous tissue	172	138 (80.2)	34 (19.8)	0.73	0.67–0.79
Diseases of the musculoskeletal system and connective tissue	449	414 (92.2)	35 (7.8)	0.72	0.68–0.76
Diseases of the genitourinary system	2,313	634 (27.4)	1,679 (72.6)	0.21	0.19–0.23
Pregnancy, childbirth and the puerperium	1,696	1,677 (98.3)	29 (1.7)	0.75	0.73–0.77
Other subgroups of ICD10	3,130	1,963 (62.7)	1,167 (37.3)	0.48	0.46–0.50

CI – confidence intervals.

Table 3

Agreement between some subgroups of International Classification of Diseases, 10th revision (ICD 10)

Subgroups of ICD10	Admission diagnostic groups (n)	Discharge diagnostic groups (n)	Agreement	Kappa	95% CI
Ischaemic heart diseases	400	312	290	0.57	0.52–0.62
Other forms of heart disease	272	242	191	0.55	0.49–0.61
Cerebrovascular diseases	269	224	201	0.57	0.51–0.63
Hypertensive diseases	220	125	96	0.33	0.30–0.36
Chronic lower respiratory diseases	340	302	259	0.51	0.45–0.57
Influenza and pneumonia	211	224	164	0.5	0.41–0.59
Other diseases of the digestive system	153	146	141	0.65	0.57–0.73
Diseases of liver	228	223	218	0.83	0.78–0.88
Hernia	221	232	211	0.9	0.85–0.95
Other diseases of intestines	193	165	155	0.69	0.62–0.76

CI – confidence intervals.

Table 4

Discharge diagnoses of International Classification of Diseases, 10th revision (ICD 10)
Kappa statistics and 95% confidences interval (CI)

Discharge diagnostic groups (ICD 10 groups)	n	Agreement n (%)	Disagreement n (%)	Kappa	95%CI
Certain infectious and parasitic diseases	772	639 (82.8)	133 (17.2)	0.63	0.59–0.67
Neoplasm	3,602	3,351 (93)	251 (7)	0.81	0.80–0.82
Diseases of the blood and blood-forming organs	330	294 (89.1)	36 (10.9)	0.66	0.61–0.71
Endocrine, nutritional and metabolic diseases	457	366 (80.1)	92 (19.9)	0.55	0.51–0.59
Mental and behavioral disorders	464	425 (91.6)	39 (8.4)	0.7	0.66–0.74
Diseases of the nervous system	921	748 (81.2)	173 (18.8)	0.7	0.67–0.73
Diseases of the eye and adnexa	391	387 (99)	4 (1)	0.95	0.92–0.98
Diseases of the ear and mastoid process	82	45 (54.9)	37 (45.1)	0.6	0.49–0.71
Diseases of the circulatory system	1,640	1,144 (69.8)	496 (30.2)	0.38	0.36–0.40
Diseases of the respiratory system	1,139	876 (76.9)	263 (23.1)	0.52	0.49–0.55
Diseases of the digestive system	1,366	1,092 (79.9)	275 (20.1)	0.55	0.53–0.57
Diseases of the skin and subcutaneous tissue	166	138 (83.1)	28 (16.9)	0.65	0.58–0.72
Diseases of the musculoskeletal system and connective tissue	488	414 (84.8)	74 (15.2)	0.54	0.50–0.58
Diseases of the genitourinary system	768	634 (82.6)	134 (17.4)	0.66	0.63–0.69
Pregnancy, childbirth and the puerperium	1,721	1,667 (96.9)	54 (3.1)	0.73	0.71–0.75
Other subgroups of ICD 10	3,865	1,967 (50.9)	1,905 (49.1)	0.36	0.35–0.37

Table 5

Order of admission and discharge diagnostic groups

ICD 10 Groups	Admission diagnostic groups (%)	Order	Discharge diagnostic groups (%)	Order
Neoplasm	18.8	1	19.5	2
Other subgroups of ICD 10	16.1	2	19.6	1
Diseases of the genitourinary system	12.7	3	3.8	9
Pregnancy, childbirth and the puerperium	9.3	4	8.5	4
Diseases of the circulatory system	7.7	5	10.8	3
Diseases of the digestive system	6.6	6	8.1	5
Diseases of the respiratory system	5.7	7	5.8	6
Diseases of the nervous system	5.4	8	4.7	7
Certain infectious and parasitic diseases	3.7	9	4.1	8
Mental and behavioral disorders	2.6	10	2.6	12
Endocrine, nutritional and metabolic diseases	2.5	11.5	3	10.5
Diseases of the musculoskeletal system and connective tissue	2.5	11.5	3	10.5
Diseases of the eye and adnexa	2.3	13	1.9	14
Diseases of the blood and blood-forming organs	1.7	14	2	13
Diseases of the skin and subcutaneous tissue	1	15	1	15
Certain conditions originating in the perinatal period	0.8	16	0.8	16
Congenital malformations, deformations and chromoso-mal abnormalities	0.3	17.5	0.4	17.5
Diseases of the ear and mastoid process	0.3	17.5	0.4	17.5

ICD 10 – International Classification of Diseases, 10th revision.

Table 6

Referral diagnoses / Discharge diagnoses (row/column)

ICD10	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	Total
I	**639**	3	0	0	1	1	0	1	1	9	7	4	0	2	0	0	1	0	1	0	1	671
II	0	**3,351**	3	8	0	2	0	0	9	9	17	1	0	22	0	0	0	2	1	0	1	3,426
III	0	2	**294**	0	1	0	0	1	6	4	4	0	2	3	1	0	0	0	0	0	0	318
IV	2	2	0	**366**	0	14	0	0	41	4	5	3	4	6	0	0	1	2	0	1	3	454
V	0	4	0	1	**425**	7	0	0	11	2	4	0	0	0	0	0	0	1	12	0	2	469
VI	2	17	0	7	9	**748**	1	2	156	2	0	0	14	0	2	0	1	3	8	0	9	981
VII	0	3	0	0	0	1	**387**	10	3	0	0	0	0	0	0	0	1	0	1	0	3	409
VIII	0	0	1	0	0	3	0	**45**	3	4	0	0	2	1	0	0	0	0	0	0	0	59
IX	1	8	4	13	9	63	0	2	**1,144**	44	23	3	6	4	0	0	0	7	7	2	63	1,403
X	22	35	2	4	1	3	1	3	57	**876**	13	3	2	4	0	0	0	1	1	0	2	1,030
XI	9	25	1	4	0	1	1	1	22	14	**1,092**	0	0	5	0	0	1	6	5	0	5	1,192
XII	3	0	1	6	0	2	0	1	6	2	3	**138**	2	0	0	1	0	1	5	1	0	172
XIII	1	4	0	2	1	8	0	0	5	5	0	0	**414**	0	0	0	1	1	2	0	5	449
XIV	2	27	11	14	0	0	0	1	24	2	3	0	1	**634**	17	0	3	1	1	0	1,572	2,313
XV	1	0	3	0	0	0	0	0	2	0	0	0	1	7	**1,667**	3	0	0	0	0	12	1,696
XVI	0	0	0	1	0	0	1	0	0	0	0	0	0	0	4	**133**	2	0	1	0	0	142
XVII	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**59**	0	0	0	0	60
XVIII	88	46	9	20	13	35	0	15	102	147	157	8	11	62	5	3	4	**384**	28	0	11	1,148
XIX	0	5	0	0	4	10	0	0	7	1	2	5	10	1	0	0	0	3	**965**	4	31	1,048
XX	1	0	0	1	0	0	0	0	13	1	0	0	0	1	0	0	0	0	3	**16**	1	37
XXI	1	70	1	10	0	23	0	0	28	12	36	1	19	16	25	26	5	1	13	2	**406**	695
Total	772	3,602	330	457	464	921	391	82	1,640	1,139	1,366	166	488	768	1,721	166	79	413	1,054	26	2,127	**18,173**

ICD10 – Interational Clasisification of Diseases 10th revision.

I – Certain infectious and parasitic diseases; II – Neoplasms; III – Diseases of the blood and blood-forming organs and ceratin disorders involving the immune mechanism; IV – Endocrine, nutritional and metabolic diseases; V – Mental and behavioural disorders; VI – Diseases of the nervous system; VII – Diseases of the eye and adnexa; VIII – Diseases of the ear and mastoid process; IX – Diseases of the circulatiory system; X – Diseases of the respiratory system; XI – Diseases of the digestive system; XII – Diseases of the skin and subcutaneous tissue; XIII – Diseases of the musculoskeletal system and connective tissue; XIV – Diseases of the genitourinary system; XV – Pregnancy, childbirth and the puerperium; XVI – Certain conditions originating in the perinatal period; XVII – Congenital malformations, deformations and chromosomal abnormalities; XVIII – Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified; XIX – Injury, poisoning and certain other consequences of external causes; XX – External causes of morbidity and mortality; XXI – Factors influencing health status and contact with health services.

Discussion

A statistic parameter which is most commonly used in determining the degree of agreement between admission and discharge diagnostic groups was the Kappa coefficient.

Using ICD10 discharge diagnostic groups as "the gold standard" has its limitations mainly related to the possibility of double coding which International Classification of Diseases provides. Similarly to our research in which dialysis as a treatment is coded as discharge diagnosis (ICD10 group XXI) with patients who have chronic renal insufficiency (ICD10 group XIV) as an admission diagnosis, the Canadian Institute for Health Research conducted a research and it also referred to a group of ICD10 XXI, specifically the diagnosis Z.54 (convalescence) [8].

The results of the research show that the degree of agreement for the whole model is satisfactory but there are also significant variations for certain diagnostic groups, even though some disagreement was expected. Similarly, in the research conducted in Canada (2006) in which 13,803 hospitalization reports were analyzed, the diagnostic group agreement was registered in 9,328 (67.6%) reports. The value of the Kappa coefficient for 50 most common diagnostic groups was $\kappa = 0.81$ (0.70 to 0.87). The value of the Kappa coefficient for the coronary artery disease was higher than in our research ($\kappa = 0.86$) [8].

In the research conducted in Brazil, there was a higher degree of agreement among the most common diagnostic groups such as primary hypertension where the Kappa coefficient value was $\kappa = 0.74$ [9]. In another research, also conducted in Brazil, the Kappa coefficient value for five leading admission diagnostic groups according to ICD was somewhat higher than in our research (from $\kappa = 0.79$ to $\kappa = 0.98$) [10].

The degree of agreement between admission and discharge diagnoses in patients with or with no diabetes, and with below-knee amputation in the Republic of Ireland (2013), shows that diagnostic group agreement with diabetes patients who had an amputation was $\kappa = 0.82$ (0.75–0.89) [11].

In the research conducted in America in the period from 2005 to 2006, among the patients older than 18 and admitted to Internal medical clinic, diagnostic group disagreement was registered in 68% of cases [12].

In 2000 in Italy analysis of 22,892 patients who came to the Emergency Center due to injuries not caused by violence, showed that in 62.2% of cases the admission diagnostic group from the Emergency Center matched the discharge diagnostic group after the period of hospitalization. It is determined that the possibility of death as an outcome was 30% higher with patients whose admission diagnostic groups did not match their discharge diagnostic groups in relation to those whose diagnostic groups matched [13].

Many factors can influence the degree of agreement between admission and discharge diagnoses. The research conducted in Singapore shows that the diagnoses disagreement occurs mainly as a consequence of the complex medical problem [14]. Other researches show that the disagreement can occur as a consequence of bad prehospitalization diagnostics, diagnostic dilemmas or mistakes such as bad information triage of primary care doctors [15]. The problem also occurs when patients simultaneously have two or more health disorders, and it is difficult to distinguish a primary illness from comorbidities. All of this leads to longer hospital length of stay and higher hospital expenses [16]. Diagnostic group agreement not only shortens hospital length of stay and reduces hospital expenses but it enables a patient to immediately get an adequate treatment without unnecessary waste of time [15].

Conclusion

The report on hospitalization is a reliable individual report on inpatient treatment, and it can be used in determining the degree of the agreement between admission and discharge diagnoses. The most frequent admission diagnostic groups according to the ICD10 match the most frequent discharge diagnostic groups, but the Kappa coefficient values are different. The most frequent diagnostic groups include neoplasm, pregnancy, childbirth and puerperium, diseases of the circulatory system, diseases of the digestive system and diseases of the respiratory system. In the reports in which discrepancies were recorded, there was a statistically significant higher number in hospital days of elderly patients, mostly males, and with a higher number of comorbidities. Defining the factors which cause the discrepancy of admission and discharge diagnostic groups within ICD 10 diagnostic groups can be the subject of a new research.

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Histomorphometric evaluation of bone regeneration using autogenous bone and beta-tricalcium phosphate in diabetic rabbits

Histomorfometrijska analiza regeneracije kosti kod kunića sa dijabetesom melitusom posle primene autotransplantata kosti i beta-trikalcijum fosfata

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Abstract

Background/Aim. The mechanism of impaired bone healing in diabetes mellitus includes different tissue and cellular level activities due to micro- and macrovascular changes. As a chronic metabolic disease with vascular complications, diabetes affects a process of bone regeneration as well. The therapeutic approach in bone regeneration is based on the use of osteoinductive autogenous grafts as well as osteoconductive synthetic material, like a β-tricalcium phosphate. The aim of the study was to determine the quality and quantity of new bone formation after the use of autogenous bone and β-tricalcium phosphate in the model of calvarial critical-sized defect in rabbits with induced diabetes mellitus type I. **Methods.** The study included eight 4-month-old Chincilla rabbits with alloxan-induced diabetes mellitus type I. In all animals, there were surgically created two calvarial bilateral defects (diameter 12 mm), which were grafted with autogenous bone and β-tricalcium phosphate (n = 4) or served as unfilled controls (n = 4). After 4 weeks of healing, animals were sacrificed and calvarial bone blocks were taken for histologic and histomorphometric analysis. Beside descriptive histologic evaluation, the percentage of new bone formation, connective tissue and residual graft were calculated. All parameters were statistically evaluated by Friedman Test and post hock Wilcoxon Singed Ranks Test with a significance of $p < 0.05$. **Results.** Histology revealed active new bone formation peripherally with centrally located connective tissue, newly formed woven bone and well incorporated residual grafts in all treated defects. Control samples showed no bone bridging of defects. There was a significantly more new bone in autogeonous graft (53%) compared with β-tricalcium phosphate (30%), ($p < 0.030$) and control (7%), ($p < 0.000$) groups. A significant difference was also recorded between β-tricalcium phosphate and control groups ($p < 0.008$). **Conclusion.** In the present study on the rabbit grafting model with induced diabetes mellitus type I, the effective bone regeneration of critical bone defects was obtained using autogenous bone graft.

Key words:
rabbitis; diabetes mellitus; bone regeneration; transplantation, autologous; beta-tricalcium phosphate.

Apstrakt

Uvod/Cilj. Mehanizam otežanog zarastanja tkiva kod dijabetesa melitusa zasnovan je na različitim promenama funkcije na tkivnom i ćelijskom nivou, usled prisutnih mikro- i makrovaskularnih promena. Kao hronično metaboličko oboljenje sa vaskularnim komplikacijama, dijabetes melitus zahvata i proces koštane regeneracije. Terapijski postupci u okviru regeneracije kosti obuhvataju primenu autotransplantata sa oseoinduktivnim delovanjem i sintetskih osteokonduktivnih materijala, kao što je i β-trikalcijum fosfat. Cilj ovog rada bio je da se ispita kvantitet i kvalitet novoformiranog koštanog tkiva posle korišćenja autotransplantata kosti i β-trikalcijum fosfata, na modelu kritičnog defekta kalvarije kunića sa eksperimentalno izazvanim dijabetesom melitusom tipa I. **Metode**. U ovo istraživanje bilo je uključeno 8 kunića (soj Činičila), starosti 4 meseca, kod kojih je dijabetes melitus tipa I bio izazvan aloksanom. Kod svih životinja hirurški je urađen defekt kritične

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veličine na kosti kalvarije (prečnika 12 mm), koji je popunjen autotransplantatom kosti i β-trikalcijum fosfatom (n = 4) ili je ostavljen da spontano zarasta kao kontrolni defekt (n = 4). Posle 4 nedelje, sve životinje su bile žrtvovane i koštani uzorci uzeti za histološku i histomorfometrijsku analizu. Pored deskriptivne histološke analize, urađena je i kvantitativna analiza novoformirane kosti, vezivnog tkiva i materijala za koštanu regeneraciju. Statistička analiza vršena je primenom Friedman-ovog testa i *post hock* Vilkoksonovog neparametrijskog testa sa stepenom značajnosti od $p < 0,05$. **Rezultati.** Histološka analiza uzoraka kosti pokazala je prisustvo novoformirane kosti na periferiji defekta, dok je u centralom delu bilo prisutno vezivno tkivo, nezrelo koštano tkivo i dobro sjedinjeni neresorbovani materijal za regeneraciju kosti. Kontrolni uzorci nisu pokazali koštano zarastanje defekata. Značajno više novoformirane kosti bilo je prisutno u defektima regenerisanim autotransplantatom (53%) u poređenju sa kontrolnim defektima (7%), ($p < 0,000$) i defektima popunjenim β-trikalcijum fosfatom (30%), ($p < 0,030$). Takođe, značajna razlika uočena je i između grupe sa β-trikalcijum fosfatom i kontrolnim koštanim defektom ($p < 0,008$). **Zaključak.** Primena autotransplantata kosti značajno povećava uspešnost regeneracije kritičnih defekata kosti kalvarije kunića sa dijabetesom melitusom tipa I.

Ključne reči:
zečevi; dijabetes melitus; kost, regeneracija; transplantacija, autologna; beta-trikalcijum fosfat.

Introduction

Diabetes mellitus (DM) is a chronic disease characterized with hyperglycemia which leads to complications of micro- and macrovascular diseases of various organs, including bone [1]. The process of bone regeneration is particularly affected in DM [2, 3]. Various animal studies showed impaired bone healing process in diabetic animals compared with non-diabetic controls [4]. There are multiple mechanism trough which diabetes may affect bone, including the expression of genes that regulate osteoblast differentiation and expression of growth factors that promote bone formation [5]. Hyperglycemic status in diabetes leads to an increase of bone resorption and a decrease of bone turnover [6]. Moreover, the delay in cell proliferation and the decrease of collagen metabolism, are direct consequences of diabetes that severely affects the tissue repair process [7, 8].

Poor blood supply and deficiency in bone marrow make rabbit calvaria the appropriate model for evaluation of bone repair and regeneration potential of different materials [9]. The rabbit calvaria model has been used extensively for the study of different bone substitutes in bone regeneration experiments because anatomical and physiological characteristics are sufficiently close to humans [10]. Bone substitute materials for regeneration of intraosseous defects should be osteoinductive, to stimulate osteogenesis, and osteoconductive, to provide a scaffold for bone deposition [11]. Autogenous bone graft remains the gold standard among bone reconstruction materials, since these requirements are adequately fulfilled. However, limited supply of bone and donor site morbidity are problematic [12]. Therefore, synthetic material, such as β-tricalcium phosphate (β-TCP), has been used in bone regeneration because its mineral composition resembles that of human bone, providing osteoconductive and biodegradable activity [13].

Currently, there is few information in the literature regarding the influence of DM on bone regeneration in the specific condition of the critical sized defect (CSD) healing [14–17]. CSD has been originally defined by Schmitz and Hollinger [9] as the smallest size intra-osseous wound in a particular bone and species that will not heal spontaneously by bone tissue, or less than 10% of bone regeneration should be observed during the life time of the animal. Recently modified by Cooper et al. [18], CSD has been also defined as the smallest size of a defect that does not heal spontaneously when left untreated for a certain period of time, except if bone regeneration therapy is used.

Since the appropriate model for the investigation of bone regeneration is still recognized by calvarial bone, especially related to bicortical type, it was of interest to evaluate success of bone regeneration in diabetic conditions. Therefore, the purpose of this interim study was to determine the quality and quantity of new bone formation after the use of autogenous bone and β-TCP in the model of calvarial-critical sized defect in rabbits with induced DM type I.

Methods

Experimental design

Eight, 4-month-old giant Chincilla rabbits (Chinchilla Chinchilla), weighing 3.5−4.0 kg, were assigned to receive alloxan in order to experimentally induce DM type I. Animal selection, housing conditions and surgical protocol were approved by the Ethical Committees of the Faculty of Veterinary Medicine and Faculty of Dental Medicine, University of Belgrade (Certification No. 36/17) and all experimental procedures were performed in accordance with the European Union regulations on the use of animals in scientific purposes. After the induction of diabetes, two circular bilateral defects (12 mm) were created on each rabbit calvarium. In 4 animals, bone defects were grafted with the following material: β-TCP (RTR® Septodont, France) and autogenous bone graft (AUTO), collected from the area of surgical site. The other 4 animals, with two bilateral defects, served as no-filled control group. The defects were analyzed 4 weeks postoperatively, after sacrificing the animals.

Induction of diabetes

During experiment, all animals were housed in separate cages with free access to food and water *ad libitum*. Diabetes mellitus type I was induced in the experimental group of rabbits with a single dose of alloxan (100 mg/kg, diluted in physiological saline solution) applied into the marginal ear vein. A solution of alloxan was prepared immediately prior

to injection. To prevent severe hypoglycemia during the critical first 24 h after injection, animals were provided with 5% glucose in their drinking water. The blood glucose level was monitored three times a day. A week after the administration of alloxan, rabbits were monitored for the development of hyperglycaemia, measured by the level of glucose in the blood taken from the marginal ear vein, for the confirmation of hyperglycemia with glucose level greater than 11 mmol/L.

Surgical procedure

The surgical procedure was done under general anesthesia which was induced by an intramuscular injection of a combination of tiletiamine and zolazepam 15 mg/kg. The surgical site was shaved and the skin washed with 70% ethanol and povidone iodine. Local anesthesia (2% lidocaine with 1/100 000 epinephrine) was administered to control bleeding of the operating area. Sagittal incision at the midline of the calvaria was made through the skin and the periosteum, from the frontal bone to the occipital bone. A full thickness flap was elevated and surgical sites were exposed. Two standardized, circular, transosseus defects (12 mm in diameter) were made in the mid-portion of each parietal bone, using a stainless-steel trephine bur with an outer diameter of 12 mm, under copious irrigation with sterile saline solution. Care was taken to avoid injury to the dura. In 4 animals, one defect was filled with β-TCP and the other one with autogenous particulate bone collected from the surgical area. In the control group (4 animals) bone defects were naturally filled with blood clot. After the surgery, soft tissue was repositioned and sutured in layers with resorbable suture material (Vicryl, Ethicon, Somerville, NJ, USA). In a 3-day postoperative period, an antibiotic (oxitetracycline 15 mg/kg) and analgesic (buforphanol 0.6 mg/kg) were administered intramuscularly to prevent infection and control pain. Four weeks after the surgery rabbits were sacrificed by the lethal dose of pentobarbital sodium, 100 mg/kg.

Histological and histomorphometric evaluation

Block samples that included original surgical defect and surrounding tissue were removed after animals sacrifice. The sections were rinsed in sterile saline and fixed in 10% buffered formalin for 10 days. All specimens were then decalcified in 10% ethylendiamintetra acetic acid (EDTA) and dehydrated in a graded series of increasing ethanol concentrations and then embedded in paraffin. Longitudinal, 5-μm thick sections were cut through the center of the circular calvarial defects. Five sections that contained the central portion were selected from each block, and stained with Goldner's Trichrome.

Histomorphometry was carried out using a light microscope (Olympus BX-51; Olympus, Tokyo, Japan). Image acquisition and stage movement were controlled by the newCAST stereological software package (Visiopharm Isofarm Integrator System, ver. 2.12.1.0; Visiopharm; Denmark – VIS) running on a personal computer. Volume density estimation was used to determine the precentage of newly formed bone, connective tissue and residual graft material.

Statistical analysis

Statistical analysis was performed using the software program (SPSS 10.0, SPSS, Chicago, IL). Histomorphometric records were presented as mean ± SD values expressed in percentages. To compare the differences among the three investigated groups, Friedman Test and *post hoc* Wilcoxon Singed Ranks Test were used. A significance for analysis was set to $p < 0.05$.

Results

During the postoperative period, healing was uneventful for all animals. No animals had been lost. There were no signs of graft exposure, allergic reaction or grafted area infection. The total number of analyzed defects was four per group, with the exclusion of previously created defects in control group.

Descriptive histology evaluation

Four weeks after the surgery, defects filled with β-TCP exhibited residual graft particles in the middle part of bone samples, mostly surrounded by the connective tissue. Newly formed bone was restricted to areas close to the margins of the surgical defect (Figure 1). The bony islands of new bone formation were found inside the porosity of β-TCP in a close proximity to the connective tissue and β-TCP. It was surrounded by a small number of osteoblasts and it was irregular woven type of bone (Figure 2).

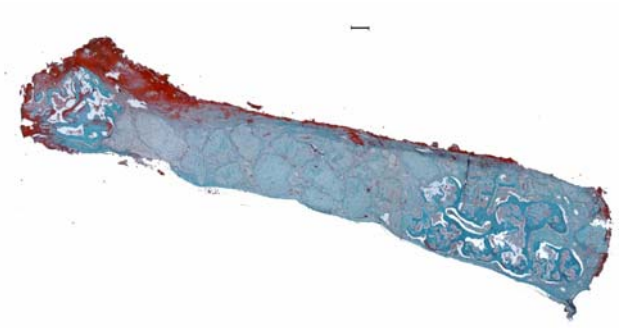

Fig. 1 − Photomicrograph of bone sample in its entirety obtained after 4 weeks of regeneration with beta-tricalcium phosphate (β-TCP). The sample contains new bone formation, residual graft and connective tissue (Goldner's Trichrome staining, bar – 400 μm, ×25 magnification).

Defects filled with autogenous graft showed areas of newly formed bone with thin immature trabeculae and wide intratrabecular spaces with collagen fibers (Figure 3). Autogenous grafts were well incorporated in new bone. The newly formed bone was a woven type. Part of autogenous graft fragments were recognized by the absence of osteocytes

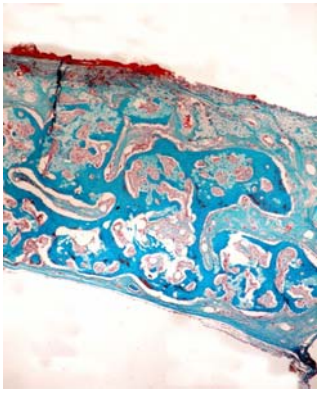

Fig. 2 − Histologic specimen from beta-tricalcium phosphate (β-TCP) grafted calvarial defects showing areas of woven bone with lining osteoblasts and small amounts of marrow spaces. The residual β-TCP is mostly incorporated inside woven and marrow bone (Goldner's Trichrome staining, bar – 200 μm, ×40 magnification).

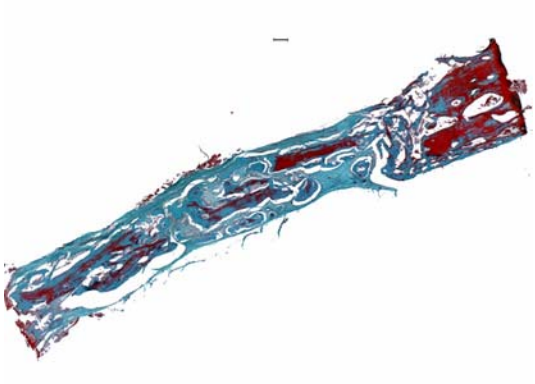

Fig. 3 − Photomicrograph of bone sample in its entirety obtained after 4 weeks of regeneration with autogenous bone graft. The sample contains newly formed mineralized and marrow bone, particulate bone graft and connective tissue (Goldner's Trichrome staining, bar – 400 μm, ×25 magnification).

in lacunas. Osteoblasts were detected on the surface of new bone (Figure 4).

In the control group, minimal amounts of new bone tissue were formed at the defect margins while no bone bridging was seen (Figure 5). The greater parts of the defects were filled with thin fibrous connective tissue layers with newly formed bone islands in the middle of bone defects (Figure 6).

Histomorphometry

Histomorphometric analysis is summarized in Table 1. The percentage of newly formed bone was significantly higher in the AUTO and β-TCP group than in the control group. A significant difference in new bone formation was detected between the AUTO and β-TCP. In the control group, the percentage of connective tissue was significantly higher comparing to the AUTO and β-TCP group. Analysis of regenerated tissue inside the treated bone defects showed significantly more new bone and grafts *vs* connective tissue in the AUTO, while this difference was seen only for new bone *vs* connective tissue in the β-TCP group (Table 1).

Discussion

Generally, the bone repair process is particularly affected in diabetic individuals. In the field of the effective bone

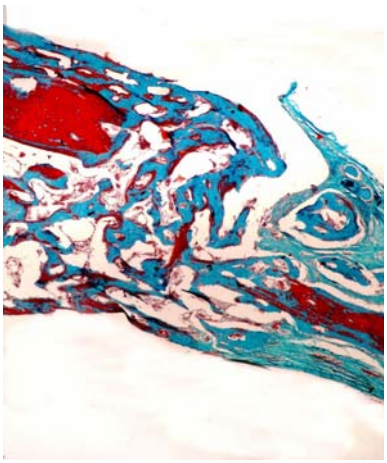

Fig. 4 − Histologic specimen from autogenous bone grafted calvarial defects showed trabecular connectivity of new bone formation associated with residual particle of autogenous graft through entire bone defect. The bone was woven type which surrounded marrow spaces and residual grafts (Goldner's Trichrome staining, bar – 200 μm, ×40 magnification).

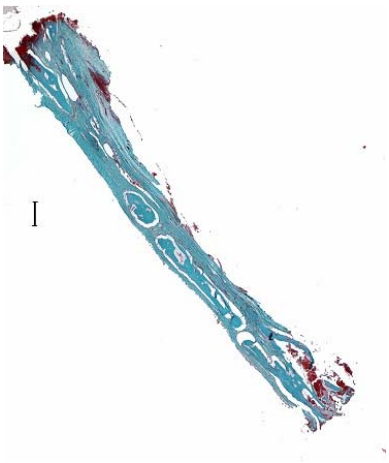

Fig. 5 − Photomicrograph of bone sample in its entirety obtained after 4 weeks of healing of unfilled control defect. The sample contains newly formed bone at the defect margin, while connective tissue filled central part of specimen (Goldner's Trichrome staining, bar – 400 μm, ×25 magnification).

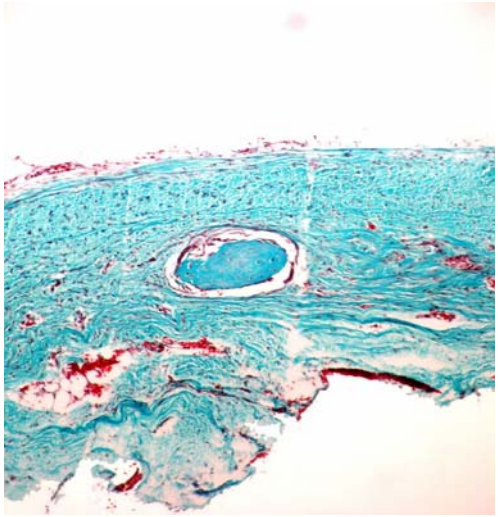

Fig. 6 − Histologic specimen of unfilled calvarial defects represents areas of fibrovascular connective tissue and reduced islands of new bone formation (Goldner's Trichrome staining, bar – 200 µm, ×40 magnification).

Table 1

Histomorphometric results (%) of critical sized defect (CSD) healing in diabetic rabbits

Spacimen	Auto	β-TCP	Control	*p*
New bone	53.15 ± 10.82	30.15 ± 5.71	7.32 ± 8.40	0.030[a] 0.008[b] 0.000[c]
Connective tissue	14.41 ± 7.24	22.39 ± 11.57	92.68 ± 5.63	0.000[b] 0.000[c]
Graft 32.44 ± 9.17	32.44 ± 9.17	47.46 ± 6.92	0	ns
p	0.004[d] 0.021[e]	0.042[e]	0.000[d]	

Values were given as mean ± SD. Statistical significance between groups (Friedman Test, *post-hoc* Wilcoxon Singed Ranks Test): [a]AUTO *vs* β−TCP; [b]β−TCP *vs* the control group; [c]AUTO *vs* the control group. Statistical significance inside the groups (Friedman Test, *post-hoc* Wilcoxon Singed Ranks Test): [d]new bone *vs* connective tissue; [e]connective tissue *vs* graft. β−TCP – beta tricalciumphosphate; Auto – autogenous bone graft.

healing, the success rate of bone regeneration should be analyzed after the use of different therapeutic approaches to improve the process of bone healing in DM. In the present study, we assessed the effectiveness of autogenous bone graft and synthetic osteoconductive bone substitute β-TCP in bone regeneration using critical-sized 12-mm defects in the calvarium of diabetic rabbits.

Histomorphometric analysis of this study showed that the treatment of critical bone defects in DM using the AUTO and β-TCP elicited more new bone formation compared to the control groups, which normally healed spontaneously with connective tissue. However, the percentage of newly formed bone was higher in the AUTO group than in the β-TCP group, probably due to osteogenesis that was taking place in the AUTO. This result is consistent with the results of Esteves et al.[16], who showed that the bone repair of surgical defects filled with bone autografts occurred earlier than that of surgical defects filled with blood clot in both control and diabetic groups. It is likely that such result is due to osteinduction effect of autogenous graft with increased regenerative potential of different growth factors and their cellular activity. In accordance with that, Mariano et al.[17] showed that the use of platelet-rich plasma in bone regeneration, as a method which express a high concentration of growth factors, significantly increased the quantity and quality of bone healing in calvarial critical-sized defects of diabetic rats.

Beside positive histomorphometric evidence of regenerative therapy in diabetic condition, histological view illustrated that the newly formed bone was well incorporated into the both autogenous bone particles and β-TCP material, suggesting the mechanisms of bone regeneration based on its osteoconductive property. This finding is consistent with the previously published data [19–21] indicating that β-TCP behaves as an osteoconductive material, which acts as a scaffold for the cell in-growth, growth factor production inside the material and subsequent increased in bone formation. Furthermore, Murai et al.[13] reported that osteoblasts and osteoid formation were present on the surfaces of β-TCP particles what was also seen in the presented histologic analysis. However, it was observed that the major part of the healing process came from the periosteal and the defect edges in the treated and untreated defects, which agrees with the previously published data in healthy animal models [22, 23]. That observation may provide evidence for the regenerative potential in the diabetic bone, which occurred using the same mechanism of healing in healthy and DM, beginning from the margin of rest bone. Nevertheless, the amount of regenerated bone in DM may be dependent on the

different proliferation rate of varying types of cells affected by DM, local trauma and the size of bone defects. Moreover, data obtained from the study of Retzepi et al. [24] demonstrated, that *de novo* alveolar bone formation can be achieved in experimentally induced DM with application of the guided bone regeneration (GBR) technique, the major strategy conducted to improve bone healing.

Concerning the fact that DM may impair the process of bone regeneration, probable related to changes in bone metabolism [25], it is interesting to note that the amount of connective tissue in the AUTO and β-TCP-treated bone defects did not exceed the quantity of connective tissue expected during bone healing in healthy individuals, especially in the early phase of healing, what was the scope of this study. In relation to this evidence, other authors have reported similar amount of connective tissue in healthy rabbits when bone defects were treated with autogenous bone grafts (6-mm calvarial defects) [23] or in unfilled defects (6-mm control tibia defects) [26]. Apparently, DM could not have any influence in the quantity of regenerated tissue, but have changed the quality of newly formed bone in the time-related manner. To support that evidence, Vieira et al.[14] showed that bone repair was slower in the diabetic group than in the control and diabetic-polytetrafluoroethylene (PTFE) membrane treated groups.

Conclusion

In the present study on the rabbit grafting model, the effective bone regeneration of critical bone defects was significantly obtained by the use of autogenous bone grafts. Further studies, which would include healthy individuals and different healing intervals, could probably clarify the mechanisms of bone healing and differences between autogenous bone grafts and other bone substitutes.

Acknowledgement

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ORIGINAL ARTICLE

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Diagnostic accuracy of the A-test and cutoff points for assessing outcomes and planning acute and post-acute rehabilitation of patients surgically treated for hip fractures and osteoarthritis

Dijagnostička tačnost A-testa i tačke preseka za procenu ishoda i planiranje rane i produžene rehabilitacije bolesnika operativno lečenih zbog preloma i osteoartritisa kuka

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Abstract

Background/Aim. The A-test is used in daily clinical practice for monitoring functional recovery of orthopedic patients during early rehabilitation. The aim of this study was to determine the accuracy of A-test and cutoff point at which the test can separate patients with and without functional disability at the end of early rehabilitation. Also, it was important to determine whether A-test has that discriminative ability (and at which cutoff points) in the first days of early rehabilitation in order to have time to plan post acute rehabilitation. **Methods.** This measurement-focused study was conducted in the Orthopedic Ward during early inpatient rehabilitation (1st−5th day after the operation) of 60 patients with hip osteoarthritis (HO) that underwent arthroplasty and 60 surgically treated patients with hip fracture (HF). For measurements we used the A-test and the University of Iowa Level of Assistance Scale (ILAS) as the gold standard. For statistical analysis we used the receiver operating characteristic (ROC) curve and the area under the curve (AUC) with 95% confidence interval for the results of A-test from the first to the fifth day of rehabilitation, sensitivity, specificity, the rate of false positive and false negative errors, positive and negative predictive value, ratio of positive and negative likelihood ratio, accuracy, point to the ROC curve closest to 0.1 and Youden index for all the cutoff points. **Results.** The AUC was 0.825 (0.744−0.905) for the first day of rehabilitation, 0.922 (0.872−0.972) for the second day of rehabilitation, 0.980 (0.959−1.000) for the third day of rehabilitation, 0.989 (0.973−1.004) for the fourth day, and 0.999 (0.996−1.001) for the fifth day of rehabilitation. The optimal cutoff for the results of A-test was: 7/8 for the first day, 29/30 for the fourth day, and 34/35 for the fifth day of rehabilitation. On the second and the third day A-test had two cutoff points, the lower point safely separated the patients with functional disability, while the upper point ruled out functional disability. On the 2nd rehabilitation day the cutoff points were 12/13 and 17/18, on the 3rd rehabilitation day cutoff points were 13/14 and 18/19. **Conclusion.** The A-test has all characteristics of an accurate tool which can be used for separating patients with and without functional disability at all stages of early rehabilitation after surgically treated hip disease or fracture. Based on the results of A-test within the first days of early rehabilitation, it is possible to make a plan for postacute rehabilitation.

Key words:
rehabilitation; recovery of function; hip prosthesis; postoperative period; predictive value of tests; serbia.

Apstrakt

Uvod/Cilj. A-test se koristi u svakodnevnoj kliničkoj praksi za praćenje ortopedskih bolesnika tokom rane rehabilitacije. Cilj ove studije bio je da se utvrdi tačnosti A-testa i odrede tačke preseka na kojima A-test odvaja bolesnike od onih bez funkcionalne nesposobnosti na kraju rane rehabilitacije. Takođe, da bi se napravio plan produžene rehabilitacije, cilj nam je bio da utvrdimo da li A-test ima tu diskriminatornu sposobnost (i na kojim tačkama preseka) i tokom prvih dana rane rehabilitacije. **Metode.** Ova studija usmerena na ispitivanje mernog instrumenta sprovedena je na Ortopedskom odeljenju tokom râne rehabilitacije (1–5. postoperativni dan) na 60 bolesnika nakon artroplastike kuka zbog osteoartritisa i 60 bolesnika nakon operativno lečenog preloma kuka. Za merenja smo koristili A-test i the *University of Iowa Level of Assistance Scale* (ILAS) kao zlatni standard. Statistička analiza obuhvatala je ROC krivu i površinu ispod krive (AUC) sa 95% intervalom pouzdanosti za rezultate A-testa od 1. do 5. dana rehabilitacije senzitivnost, specifičnost, stopu lažno pozitiv-

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ne i lažno negativne greške, pozitivnu i negativnu prediktivnu vrednost, odnos pozitivnog i negativnog odnosa verovatnoće, tačnost, tačku na ROC krivi najbližu (0.1) i Youden index za sve tačke preseka. **Rezultati.** Vrednosti AUC iznosile su: 0,825 (0,744−0,905) za 1. dan rehabilitacije, 0,922 (0,872−0,972) za 2. dan, 0,980 (0,959−1,000) za 3. dan, 0,989 (0,973−1,004) za 4. dan i 0,999 (0,996−1,001) za 5. dan rehabilitacije. Optimalna tačka preseka za rezultate A-testa bila je: 7/8 za 1. dan, 29/30 za 4. dan i 34/35 za 5. dan rehabilitacije. Drugog i 3. dana rehabilitacije A-test imao je dve tačke preseka, a nižom tačkom se pouzdano odvajaju bolesnici sa funkcionalnom nesposobnošću, dok se višom tačkom odbacuje postojanje funkcionalne nesposobnosti. Drugog dana rehabilitacije tačke preseka bile su 12/13 i 17/18, a 3. dana 13/14 i 18/19. **Zaključak.** A-test ima karakteristike dijagnostički tačnog testa koji može da odvoji bolesnike sa i bez funkcionalne nesposobnosti u svim fazama rane rehabilitacije bolesnika koji su operativno lečeni zbog oboljenja ili preloma kuka. Na osnovu rezultata A-testa tokom prvih dana rane rehabilitacije moguće je napraviti plan za produženu rehabilitaciju.

Ključne reči:
rehabilitacija; funkcija, povratak; kuk, proteza; postoperativni period; testovi, prognostička vrednost; srbija.

Introduction

Due to injuries or planned surgery, all patients admitted to the Clinic for Orthopedic Surgery and Traumatology (COST) experience a lower or higher degree of functional disability. Surgical treatment allows early mobilization of patients. The goals of early initiation of rehabilitation are to maintain or reduce the loss of functioning and to accelerate recovery and patients early autonomy [1]. The ultimate goal is to prevent disability and to avoid the need for long-term care [1]. For most patients, changes in functional status occur from day to day. From the condition of functional disability, some patients come to the state of greater or lesser functional independency for several days. Some of patients progress slowly, so they more or less dependend on the therapist's help in performing basic activities at discharge. Our rehabilitation team at the COST is a witness of these changes. But in routine work we have a problem to record the changes that occur from day to day in a simple and easy way. As in all other areas of physical medicine and rehabilitation, assessing and measuring outcomes are essential in early rehabilitation. Assessment is important for four reasons: decision on further treatment of patients, monitoring and verification of the process of rehabilitation, clinical research and better planning of health services [2]. However, it seems that it is still not easy to find or develop a suitable measure for functioning [3].

Following the early rehabilitation program, we created the A-test to assess 10 basic activities a patient needs to regain in this period. For each activity, the patient receives a score from 0 to 5 depending on the degree of autonomy. The tenth item of the test is walking endurance, and it is graded from 0 to 5 according to the distance that the patient is able to cross. Total scores can range from 0 to 50, or from inability to perform any activity with the help of the therapists to complete independence and safety in performing all activities (Appendix 1). The test was called the A-test (A−for assessment or activity).

First, we used the A-test in a study to assess the effects of preoperative physical therapy and education of patients scheduled for hip arthroplasty [4]. Then we continued to use A-test in everyday practice to make it easier to monitor the process of rehabilitation of each patient from day to day. The A-test is not an additional obligation to the therapists because each activity in the test is an integral part of early rehabilitation program and has been practiced in the COST for years. After the session, the therapists recorded the degree of independency which each patient achieved for a particular activity from the early rehabilitation program. It takes less than 1 minute to the physiotherapist to complete A-test form for each patient. Also, we find that A-test is valid and reliable measurement tool for assessment of functional recovery during early rehabilitation of patients in the Orthopedic Ward [5, 6].

We believe that A-test could help us in making decisions about further treatment and planning health services. The pressure to shorten the stay in the surgical units is ubiquitous, and we are not an exception to this phenomenon. On the other hand, the problem is the small capacity of the rehabilitation department. Candidates for transfer to the rehabilitation department are patients who, until discharge from the COST, did not achieve a satisfactory degree of independence in basic activities. The importance of A-test in this case would be to separate these patients from the group of patients who achieved a satisfactory degree of independence and can be discharged home to continue rehabilitation. So, the aim of this study was to determine which is the most appropriate cutoff for separating these two groups of patients. There was another clinical dilemma, however, thas we expect the A-test to help us in solving it. We usually discharged patients from the COST on the seventh day of the operation. The plan for transfer should be made several days earlier. In the first days after the surgery the A-test score is much lower for most patients, and in that case the cutoff should also be set lower than on discharge, because we would, otherwise, transfer almost all patients from the COST to the rehabilitation department. Therefore the aim of this study was also to determine the cutoff point for solving the transfer plan in the first days after surgery. In addition to determining the cutoff points of A-test during early rehabilitation, the aim of this study was to evaluate the A-test in terms of other features of test accuracy, as well.

Methods

This prospective study was conducted in the COST of Military Medical Academy, Belgrade, on 120 patients of both sexes: 60 patients with acute hip fracture and 60 pati-

ents who underwent hip arthroplasty due to osteoarthritis. Patients with hip fracture were able to walk or without aids and up-and down stairs (help of another person was allowed for this activity) before the injury. This study did not include patients with dementia, pathological hip fractures, bilateral hip fractures, concurrent fracture in any other part of the body, and patients to whom surgical treatment is contraindicated. Patients who underwent hip arthroplasty due osteoarthritis were, also, without significant mental disability, and were able to walk with or wihout aids, and up- and downstairs (help of another person was allowed for this activity) before the operation.

Exclusion criteria during the study were the occurrence of intraoperative or postoperative complications that prevented or delayed the start of rehabilitation, lethal outcome immediately after surgery and incomplete collected data for individual patient.

All the patients were treated surgically. The modality of treatment depended on the type of fracture: osteosynthesis with a dynamic hip screw was applied to patients with intertrochanteric fracture, and arthroplasty was performed in patients with fractures of the femoral neck (partial arthroplasty for older than 70 and total arthroplasty for younger than 70). All the patients admitted with arthritis of the hip were underwent arthroplasty.

After the surgery, all the patients had the same rehabilitation treatment, which involved early mobilization of the patient at the bedside (from the first postoperative day, unless the general condition of the patient did not allow), progressive verticalization (in accordance with the possibilities of the patient), walking with aids on the flat as well as up- and downstairs, practicing the basic activities of daily living (using the toilet, sitting down in a chair). Daily physical therapy treatment lasted 30 minutes, and it was applied every working day (from Monday to Friday). The allowable weight bearing when walking depended on the modality of surgery.

Data on comorbidity and the used drugs, mental and functional status before injury (for the patients with hip fracture) and on admission (for the patients with hip osteoarthritis) (walking distance, the ability to walk up- and downstairs, use of walking aids, carrying out basic and instrumental activities), as well as socio- epidemiological data (marital status, housing conditions) were collected from all the patients on admission. Assessment of the mental status was made using the Serbian version of the shortened mental test score [7], while the functional status before injury was assessed by the New Mobility Score [8].

In the postoperative period, from the first day of rehabilitation until discharge, each patient's functional status was assessed by using the A-test and The University of Iowa Level of Assistance Scale (ILAS) [9, 10].

By the protocol, postoperative complications that occurred and slowed down the course of rehabilitation, the number of days of treatment, duration of hospitalization after the surgery, and destination after discharge were recorded.

We conducted this research with the approval of the competent local Ethics committee.

The diagnostic test accuracy and the best position of the cutoff point were determined using the receiver operating characteristic (ROC) curve [11–13]. The ROC curve was determined for the first five days of rehabilitation in the SSPS 10.0 program. Based on the score of ILAS on the fifth day of rehabilitation (the seventh day after the surgery) the patients were divided into two groups: patients with a score above 10 were considered to require inpatient rehabilitation, while patients with a score of 10 and less could continue rehabilitation at home.

The fifth day of rehabilitation (the seventh day after surgery) was chosen because hospital stay after the surgery took usually 7 days. The cutoff point for ILAS was arbitrarily defined and we were guided by the following principles: the patient is supposed to get out of bed and walk independently or under supervision of the therapist, but without support (maximum 3 points for these 3 activities), holding by the therapist was allowed while walking up- and downstairs if the patient performed this activity before admission to the hospital with the help of another person (maximum 2 points), and the patient should cross the length of 13.4 m for no more than 70 seconds (maximally 5 points).

The diagnostic accuracy of the A-test was estimated by the value of the area under the curve (AUC). The AUC greater than 0.9 was considered the distinction of high accuracy, while 0.7−0.9 indicated moderate accuracy and the values of 0.5 to 0.7 were associated with low accuracy [14]. Standard error, significance level and 95% confidence intervals were presented with the value of the AUC.

For each cutoff point the following parameters were calculated by using standard statistical procedures: sensitivity, specificity, false positive error rate, the rate of false negative errors, positive and negative predictive values.

Also, for each cutoff point we calculated the positive likelihood ratio (LR+), as the ratio of sensitivity and false positive error rate, negative likelihood ratio (LR-), as the ratio of false negative errors and specificity, and the ratio LR+/ LR-. The ratio of LR+ and LR- which was about 50, we considered the feature of precise test [15].

The accuracy of the test was calculated as a proportion of all patients who were correctly diagnosed by this test: (true positives + true negatives)/ total number of examined patients [16].

To determine the optimal cutoff point, we used two previously described methods: point on the ROC curve closest to 0.1 and the Youden index(j) [11, 12].

The first method assumes that the best cutoff point for balancing the sensitivity and specificity of the test is the point on the curve closest to the 0.1 point. In this method, optimal sensitivity and specificity are defined as those yielding the minimal value for $(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$. The cutoff point corresponding to these sensitivity and specificity values is the one closest to the 0.1 point and is taken to be the cutoff point that best differentiates between people with disease and those without disease [11].

The Youden index is defined as the maximum vertical distance between the ROC curve and the diagonal or chance line and is calculated as J = maximum {sensitivity + specificity −1}. Using this measure, the cutoff point in the ROC curve which corresponds to J, that is, at which (sensitivity + specificity − 1) is maximized is taken to be the optimal cutoff point [11].

Results

Out of the 120 patients included in the study, 15 patients (10 with hip fracture and 5 with osteoarthritis of the hip) were excluded during the study: 2 patients with intertrochanteric fracture were excluded due to poor operative stabilization of the fracture and orthopedic surgeon recommendations to rest after surgery, 2 patients with hip fracture were excluded due to cardiac disorders and recommendations of cardiologists to delay mobilization, 3 patients (2 with hip fracture and one with osteoarthritis) were excluded because of the debilitating diarrhea, severe electrolyte imbalances and extreme hypotension so physiatrist recommended postponing initiation of early rehabilitation, in 1 patient with hip fracture and with symptoms of pulmonary embolism, early rehabilitation was interrupted in the first days after the surgery as recommended by the pulmonologists, 4 patients died in the first days after surgery (3 patients with hip fracture and one with osteoarthritis of the hip), 3 patients with osteoarthritis had no completely collected data (hospital discharge was performed before the seventh day after surgery).

Complications that occurred in other patients, because of which we did not delay the start of early rehabilitation were: confusion, gastric complaints, hypotension, urinary tract infection, short-term diarrhea, the occurrence of pressure ulcers in the sacral region and on the feet, vomiting.

Demographic characteristics, numbers of concomitant diseases and used medications, mental and functional state, socioepidemiological data, hospital stay and rehabilitation duration are shown in Table 1. The patients with hip fracture had occasional mild mental problems before the injury, mainly related to the recall of new information, while patients scheduled for arthroplasty had perfectly satisfactory mental state. It can be observed from the data that the patients with hip fracture had plenty of good mobility before the injury. In the group of patients with hip fracture there was a greater proportion of people whose spouse died and who lived alone. After discharge home, a large percentage of patients (33% in the group with osteoarthritis and 28% in the hip fracture) encountered an obstacle, because they lived in an apartment without the elevator.

The distribution of the values of A-test results for all patients who were followed from the first to the fifth day is given in Figure 1. From the first to the fifth day of rehabilita-

Table 1

Demographic characteristics, comorbidity, mental and functional status before admission/injury, socioepidemiological data, hospital stay and rehabilitation duration

Patients' characteristics	The group of patients with osteoarthritis of hip (n = 55)	The group of patients with hip fracture (n = 50)	*p*
Age (years)[1]	65 ± 12; 53 (32–85)	75 ± 10; 76 (47–89)	0.000*
female[2]	32 (58)	37 (74)	0.088†
Number of comorbid diseases[1]	1 ± 1; 1 (0–4)	2 ± 1; 2 (0–4)	0.005*
Number of used drugs[1]	2 ± 2; 2 (0–8)	3 ± 2; 3 (0–9)	0.083*
Shortened mental test score (Serbian version) [1]	10 ± 0; 10 (10–10)	9.84 ± 0.51; 10 (8–10)	0.017‡
Occasional confusion[2]	0 (0%)	3 (6%)	
New Mobility Score[1]	7 ± 2; 6 (2–9)	7 ± 2; 9 (1–9)	0.009‡
Limited walking distance[2]	41 (74.5)	26 (52)	0.016†
Aids when walking[2]	28 (51)	16 (32)	0.050†
Up and down stairs with difficulty[2]	51 (93)	32 (64)	0.000†
Lives in the flat without elevator[2]	18 (33)	14 (28)	
Lives alone[2]	7 (13)	10 (20)	
A widow / widower[2]	14 (26)	23 (46)	
Hospital stay (day)[1]	7.44 ± 1.08, 7 (7–12)	8.52 ± 3.40, 7 (7–24)	0.035*
Rehabilitation (day)[1]	5.25 ± 0.78, 5 (5–10)	6.20 ± 2.28, 5 (5–16)	0.007*
5 days of rehabilitation[2]	46 (84)	33 (66)	

[1]x̄ ± SD, median (range); [2]n (%); **t*-test; †Pearson χ^2; ‡Mann Whitney test.

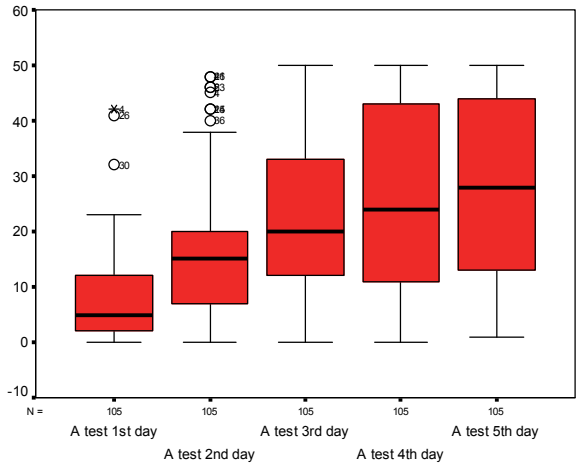

Fig. 1 – Distribution of A- test scores from the first to the fifth day of rehabilitation.

tion pronounced dispersion parameters were found. The parameters of central tendency and dispersion [mean ± SD, mediana range (minimum-maximum)] for the first day of rehabilitation were: 8 ± 8; 5; (0–42), for the second day of rehabilitation: 16 ± 12; 15; (0–48), for the third day of rehabilitation: 22 ± 14; 20; (0–50), for the fourth day of rehabilitation: 26 ± 16; 24; (0–50) and for the fifth day of rehabilitation: 28 ± 16; 28; (1–50).

Based on the ILAS score on the fifth day of rehabilitation, all the patients were divided into two groups: 46 patients with the score of ILAS smaller or equal to 10 were classified in the group without functional disability (37 patients after hip arthroplasty due to hip osteoarthritis, and 9 patients after surgically treated hip fracture), while 59 patients with the score of ILAS greater than 10 were classified in the group with functional disability (18 patients after hip arthroplasty due to hip osteoarthritis, and 41 patients after surgically treated hip fracture).

In Figure 2, ROC curves were plotted from the first to the fifth day of rehabilitation. Day after day, the ROC curve approached the upper left corner of the diagram. On the fifth day of rehabilitation the ROC curve almost reached the upper left corner, which was one of the features of high accuracy of the test.

The AUC indicated a high-accuracy of A-test from the second to the fifth day of rehabilitation (Table 2). Only on the first day of rehabilitation the AUC was slightly smaller, indicating moderate accuracy.

For the first day of rehabilitation, all parameters that determined the optimal cutoff point indicated that it was 7/8. The highest values of LR+/LR-, accuracy and the Youden's index, and the minimum value of point of ROC curve closest to (0.1) are related to this point (Table 3).

On the second day of rehabilitation the lower cutoff po-

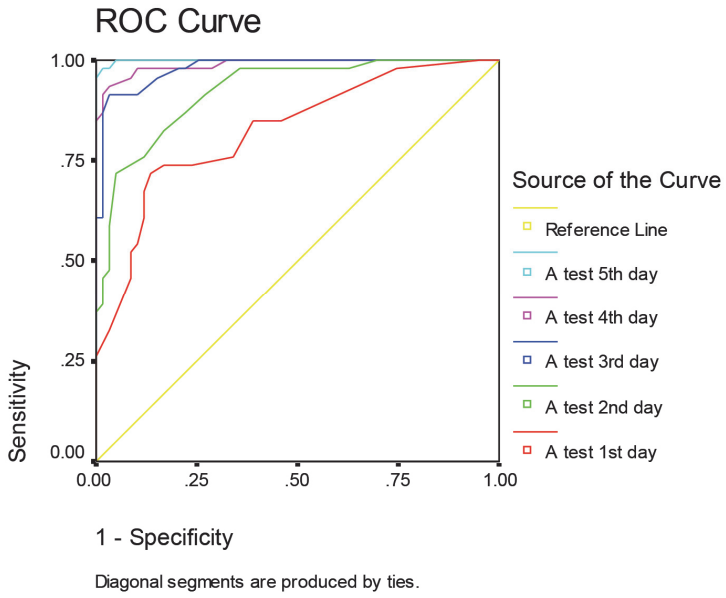

Fig. 2 – The receiver operating characteristic (ROC) curve for A-test from the first to the fifth day of rehabilitation.

Table 2

The area under the curve (AUC): A-test form the first to the fifth day

Postoperative days	Area	Std. error	Asymptotic sig.	Asymptotic 95% CI	
				lower bound	upper bound
1st	0.825	0.041	0.000	0.744	0.905
2nd	0.922	0.025	0.000	0.872	0.972
3rd	0.980	0.010	0.000	0.959	1.000
4th	0.989	0.008	0.000	0.973	1.004
5th	0.999	0.001	0.000	0.996	1.001

Table 3

A-test – cutoff points for the first day of early rehabilitation and related sensitivity, false positive rate of error (1-specificity), positive and negative predictive value, positive and negative likelihood ratio, the ratio of positive and negative, accuracy, minimum 0.1 point and the Youden's index

Cutoff	Sensitivity	1-specificity	PPV	NPV	LR+	LR-	LR+/LR-	Accuracy	Min 0.1 point	Youden index
5/6	0.76	0.26	0.79	0.71	3.22	0.35	9	0.75	0.12	0.50
6/7	0.83	0.26	0.80	0.77	4.92	0.35	14	0.79	0.10§	0.57
7/8*	0.86	0.28	0.80	0.80	6.35	0.39	16†	0.80‡	0.10§	0.58‖
8/9	0.88	0.33	0.78	0.82	7.40	0.48	15	0.79	0.12	0.56

***Selected cutoff point; †maximal LR+/LR-; ‡maximal accuracy; §minimal value of point of receiver operating characteristic (ROC) curve closest to (0.1); ‖maximal Youden's index; PPV – positive predictive value; NPV – negative predictive value; LR+ – positive likelihood ratio; LR- – negative likelihood ratio.**

int was 12/13 and the upper point 17/18. LR +/LR-ratio indicated lower point, while the accuracy and the Youden index indicated upper point (Table 4).

On the third day of rehabilitation the situation was similar, only, the cutoff points were slightly higher. The lower cutoff, indicating the slow progress in functional recovery of the patient, was 13/14. The upper cutoff, which we could use to reject a problem in functional improvement, was 18/19 (Table 5).

On the fourth rehabilitation day, parameters that defined the optimal cutoff point indicated that it could be 29/30 (Table 6). The highest values of LR+/LR-, accuracy and Youden's index, and the minimum value of the point of ROC curve closest to 0.1 were related to this point (Table 6).

On the fifth day of rehabilitation, optimal cutoff could be 34/35. The greatest value of LR+/LR-, accuracy and Youden's index, and the minimum value point of the ROC curve closest to 0.1 were related to this point (Table 7).

Table 4

A-test – the second day of early rehabilitation: cutoff points and related parameters

Cutoff	Sensitivity	1-specificity	PPV	NPV	LR+	LR-	LR+/LR-	Accuracy	Min 0.1 point	Youden index
11/12	0.63	0.02	0.97	0.67	1.68	0.02	75	0.78	0.14	0.61
12/13*	0.64	0.02	0.97	0.68	1.81	0.02	80†	0.79	0.13	0.62
13/14	0.73	0.09	0.91	0.72	2.69	0.10	28	0.81	0.08	0.64
14/15	0.78	0.13	0.89	0.76	3.55	0.15	24	0.82	0.07	0.65
15/16	0.83	0.17	0.86	0.79	4.92	0.21	23	0.83	0.06§	0.66
16/17	0.88	0.24	0.83	0.83	7.40	0.31	24	0.83	0.07	0.64
17/18*	0.95	0.28	0.81	0.92	18.61	0.39	47	0.85‡	0.08	0.67‖
18/19	0.97	0.41	0.75	0.93	28.41	0.70	40	0.80	0.17	0.55

***Selected cutoff point; †maximal LR+/LR-; ‡maximal accuracy; §minimal value of the point of the receiver operating characteristic (ROC) curve closest to (0.1); ‖maximal Youden's index; PPV − positive predictive value; NPV − negative predictive value; LR+ − positive likelihood ratio; LR- − negative likelihood ratio.**

Table 5

A-test – the third day of early rehabilitation: cutoff points and related parameters

Cutoff	Sensitivity	1-specificity	PPV	NPV	LR+	LR-	LR+/LR-	Accuracy	Min 0.1 point	Youden index
12/13	0.63	0.02	0.97	0.67	1.68	0.02	75	0.78	0.14	0.61
13/14*	0.64	0.02	0.97	0.68	1.81	0.02	80†	0.79	0.13	0.62
14/15	0.73	0.09	0.91	0.72	2.69	0.10	28	0.81	0.08	0.64
15/16	0.78	0.13	0.89	0.76	3.55	0.15	24	0.82	0.07	0.65
16/17	0.83	0.17	0.86	0.79	4.92	0.21	23	0.83	0.06§	0.66
17/18	0.88	0.24	0.83	0.83	7.40	0.31	24	0.83	0.07	0.64
18/19*	0.95	0.28	0.81	0.92	18.61	0.39	47	0.85‡	0.08	0.67‖
19/20	0.97	0.41	0.75	0.93	28.41	0.70	40	0.80	0.17	0.55

***Selected cutoff point; †maximal LR+/LR-; ‡maximal accuracy; §minimal value of the point of the receiver operating characteristic (ROC) curve closest to (0.1); ‖maximal Youden's index; PPV − positive predictive value; NPV − negative predictive value; LR+ − positive likelihood ratio; LR- − negative likelihood ratio.**

Table 6

A-test – the fourth day of early rehabilitation: cutoff points and related parameters

Cutoff	Sensitivity	1-specificity	PPV	NPV	LR+	LR-	LR+/LR-	Accuracy	Min 0.1 point	Youden index
24/25	0.90	0.02	0.98	0.88	8.80	0.02	391	0.93	0.01§	0.88
25/26	0.92	0.04	0.96	0.90	10.76	0.04	240	0.93	0.01§	0.87
27/28	0.97	0.07	0.95	0.96	28.41	0.07	409	0.95‡	0.01§	0.90‖
29/30*	0.98	0.09	0.94	0.98	57.82	0.10	607†	0.95‡	0.01§	0.90‖
30/31	0.98	0.11	0.92	0.98	57.82	0.12	473	0.94	0.01	0.87

***Selected cutoff point; †maximal LR + / LR-; ‡maximal accuracy; §minimal value of point of receiver operating characteristic (ROC) curve closest to (0.1); ‖maximal Youden's index; PPV − positive predictive value; NPV − negative predictive value; LR+ − positive likelihood ratio; LR- − negative likelihood ratio.**

Table 7

A-test – the fifth day of early rehabilitation: cutoff points and related parameters

Cutoff	Sensitivity	1-specificity	PPV	NPV	LR+	LR-	LR+/LR-	Accuracy	Min 0.1 point	Youden index
29/30	0.95	0.00	1.00	0.94	18.61	0.00	-	0.97	0.00§	0.95
31/32	0.97	0.02	0.98	0.96	28.41	0.02	1263	0.97	0.00§	0.94
34/35*	0.98	0.02	0.98	0.98	57.82	0.02	2571†	0.98‡	0.00§	0.96‖
36/37	1.00	0.04	0.97	1.00	-	0.04	-	0.98‡	0.00§	0.96‖

***Selected cutoff point; †maximal LR+/LR-; ‡maximal accuracy; §minimal value of point of receiver operating characteristic (ROC) curve closest to (0.1); ‖maximal Youden's index; PPV − positive predictive value; NPV − negative predictive value; LR+ − positive likelihood ratio; LR- − negative likelihood ratio.**

Discussion

This study investigated diagnostic accuracy of the A-test in the assessment of functional recovery of patients treated surgically due to hip fracture and osteoarthritis in the Orthopedic Ward. We determined cutoff points to separate patients with from those without disabilities from the beginning to the end of early rehabilitation. Early rehabilitation of these patients is a dynamic process of short duration. Changes in functional status occur from day to day and their recording is essential for monitoring the recovery of patients, but also for planning their further rehabilitation.

The population of patients admitted to the COST was heterogeneous. However, two clinical entities were the most numerous: fractures of the hip and hip osteoarthritis. Therefore, our study included patients with these admission diagnoses. The patients with hip osteoarthritis who were scheduled for arthroplasty usually recover quickly after surgery. On the other hand, the patients with hip fractures often have a delayed recovery and occurrence of complications changes the flow of rehabilitation. But in both groups of patients, there were those who would deviate from the expected pace of recovery.

It can been seen in the chapter on the results that all parameters that determined the optimal cutoff point indicated that it was 7/8 for the first day of rehabilitation. In practice this means that patients who get out of bed on the first day with the help of the therapists, walk around the room and out into the hallway (A-test score of 8 and higher) will not easily fall into the group of patients with functional disability as the rate of false-negative error is quite low (0.14). The negative predictive value is quite high (0.80), which could mean that 80% of these patients will have no need for inpatient rehabilitation after the fifth day of rehabilitation. However, the LR+/LR- is quite low, the maximum value is 16. Also, it should be noted that the AUC is 0.825, and on the next day the area is higher. Therefore, we recommend that special attention should be paid to all patients with the A-test score less than 8 on the first day of rehabilitation, and that the final decision on further rehabilitation should be left for the next day. A study of Hulsbæk et al. [17], also shows that patients undergoing hip fracture surgery, who are not able to complete physiotherapy on the first post-operative day, are at a greater risk of not regaining basic mobility during hospitalization.

The results obtained for the second and the third day after rehabilitation were interesting for interpretation. The AUC indicate a high accuracy for both test days. This would mean that as early as then we could make a plan for the transfer of patients to the rehabilitation unit based on the score of A-test. And it is very important from the clinical point of view. However, when you look at other parameters that determine the best cutoff point and confirm the accuracy of the test it is easy to notice a discrepancy. We therefore consider that for these two days, in fact, there were two cutoff points for each curve: upper to rule out functional disability with high probability and lower to rule in functional disability with high probability [15].

As noted above, on the second day of rehabilitation the lower cutoff point was 12/13 and the upper point was 17/18. From the clinical point of view this would mean that we would not be (much) wrong if we planned patients with A-test score of less than 13 for the transfer as the rate of false positive error is minimum 0.02. Also, it would be not a (big) mistake to predict that patients with the score of 18 and more will become independent until the fifth day of rehabilitation, as the rate of false negative errors is small 0.05 and negative predictive value is great 0.92. The patients who had the A-test score from 13 to 17 on the second day of rehabilitation should be followed in the coming days. The probability to make the mistake is higher as the rate of false positive and false negative error is higher.

On the fourth rehabilitation day, parameters that define the optimal cutoff point indicated that it could be 29/30. This means that patients whose A-test scores are 30 and higher can be discharged home after five days of rehabilitation, because most activities are performed independently, and the importance of the therapist's presence is limited to verbal suggestion. The rate of false-negative error related to this cutoff point is small (only 0.02). In this study this is one patient. By analyzing the results of A-test in the patients we found that the patient performed all activities quite independently, except walking up- and downstairs. The patient even refused to attempt this activity because it was irrelevant to her everyday life (she lived in the apartment with the elevator). Therefore, her A-score test was greater than 30, and the score of ILAS-a greater than 10. Patients with A-test score of 29 or less require inpatient rehabilitation longer than 5 days in 94% of cases, which indicates a positive predictive

value. The rate of false positive error was 0.09, which ment that rehabilitation facilities would be burdened with 4 patients who could have been discharged home.

On the fifth day of rehabilitation, the optimal cutoff could be 34/35 and that is acceptable from the clinical aspect. But if we want to avoid a false positive error, the cutoff could be 29/30. However, the optimal cutoff point 34/35 is characterized by the following features: high sensitivity, which means that, with the help of A-test, we can detect the existence of functional dependence in performing basic activities when it actually exists in 98% of cases, a low rate of false negative errors (in 2% of cases, this test fails to detect the existence of a functional dependency), high specificity, which shows that in 98% of cases, the A-test show that there is no functional dependence when it is really so, and low rate of false positive errors which shows us that the A-test fails to diagnose the functional dependence when it is present in only 2% of cases. The positive predictive value is very high on this cutoff point, which means that 98% of respondents with positive result are truly functionally dependent. The negative predictive value was also high, revealing that 98% of respondents with a negative result had no significant functional disability.

Measurements of mobility on the second day after the surgery are significant and reliable predictors of independence on transfers and ambulation [18]. In patients with hip fractures, The Cumulated Ambulantion Score of 10 and more for the first three days after the surgery, predicts whether a patient will be discharged home within 2 weeks in 76% of cases [19]. Our clinical experience suggests that a patient who gets out of bed, leaves the room and walkes in the hall department on the first day of rehabilitation with the help of a therapist has a great opportunity to be found in the group of patients who after the fifth day of rehabilitation can be discharged home. In this analysis, the "optimal" cutoff points are presented. A patient who has A-test score 8 and more on the first day, 18 and more on the second day, 30 or more on the fourth day of rehabilitation has a good pace of recovery and will be found in the group of patients who can be discharged home on the fifth day (score 35 and more).

Now we know that we will pay special attention to patients who achieve a score of less than 8 on the first day of rehabilitation. If they do not make a significant functional improvement on the second day of rehabilitation (their A-test score is less than 13), as early as then we can plan them for continuing inpatient rehabilitation.

By monitoring the patients from day to day, we can easily notice stagnation in the functional recovery and immediately take some of the available measures. Let's say that a patient achieved A-test score 12 on the first rehabilitation day, but the score remained the same on the second day of rehabilitation. If this stagnation is not associated with the appearance of some of the complications, the first measure would be to intensify physical therapy. Intensive physical therapy during this period will accelerate functional recovery [20–22] and reduce hospital stay [23]. We do not have the capacity to implement physical therapy in two or more terms for all patients, but adding the term target for patients with delayed recovery can always be arranged. Based on the A-test score, we can specifically and accurately plan additional physical therapy for patients who really need it. It would certainly be a contribution to a better use of health resources.

Based on the analysis we obtained the value of the cutoff point that will be the criterion for further in-patient rehabilitation. The patients with a score of less than 35 on the fifth day of rehabilitation should move to the rehabilitation unit because they need help of a physiotherapist when performing certain activities, while patients with a score of 35 and higher can be discharged home because they can perform most activities independently, a therapist help is limited to verbal suggestion.

The ROC curve is a useful method for assessing responsiveness [24]. It provides a very useful overview of the relationship between a measure and an external indicator of change [24]. The appearance of ROC curves and almost maximum AUC of the fifth day of rehabilitation call attention. Obviously, the ILAS and A-test almost identically assess patient's functional ability/disability. Although the ILAS estimates a smaller number of functions, A-test is much more convenient for everyday work. And from this analysis we see how important it is to assess the outcome of each day, not only at the end of early rehabilitation.

In the presented study, we were concentrated on patients with hip fractures and osteoarthritis who were treated surgically in the COST. From our experience, we expected to find a proportional number of patients in our sample to be discharged home and those who should continue in-patient rehabilitation, which was essential for statistical analysis. Testing should be extended to patients with injuries and disease of other segments of the lower extremities and check the diagnostic accuracy of the A-test in these situations.

Regardless the number of premorbid predictive factors to be taken into consideration when predicting the recovery of the patient [25], early rehabilitation outcome is often unpredictable. Therefore, we emphasize that daily monitoring of functional recovery after the surgery is very important. And if an instrument should be used in clinical practice it has to be simple and should not further burden personnel or patients. Also, an instrument like that has a greater potential to be applied in randomized studies [26].

Conclusion

The A-test has characteristics of an accurate tool for separating patients with from those without functional disability at all stages of early rehabilitation after surgically treated hip disease or fracture. Based on the results of A-test in the first days of early rehabilitation it is possible to make a plan for postacute rehabilitation.

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Appendix 1.

The A-test form

No	Parameters	Day of rehabilitation				
		1st	2nd	3rd	4th	5th
1	From supine to side lying					
2	From supine to sitting					
3	From sitting to standing					
4	Standing					
5	Back to bed					
6	Walking with aides					
7	Use of toalet					
8	Sitting on chair					
9	Walking up and down stairs					
10	Endurance while walking					
	SUMM					

The assessment of patient's ability to perform activity: 0 – if patient didn't perform activity, 1 – if patient was absolutely dependent of therapist help, 2 – if patient performed activity with little therapist help, 3 – patient needed therapist' verbal suggestion while performing activity, 4 – patient performed activity independently but insecurely (needed presence of another person, member of family for example), 5 – patient performed activity independently and securely.	The assessment of patient's endurance while walking: 0 – didn't walk 1 – walked 5 meters (in bed room) 2 – walked 15 meters 3 – walked 50 meters 4 – walked 100 meters 5 – walked more than 100 meters The optimal cut-off for the results of A-test: 1st day of rehabilitation: 7/8, 2nd day: lower – 12/13, upper – 17/18, 3rd day: lower – 13/14, upper – 18/19, 4th day of rehabilitation: 29/30, 5th day of rehabilitation: 34/35.

The lower point of the A-test safely separates the patients with functional disability, while upper point rules out functional disability.

GENERAL REVIEW

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Risk factors for cardiovascular disease in children on chronic hemodialysis – Traditional (general) risk factors, Part I

Faktori rizika od nastanka kardiovaskularnih bolesti kod dece na hroničnoj hemodijalizi: tradicionalni (opšti) faktori rizika, I deo

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Key words:
renal insufficiency, chronic; child; cardiovascular diseases; risk factors; hypertension; dyslipidemias; atherosclerosis; obesity.

Ključne reči:
bubreg, hronična insuficijencija; deca; kardiovaskularne bolesti; faktori rizika; hipertenzija; dislipidemije; ateroskleroza; gojaznost.

Introduction

Cardiovascular complications are almost always present in children with chronic kidney diseases (CKD) who are being treated with hemodialysis (HD). As in adults, the risk factors responsible for the onset of cardiovascular diseases in childen with CKD can be divided in two basic groups: traditional (general) risk factors related to the arteriosclerosis and nontraditional (uremia-related) risk factors, which are especially present in HD patients.

Arterial hypertension

Arterial hypertension is one of the main risk factors in patients with CKD who are being treated with HD. The presence of hypertension increases the risk of cardiovascular mortality in patients on HD. In end-stage renal disease (ESRD), almost all children and adolescents are hypertensive. In the first weeks or months after the commencement of treatment, HD blood pressure rapidly decreases and it is being reduced by the use of antihypertensive therapy, however, in many children on HD the hypertension persists [1,2].

Apart from that, it is difficult to precisely define the hypertensive status in children on dialysis, due to the frequent changes in the volume of the circulating fluid and the changes in the circadian rhythm [3,4].

Most would argue that the two major pathogenetic mechanisms of hypertension are hypovolaemia and vasoconstriction. Preload volume (preload) is responsible for many cases of hypertension. This is related to the retention of salt and water, as well as to the reduction in the mechanism for excretion of sodium. Insufficient elimination of the fluids (body weight above the "dry weight") leads to the state of chronic hyperhydration. Chronic salt and water retention in children with chronic renal insufficiency leads towards hypertrophy and dilatation of the left ventricle [5–7]. The presence of an arteriovenous fistula (AVF) in dialysis patients increases the venous flow to the heart and it increases the pressure of the volume. AVF additionally increases the preload, since it has the effect of the left-to-right shunt, thus the chronic hypovolaemia is almost always present in patients on HD, even when the "dry weight" is reached at the end of the dialysis treatment [4,5].

Vasoconstriction or rather the increase in peripheral vascular resistance may occur as the result of the activation of the sympathetic nervous system or due to the vasoconstrictor ofthe endothelial origin (e.g. endothelin-1). Another possible mechanism is the reduction in the production of vasodilators (e.g. nitric oxide, NO). Increased vascular rigidity may occur due to the increase in collagen glycosylation, which leads to a reduced compliance of the arteries and the increase of intracellular calcium (e.g., hyperparathyroidism) [6,7].

The incidence of arterial hypertension

According to various sources, the prevalence of hypertension in children on dialysis ranges from 40% to 90% [2–4]. A study by Tkaczyk et al. [8] shows that the prevalence of hypertension is 55% in the entire group of children on HD in Poland. In our study, conducted at the University

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Children's Hospital in Belgrade on 20 patients on HD, hypertension was present in 60% of the patient [5, 6, 9]. In a recent research, Mitsnefes et al. [4] have shown that the prevalence of hypertension is 76.6% of cases in the pediatric population (period 1992–2004). Other recent studies have shown a high prevalence of hypertension in children on HD (Halbach et al. [10] 67.9%, Hölttä et al. [11] 52%, Lingens et al. [12] 47%). The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) [13] shows that hypertension develops early in chronic renal disease. The increase in the incidence of hypertension from 48% in the early stage of CKD to 50–75% in terminal renal failure is very significant. Recent data from a study Chronic Kidney Disease in Children (CkiD) [4], on 586 children aged 1–16 years, from 46 pediatric nephrology centers in North America, show that the frequency of hypertension is 54% in patients in the early stages of CKD, with an increase by 25% in the end-stage renal disease and those treated with chronic HD (Table 1). The fact that children on antihypertensive therapy continue to have high blood pressure in 48% of cases is even more worrisome [14, 15].

tors are chosen as the first choice drugs for the treatment in the majority of children on HD. Besides, these two drugs present the most popular combination in most countries. Previous studies clearly describe that in patients on chronic HD, the antihypertensive therapy is often inefficient (60–70%). Most authors suggest that for this population, the selection of medication is not the only thing which is important, but also other non-pharmacologic therapies, for instance dietary salt restriction, maintaining adequate volemia and dry weight [15].

Control volume overload in patients on HD is essential in controlling hypertension. Experiences in adult patients on chronic HD are that significant help in controlling hypertension, and left ventricular hypertrophy regression can achieve HD every day, or the introduction of HD during the night 5–7 nights a week [16] (although data for children are still limited).

Recent data from the CkiD [4] emphasize the importance of the presence of masked hypertension, which was present in 25% of patients with CKD [2–4]. The presence of camouflage hypertension doubles the incidence of left ventricular hypertrophy. Therefore, routine ambulatory blood pressure

Table 1

Traditional risk factors for the onset of cardiovascular disease (CVD) in children with chronic kidney disease (CKD) [4]

Risk factors	CKD (%)	Dialysis (%)	Transplant (%)
Hypertension	47–54	52–75	63–81
Dyslipidemia	45	33–87	55–84
Obesity	15	8-11	12–22
Hyperglycemia	4	11	22

Note: Data are from the Chronic Kidney Disease in Children (CkiD) study.

Apart from that, arterial hypertension persists in most patients on HD, despite of the fact that nephrologists are aware of this problem. It is possible that high blood pressure in patients treated with dialysis is the consequence of untreated or inadequately treated ones. According to the European Dialysis and Transplant Association 55% of children on chronic HD receive antihypertensive therapy. Despite of this treatment, one third of all patients maintain the level of blood pressure 10 mmHg or more, above the 95th percentile for their age, gender and height [15, 16].

After kidney transplantation, hypertension is more frequently present in children than in adults. Successful transplantation leads to a significant improvement of the renal function and elimination of many risk factors, however, even after transplantation the prevalence of hypertension remains in 50–80% of patients [14].

Treatment of arterial hypertension

In most of recent studies, the majority of hypertensive children on chronic HD were treated with two or more medications. Lately, the majority of pediatric nephrologists tend to treat aggressively hypertension in their dialysis patients to achieve better control of hypertension. Calcium channel antagonists and angiotensin-converting enzyme (ACE) inhibi-

monitoring in children with CKD should begin at an early stage of CKD. This can significantly improve the control of hypertension [17].

Dyslipidemia

It is well-known that children on HD have an abnormal lipid status. It is reflected in the increase of the levels of lipoprotein of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) and of the total cholesterol and in the decrease of the levels of high density lipoprotein (HDL). The level of triglycerides is usually elevated, especially in patients with terminal renal insufficiency who are treated with HD and in patients after kidney transplantation [18].

It is believed that the basic mechanisms for the onset of dyslipidemia in CKD is a disorder in the break down of triglycerides which is associated with the increased levels of apolipoprotein C-III (an inhibitor of lipoprotein lipase) and with a decrease of the insulin sensitivity in the vascular endothelium of the blood vessels in skeletal muscle and adipose tissue. In children with CKD and those who are treated with HD, in almost half of the cases a combined dyslipidemia is present while in general population it is present in 20% of patients [18, 19].

The NAPRTCS shows that the prevalence of dyslipidemia is 70–90% in children on HD [16], however, dyslipidemia is common in the earlier stages of CKD (stages 2–4) [20]. The data of the aforementioned CKiD study show that dyslipidemia was found in 45% of children with CKD [5].

The CKiD study shows that 21% of children with CKD had total cholesterol greater than 200 mg/dL, in 21% HDL cholesterol was lower than 40 mg/dL and in 16% the VLDL cholesterol was greater than 160 mg/dL. Disorders of the lipid fraction may remain after kidney transplantation [19].

The treatment of dyslipidemia

The latest guidelines for monitoring and treatment of lipid status in children over 8 years of age and adults with diabetes show that the statin therapy should be considered in patients with LDL cholesterol ≥ 130 mg / dL [20]. Although these guidelines do not refer exclusively to children with CKD, they can be applied to them. Since 2006 the American Heart Association has been placing these patients in the high CV risk group, like patients with diabetes mellitus or a heart transplant. One could say that statin therapy will be a standard part of care for patients with chronic renal insufficiency older than 8 years, with LDL cholesterol ≥ 130 mg/dL, although this is not explicitly stated in the guidelines [21]. There is a few data on the improvement of long-term outcomes in children with CKD, if good control of the lipid status is performed [22, 23]. It is believed that statin therapy reduces the incidence of transplant vasculopathy. Serón et al. [24] show a significant reduction in the incidence of vasculopathy after kidney transplantation (33% compared to 7%) in the first 6 months after transplantation with statin therapy as compared to placebo. Recently, two large clinical studies (SHARP and AURORA) [25, 26] have tried to answer the question whether statin therapy benefits the patients with CKD and ESRD. The results of the AURORA study indicate that rosuvastatin has no effect on the development of atherosclerotic lesions and no significant effect on the reduction of mortality in patients undergoing HD, while the SHARP study is still in progress. Based on the data regarding the prevalence of the dyslipidemia in children, which was published as a part of the CKiD study, the NKF / KDOQI in 2008 issued the guidelines for the necessary screening of dyslipidemia in children with CKD since the beginning of puberty [27].

Atherosclerosis

Accelerated atherosclerosis in children with CKD is likely. There are indirect and direct evidence for the onset of atherosclerosis in childhood. Indirect evidence is that most of these patients have an increased *c*arotid intima-media thickness (cIMT), which is a basic sign of atherosclerosis in adults. In the group of young adults who had ESRD in childhood, cIMT was increased compared to the control group and it was correlated with the duration of end-stage renal disease, duration of HD and with the increase of the serum calcium, phosphorus and products of calcium x phosphorus [28–30].

Mönckeberg sclerosis is a special form of arterial calcification of the *tunica media* that is seen in patients with CKD.

This form of sclerosis is regulated by the ratio of promoters and inhibitors of calcification. Mönckeberg sclerosis involves calcification of *tunica media*, but does not include calcification of the *tunica intima*. Calcification of *tunica media* and *tunica intima* can coexist together, or calcification of the *tunica media* is dominated, especially in young children and in patients in stage 4 or 5 CKD (before dialysis). It is important that the clinical manifestation of calcification determines localization in the blood vessel wall. Furthermore, calcification of the *tunica intima* increases the frequency of mortality compared to the calcification of the *tunica media*. Also, dialysis patients may develop uremic arteriolopathy, which is a special form of calcification of the *tunica media* of blood vessels of the skin and heart valves [28].

To detect calcification of blood vessels commonly used measurement calcification using high-resolution ultrasound or direct evidence of coronary artery calcification using CT (multislice CT scan). Functional changes in blood vessels due to an increase in the stiffness of the vessel wall or decrease in the compliance of the vessel wall can be monitored by pulse Doppler during ultrasound examination [5, 30].

Direct evidence suggesting that significant atherosclerosis may occur in young patients with CKD is shown in the study of Naiyr et al. [31] who did a biopsy of the iliac artery in 12 patients on HD, aged 11–17 years. Out of 12 histologically examined arteries, in 7 (58.3%) there were atherosclerotic changes found, while in 5 (41.6%) they found intimal fibroelastic thickening and in 2 (16.6%) cases they found atheromatous plaques. Also, the duration of the acute renal failure is an important factor in the onset of the atherosclerosis. It is interesting that the values of blood pressures were similar regardless of the cause of end-stage renal disease, but the patients with congenital causes of CKD had elevated levels of phosphate and products of calcium-phosphorus – bigger than those with acquired diseases such as glomerulonephritis. This points out the fact that vascular diseases in young patients with CKD have multifactorial origin, with dyslipidemia as just one of many factors involved in the pathogenesis [28, 29].

Obesity

Lately, the presence of obesity was noted in children with CKD. The trends of the increased prevalence of obesity, with the rates from 8% (1995) to 12% (2002) are shown in HD patients (the NAPRTCS) [32]. The survey data on the adult definition suggests that the longer survival in patients with higher body mass (128–130 kg), however, it seems that the body and muscle mass should not exceed 133 kg. According to the recommendation of the NKF/KDOQI 32 weight loss in patients should be approached cautiously, with constant monitoring by dietitian and exercise physiologist. After kidney transplantation in children obesity is associated with higher rates of graft rejection or graft dysfunction. There is evidence that obesity in children on HD reduces the efficiency of antihypertensive therapy.

Obesity is becoming present in children even after the kidney transplantation. Approximately, the prevalence ranges from 15% to 30% in the first year following transplantation (Table 1).

Disorders of glucose and insulin metabolism

Disorder of the glucose and insulin metabolism in children with CKD on therapy with HD, and especially after kidney transplant is becoming more frequent [5]. Small studies on children with CKD, stage 2–4 or on HD, show that the prevalence of hyperinsulinemia is 33% and the prevalence of insulin resistance disorder is up to 16% (measured by homeostasis model assessment-estimated insulin resistance –HOMA IR) [33, 34]. It appears that abnormalities in glucose and insulin metabolism are more prevalent than they were deemed to be. Recent publications featuring studies on children after kidney transplantation have shown that the frequency of disorders of glucose and insulin metabolism is from 16% to 18% [33, 35].

Seating lifestyle

In adults with CKD, seating lifestyle and low levels of physical activity are well documented. Painter et al. [36] show that children with CKD (specifically, those on HD) are fairly inactive as they spend less than 10% of time in extracurricular physical activities. Also, aerobic capabilities have already been reduced in children and adolescents in the early stages of CKD (stage 3) and the improvement does not come even after transplantation. Due to the evidence that regular physical activity can decrease the risk of CVD in the general population, it is reasonable to question whether increased physical activity can reduce cardiovascular risk in children with CKD [37].

Conclusion

Early recognition of the traditional risk factors and treatment of patients with asymptomatic cardiovascular changes is the key for the reduction of the mortality and morbidity of dialysis patients with a developed cardiovascular disease during childhood.

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CURRENT TOPIC

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Classification and the diagnostics of abnormal uterine bleeding in nongravid women of reproductive age: the PALM-COEIN classification system adopted by the International Federation of Gynecology and Obstetrics

Patološko krvarenje iz uterusa kod žena u reproduktivnom dobu: PALM-COEIN klasifikacija Internacionalne federacije ginekologa i opstetričara

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Ključne reči:
metroragija; klasifikacija; dijagnoza; žene; terminologija.

Introduction

A nomenclature applied invariably and non-standardized investigation methods for abnormal uterine bleeding (AUB) complicate comparison of studies by different researchers. To design a universally accepted system for describing bleeding problems in reproductive-aged women, a new classification system was created named for the acronym PALM-COEIN (**p**olyp, **a**denomyosis, **l**eiomyoma, **m**alignancy and hyperplasia; **c**oagulopathy, **o**vulatory dysfunction, **e**ndometrial, **i**atrogenic and **n**ot yet classified). The International Federation of Gynecology and Obstetrics (FIGO) adopted and published this classification in 2011 [1], and the American College of Obstetricians and Gynecologists adopted it in 2012 [2]. Subsequently, all publications, recommendations and guidelines have used the PALM-COEIN classification in their methodology [3–5]. Journal editors and editorial boards are advised to request that the materials, methods and reporting sections of the manuscripts dealing with AUB be designed in accordance with the PALM-COEIN classification [1]. The Association of Gynecologists and Obstetricians of Serbia, Montenegro and the Republic of Srpska presented the information about this new classification at the Congress in Belgrade in 2015 [6].

The aim of this paper was to present the PALM-COEIN classification and the recommendations referring to non-pregnant women in their reproductive age suffering from abnormal uterine bleeding in relation to the currently used classification in Serbia.

Investigations used to determine the patient's condition and the cause of AUB

The following investigations are used to assess the causes of AUB in the affected woman: duration and quantity of flow, measurement of hemoglobin and hematocrit, assessment of coagulopathies, evaluation of the uterus for leiomyomas by ultrasound, assessment of endometrial cavity by any method, assessment of ovulation. These data are clinical, ultrasound and laboratory measures used in the clinical practice for AUB evaluation, and they have also been retained in the PALM-COEIN classification.

Acute, chronic, intermenstrual and heavy menstrual bleeding – new terminology, first-time adopted in the PALM-COEIN classification

Available classifications used before the PALM-COEIN did not make a distinction between acute and chronic AUB in non-pregnant women. In everyday practice, most clinicians record if a patient requires immediate volume re-

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placement as the first step in clinical examination. However, they formally described the patient's condition as hemodynamically unstable, shocked, or seriously anemic.

The PALM-COEIN defines acute AUB as an episode of heavy bleeding, which demands immediate intervention in order to prevent copious blood loss. After the immediate intervention, a following examination will reveal the underlying cause of the acute episode of AUB.

By contrast, chronic AUB is characterized by bleeding that lasted for the bulk of the past six months. Bleeding is also unexpected and abnormal in volume and frequency. Chronic AUB, in a clinician's opinion, does not require immediate intervention.

The intermenstrual bleeding (IMB) by the PALM-COEIN, occurs between two regular menses. This term is to replace the word metrorrhagia, which should be abandoned [1].

Terms from the previous classifications that should be abandoned according to the PALM-COEIN classification

The term metrorrhagia has been replaced by the term intermenstrual bleeding and the term menorrhagia by the term heavy menstrual bleeding (HMB). It is recommended that the previously used term dysfunctional uterine bleeding should also be abandoned. This term was previously used in the diagnosis when there were no systemic or locally definable structural causes of AUB; therefore, it is not used in the PALM-COEIN classification. Women diagnosed according to the previous classification of dysfunctional uterine bleeding have a disorder of ovulation, or primary endometrial disorder or coagulopathy. What is common for all these states is that there are no systemic or locally definable structural causes for uterine bleeding. Thus, the abandoned terms dysfunctional uterine bleeding, metrorrhagia and menorrhagia are not part of the PALM-COEIN system [1].

Definition and nomenclature in the PALM-COEIN classification

The PALM-COEIN classification has 9 main categories and the first letter of each category makes the acronym PALM-COEIN. The main categories are the following: polyp, adenomyosis, leiomyoma, malignancy and hyperplasia; coagulopathy, ovulatory disfunction, endometrial, iatrogenic and not yet classified. The PALM comprises groups where the etiology of bleeding is a structural disorder that can be determined by imaging techniques and/or histopathology, whereas the COEIN comprises groups which have dysfunctional disorders in the etiology of bleeding and they cannot be defined by imaging techniques (Table 1) [1].

P – polyp

Polyps are not a frequent cause of AUB, however, some can be included in its etiology. Polyps are endometrial proliferations and they are composed of a variable quantity of glandular, connective and vascular tissue components. They are diagnosed by ultrasound, or by ultrasound combined with hysteroscopic imaging, which is also crucial for excluding polypoid – appearing endometrium (considered to be a variant of normal) from this category. Histopathology may or may not be part of the diagnostics that will determine the presence of a polyp.

Table 1

PALM-COEIN: Basic Classification System [1]

P	Polyp
A	Adenomyosis
L	Leiomyoma
	submucosal
	other
M	Malignancy and hyperplasia
C	Cogulopathy
O	Ovulatory dysfunction
E	Endometrial
I	Iatrogenic
N	Not yet classified

For the basic classification system, polyps are categorized as being either present or absent [1].

A – adenomyosis

The PALM-COEIN accepted the sonographic criteria as the minimum requirements for the diagnosis of adenomyosis. Transvaginal ultrasonography (TVUS) has proven to be useful for detecting adenomyosis, but the technique depends highly on the operator. The magnetic resonance imaging (MRI) is the most accurate, non-invasive diagnostics procedure for determining adenomyosis; however, it is not widely available, so that is why it is not mandatory.

In compliance with the PALM-COEIN publication document, adenomyosis, similar to leiomyomas, is a disorder that should have its own subclassification system [1]. To our knowledge, there is no new data related to the adenomyosis subclassification, which is still being researched.

The project dealing with adenomyosis subclassification suggests that any subclassification of adenomyosis must begin with an evaluation of the myometrium underlying the endometrium, the so-called junctional zone (JZ). The imaging of the homogeneous thickening of the JZ has become the standard criterion for non-invasive diagnosis. The MRI is currently the most precise technique for detecting a whole spectrum of lesions, which is also the basis for subclassification, ranging from increased thickness of the JZ to overt adenomyosis and adenomyomas [7].

The absolute presence of heterotopic endometrial tissue in the myometrium and the myometrial hypertrophy are the two main components of the sonographic appearance of adenomyosis.

The 3-dimensional reconstruction of the uterus in the coronal plane has recently provided a different view of the JZ. In relation to the standard 2-dimensional imaging, the 3-dimensional TVUS coronal and multiplanar views of the uterus provide an improved view of the lateral and fundal aspects of the uterine cavity and JZ. It can be concluded that 3-dimensional TVUS evaluation of the JZ is more accurate in detecting adenomyosis than the conventional 2-dimensional evaluation [8].

L – leiomyoma

The term leiomyoma has been selected out of several terms that are in use as the most adequate for defining benign fibromuscular tumors in the PALM-COEIN system. Leiomyomas are frequently present in women of reproductive age, however, as with polyps they are rarely the cause of AUB.

Several issues are taken into consideration when making the classification system: the relation of the leiomyoma to the endometrium and serosa; the uterine location of the leiomyoma (upper segment, lower segment, cervix, anterior, posterior and lateral); the size of the lesions; the number of the lesions and the existing leiomyoma classification system [9].

In addition to the primary classification system, which reflects only the presence or absence of one or more leiomyomas, there are also secondary and tertiary classification systems. In the secondary system, leiomyomas involving the endometrial cavity (submucosal, marked as SM) must be distinguished from the others (marked as O), because submucosal lesions are generally considered to be the most common contributors to the genesis of AUB. The root of the tertiary classification system is a design for subendometrial or submucosal leiomyomas that was originally submitted by Wamsteker et al. [10] and subsequently adopted by the European Society for Human Reproduction and Embriology (ESHRE). The system has been in use worldwide for more than 15 years and is considered important in the design of the PALM-COEIN system. As a result, the PALM-COEIN system involves the categorization of intramural and subserosal leiomyomas; when the leiomyoma abuts or distorts the endometriom and the serosa, it is categorized initially by the submucosal classification, followed by the subserosal classification – within the two values separated by a hyphen. This tertiary classification (Table 2) is believed to be the most useful to clinical investigators, but clinicians, particularly those who perform resectoscopic or hysteroscopic myomectomy may find immediate clinical use [1] for it.

The relationship between AUB and leiomyomas has not been completely understood [11]. Previous postulated theories included an increased endometrial surface area and the presence of fragile and engorged vasculature in the perimyoma environment. The increase in vascular flow observed along with these enlarged vessels can overcome platelet action. The increasing knowledge reveals very complex cellular and molecular changes associated with leiomyomas, with an impact on angiogenesis, an alteration in vasoactive substrates and growth factors, as well as an alteration in coagulation [12]. The effect of leiomyomas on endometrial function is now thought to represent a field change within the uterine cavity rather than just being limited to regions overlying the leiomyoma(s). Some of these changes may have an impact on endometrial receptivity and implantation as well as on AUB [13].

M – malignancy and hyperplasia

In reproductive-aged women, atypical hyperplasia and malignancy are a relatively uncommon, but very significant potential cause of AUB. These etiologies classified as AUB-M are further subclassified using the appropriate World Helth Organization (WHO) and FIGO classification system. Thus, the PALM-COEIN classification system is not created to replace the present FIGO and WHO categorization of malignant diseases [1, 14, 15]. It should be noted that malignancy, as an etiology of AUB, must be considered in all women of reproductive age.

C – coagulopathy

High quality evidence demonstrates that approximately 13% of women with HMB have biochemically detectable systemic disorders of hemostasis, most often Von Willebrand disease [16]. It is important that considering systemic hemostasis becomes a part of everyday practice, particularly in the differential diagnosis of HMB, partly because such disorders do contribute to some cases of AUB, and partly because evidence shows that few clinicians consider systemic hemostasis disorders as an etiology of AUB.

Although chronic anticoagulation therapy could be considered iatrogenic, and classified accordingly, the PALM-COEIN designers determined that it would be more appropriate to classify the affected women as having coagulopathy (AUB-HMB-C).

Table 2

Leiomyoma subclassification system [1]

SM – Submucosal	0	Pedunculated intracavitary
	1	< 50% intramural
	2	≥ 50% intramural
O – Other	3	Contacts endometrium, 100% intramural
	4	Intramural
	5	Subserosal ≥ 50% intramural
	6	Subserosal < 50% intramural
	7	Subserosal pedunculated
	8	Other (specify e.g. cervical, parasitic)
Hybrid leiomyomas (impact both endometrium and serosa)	Two numbers are listed separated by a hyphen. By convention, the first refers to the relationship with the endometrium while the second refers to the relationship to the serosa. One example is below:	
	2–5	Submucosal and subserosal, each with less than half the diameter in the endometrial and peritoneal cavities, respectively.

O – ovulatory dysfunction

Ovulatory dysfunction can contribute to the genesis of AUB and is usually manifested as a combination of unpredictable timing of bleeding and variable amount of flow, which can result in HMB in some cases. Ovulation disorders may be present as a spectrum of menstrual abnormalities – ranging from amenorrhea, through extremely light and infrequent bleeding to episodes of unpredictable and extreme HMB requiring medical and surgical intervention [17]. Ovulatory dysfunction comprised a vast majority of cases encompassed by the now discarded term dysfunctional uterine bleeding.

Endocrinopathies should be considered in the etiology of most ovulatory disorders (e.g., polycystic ovary syndrome, thyroid diseases, hyperprolactinemia, adrenal gland diseases, weight related disturbances such as obesity, anorexia, weight loss, and mental and physical stress experienced during extreme exercise associated with elite athletic training) [18]. In transitional periods, such as adolescence and perimenopause, ovulatory disorders are frequent and expected. The etiology of some disorders cannot be adequately determined [19, 20].

E – endometrial

When the menstrual periods are predictable but heavy, and no other causes are identified, this can indicate to a case of primary disorder of endometrium. In such cases the mechanisms regulating local endometrial hemostasis are changed, which can result in HMB. In fact, high quality evidence has demonstrated deficiencies in the local production of vasoconstrictors, such as endothelin1 and prostaglandin F2α, or accelerated lysis of endometrial clot, because of the excessive production of plasminogen activator in addition to the increased local production of substances that promote vasodilatation, such as prostaglandin E2 and prostacyclin [21].

HMB can be a manifestation of the primarily changed molecular mechanisms of endometrial repair. Such disorders of endometrial repair may be primary or secondary, caused by endometrial inflammation or infection. The consistent relationship between the histological diagnosis of endometritis and the presence of AUB has not been demonstrated, but the studies that published these data are retrospective and included a small cohort of women. There is a well-defined relationship between AUB and subclinical infection with *Chlamydia trachomatis*.

Endometrial disorders in reproductive-aged women should be diagnosed after a normal ovulatory function and excluding other identifiable abnormalities.

I – iatrogenic

The major component of AUB-I classification is abnormal endometrial bleeding that occurs during gonadal steroid therapy. Such type of bleeding is termed as breakthrough bleeding. In a pooled study, 35% of women with large follicles that produce endogenous estradiol had breakthrough bleeding. Other potential causes of reduced levels of circulating estrogens include agents, such as anticonvulsants and antibiotics [22, 23].

Systemic agents that interfere with dopamine metabolism have the potential to cause ovulation disorders, and AUB secondary to ovulation disorders can be the result of medication use that impacts dopamine metabolism. Within this group of medicaments, the tricyclic antidepressants are often present and they impact the dopamine metabolism indirectly by reducing the serotonin uptake, which further reduces the inhibition of prolactin release.

Anticoagulant drugs (warfarin, heparin and low molecular weight heparin) often induce HMB. The manifestations are similar to those in inherited hemostasis disorders. By convention, it was determined that this type of iatrogenic AUB should be placed in the AUB-C category [1].

N – not yet classified

Various other uterine conditions and disorders may play a role in the AUB occurrence, but specific diagnostic assays are needed to determine this. These entities include chronic endometritis, arteriovenous malformations, as well as myometrial hypertrophy. Such conditions have not been adequately identified and examined yet.

Notation

The formal approach follows the WHO tumor, nodus, metastasis (TNM) staging of malignant tumors, with each component addressed for all patients. The presence or absence of pathology in each category is noted using 0- if absent, 1- if present, or ? - if not yet assessed. For example, if submucosal leiomyoma is present, the notation will be: Po Ao L1SM Mo- Co OoEo Io No. Since the full notation might be considered cumbersome in clinical practice, an abbreviation option has been developed, which will record only positive findings [1]. Therefore, the abbreviation option for our example will be L1SM.

Guidelines for investigation

Women with AUB may have zero, one, or multiple identifiable factors contributing to the genesis of abnormal bleeding. There may also be a pathology present, but one that is believed not to contribute to AUB [1].

Firstly, a clinician faced with AUB has to ensure that bleeding is not related to an undiagnosed pregnancy and that emanates from the cervical canal, rather than from another location. A pregnancy may be reliably determined by a beta-subunit of human chorionic gonadotrpin (HCG) assay. For the first distinguishing, the cervix is visualized by gynecological examination. Women with both acute and chronic AUB should be tested for anemia; preferably full blood count should be taken.

Once the bleeding is confirmed to be of uterine origin, the clinician will follow the schedule designed for dealing with each of the components of the classification system [1].

Endometrial and uterine cavity assessment

If good ultrasonic images are obtained and there are no findings indicative of endometrial polyps and submucosal leiomyomas, the endometrial cavity may be considered normal in relation to lesions causing AUB. However, if the exam is suboptimal, if there are imaging features indicative of

endometrial polyps, and if there are leiomyomas that may be encroaching on the endometrial cavity, imaging with other, more sensitive techniques is recommended – usually saline infusion sonohysterography (SIS) (also called sonohysteroscopy or hysterosonography) or hysteroscopy, whichever is available to the clinician. If office hysteroscopy is available, there may be an additional benefit should polyps be identified as they could be removed in the same setting.

When vaginal access is difficult (adolescents and virginal women), the MRI or alternatively hysteroscopic examination under anesthesia may be the best approach.

With the PALM-COEIN classification, P-polyp is confirmed only with the documentation of 1 or more clearly defined polyps, generally either with SIS or hysteroscopy. A patient may be categorized as AUB L1SM with either SIS or hysteroscopy, but with care as not to infuse the distending medium with such pressure that the natural relationships of the leiomyoma with the endometrium and myometrium are distorted.

Myometrial assessment

Myometrium is assessed primarily with the combination of TVUS and transabdominal ultrasonography to identify the presence of leiomyomas (primary subclassification), if there is a lesion leading to L1 assignment. A combination of imaging tools (TVUS, SIS, hysteroscopy or MRI) is necessary for the secondary subclassification. The relationship of leiomyoma with the endometrium, myometrium and serosa is required for the tertiary subclassification of the leiomyoma type. In clinical terms, this would require the use of an MRI.

It is important to distinguish between leiomyomas and adenomyomas. An assignment of A1 requires three of the criteria to be met; otherwise the patient is classified as Ao. If available, an MRI may be used to distinguish between leiomyomas and adenomyosis and to measure the myometrial extent of submucosal leiomyomas. MRI use is not obligatory according to the PALM-COEIN classification, because it is not part of everyday clinical practice in many healthcare systems.

The diagnostic procedure in chronic AUB patients in clinical settings

Initial evaluation – the patient has experienced one or a combination of unpredictability, excessive duration, abnormal volume or abnormal frequency of bleeding for at least 3 months. Structured history will determine ovulatory function, potentially related medical disorders, medications and lifestyle factors that may contribute to AUB. For those with HMB, the structured history should include specific questions. Patients with a positive screen are those with HMB associated with one of these symptoms: *post partum* hemorrhage, surgical – related bleeding or bleeding after dental work; or patients with HMB associated with two of these symptoms: bruising 1–2 times per month, epistaxis 1–2 times per month, frequent gum bleeding and a family history of bleeding. Physical examination will explore the appearance of the visible part of the cervix.

The proposed ancillary investigations are: determination of the beta- subunit of HCG as a reliable diagnostic tool for pregnancy exclusion, complete blood count, testing for endocrinopathies – thyroid function, serum prolactin and serum androgens, if there is oligoanovulation. Measurement of the serum progesterone has to be timed to the best estimate of the mid-luteal phase and may provide evidence of ovulation presence in the given cycle. The frame of the therapy depends on the future fertility desires, which have to be discussed beforehand.

Examination is guided by the patient's history and clinical situation, such as age, chronic ovulatory disorder, risk factors for endometrial hyperplasia and malignancy. Uterine examination begins with TVUS.

Selection of patients for endometrial sampling is based on the combination of factors that indicate to the risk for atypical hyperplasia or carcinoma. Several guidelines use the same combination of age, personal and genetic risk factors and TVUS screening for endometrial echo-complex thickness. According to some studies, age is not important as the independent factor. However, most of them suggest that endometrial sampling should be considered after a certain age, usually after the age of 45. Endometrial biopsy is advised for those at increased risk. Evaluation of the uterus should include imaging, if there is the risk of: structural disease, previous endometrial sampling has not provided an adequate specimen or previous medical therapy has been unsuccessful. Endometrial sampling is necessary for persistent, unexplained AUB or AUB which does not react adequately to treatment. If it is possible, hysteroscopic evaluation of the uterine cavity should accompany the sampling procedure in these situations [1]. Similarly, hysteroscopic evaluation of the uterine cavity is useful for endometrial evaluation in cases of virginal girls or women, and it is more convenient in an anesthetized environment. Finally, given the apparent relationship between a chlamydial infection of the endometrium and AUB, it is advisable to consider testing for *Chlamydia trachomatis* in cervical smears [1].

Worldwide implementation of the PALM-COEIN classification

After the adoption of the PALM-COEIN classification system, some publications compared the classic with the new terminology. One of the publications found that in a total of 471 women included, the term "hypermenorrhea" covered 15 different pathology combinations, "menorrhagia" nine, "metrorrhagia" 14, and "menometrorrhagia" 18, according to the old classification [24].

The suggested diagnostic methodology and protocols in PALM-COEIN do not differ from those used so far in Serbia. According to the classification, the minimal necessary diagnostic tools are standardized for each PALM-COEIN group and defined to be widely applicable worldwide.

Conclusion

A multinational group of clinicians and researchers have created a classification system to facilitate a multi-institutional investigation of the epidemiology, etiology and

treatment of women with acute and chronic AUB. The classification system is based on group formation done according to the etiology and clinical valuation of the amount of blood loss. The PALM-COEIN classification immediately points to the etiology of bleeding, and accordingly, it has very precise, non-descriptive terminology. Descriptive terms widely used in the old terminology are not used in the PALM-COEIN classification, which can make difficulties for some clinicians when they encounter them for the first time. The agreement process created a practical system, which contains recommendations for the minimal necessary diagnostic procedures that could be used to classify patients with AUB by clinicians in most countries worldwide. The task of national professional associations is to present and enable the adoption of the PALM-COEIN classification at national level, with the aim of allowing clinicians to compare diagnostics strategies and therapies in patients with AUB with gynecologists worldwide.

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CASE REPORTS

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Bullous lung diseases as a risk factor for lung cancer - A case report

Plućna bula kao faktor rizika od karcinoma pluća

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Abstract

Introduction. A possible association between lung cancer and bullous lung disease has been suggested and recently supported by the results of genetic studies. **Case report.** A previously healthy 43-year-old man, smoker, was diagnosed with bullous lung disease at the age of 31 years. He was followed up for 12 years when lung cancer (adenocarcinoma) was found at the site. In the meantime, he was treated for recurrent respiratory infections. **Conclusion.** There is the need for active approach in following up the patients with pulmonary bulla for potential development of lung cancer.

Key words:
lung neoplasms; risk factors; bronchopneumonia; diagnosis; tomography, x-ray, computed; disease progression.

Apstrakt

Uvod. Rezultati skorašnjih genetskih istraživanja ukazuju na moguću etiološku povezanost karcinoma pluća i plućne bule. **Prikaz bolesnika.** Bolesniku, starom 43 godine, pušaču, postavljena je dijagnoza gigantske plućne bule kada je imao 31 godinu. Praćen je pulmološki 12 godina, kada je na tom mestu otkriven karcinom pluća (adenokarcinom). U međuvremenu je lečen od ponavljanih respiratornih infekcija. **Zaključak.** Neophodan je aktivni pristup u praćenju bolesnika sa plućnom bulom zbog mogućeg nastanka karcinoma pluća kod tih bolesnika.

Ključne reči:
pluća, neoplazme; faktori rizika; bronhopneumonija; dijagnoza; tomografija, kompjuterizovana, rendgenska; bolest, progresija.

Introduction

A bulla is a sharply demarcated, air-containing space of 1 cm or more in diameter that possesses a smooth wall of 1 mm or less thickness. Bullae may be large enough to compress the adjacent lung parenchyma and may occupy most of a lung. Bulla may be isolated or a part of diffuse emphysematous lung disease [1–3].

Previous reports have suggested that lung cancer may develop in patients with bullous lung disease [4, 5]. Some of the reports provide retrospective data suggesting that bullous lung disease may predispose to the development of lung cancer in smokers [6, 7]. It seems that lung cancer in this population occurs more frequently at younger age compared to general population. It may be worthwhile to target this group in an attempt to diagnose occult lung cancer [8].

Case report

A 31-year-old man with negative past medical hystory, smoker for the last 15 years (20 pack/year), had been physicaly extremely active, including diving. Dyspnea and productive cough started at the age of 31 years. Physical examination showed decreased breath sound with prolonged expiratory phase. Pulmonary function test parameters showed severe degree of the mixed type of ventilation insufficiency; diffusing capacity of the lung for carbon monoxide and transfer factor of the lung for carbon monoxide/ alveolar volume were significantly decreased (Table 1). Arterial blood gas analysis showed normal values (P_aO_2: 12.07 kPa, P_aCO_2: 5.05 kPa, pH: 7.40; HCO^-_3: 23.6 mmol/L; SaO_2: 96.9%).

Chest radiography and computed tomography (CT) scan showed bilateral diffuse massive pneumatoceles with minimal normal surrounding lung parenhyma (Figures 1a and b).

Perfusion scan showed massive perfusion defects in the upper and lateral parts of both lungs. Concentration of α-1 antitripsin was 2.29 g/L, phenotipe Pi M_1. The patient did not accept a suggested surgical intervention.

The patient was symptom free for the next three years and then, at the age of 34, was hospitalized again due to

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Table 1

Lung function test results on admission in the patient

Paremeter	Actual	Predicted	Percentages of predicted a/p
FVC (L)	3.59	5.49	65
FEV1 (L)	1.8	4.38	41
FEV1/FVC (%)	50	81.8	61
$D_{L,}CO$	3.72	11.7	32
TLC (L)	5.48	7.3	75
$T_{L,CO}$/VA mmol/min/kPa/L	0.75	2.1	34
RV/TLC	34.4	25.7	134
RV (L)	1.89	1.79	106

FVC − forced vital capacity; FEV_1 − forced expiratory volume in 1 s; $D_{L,}CO$ − diffusing capacity of the lung for carbon monoxide; TLC − total lung capacity; $T_{L,CO}$/VA − transfer factor of the lung for carbon monoxide/ alveolar volume; RV − residual volume; a – actual; p – predicted.

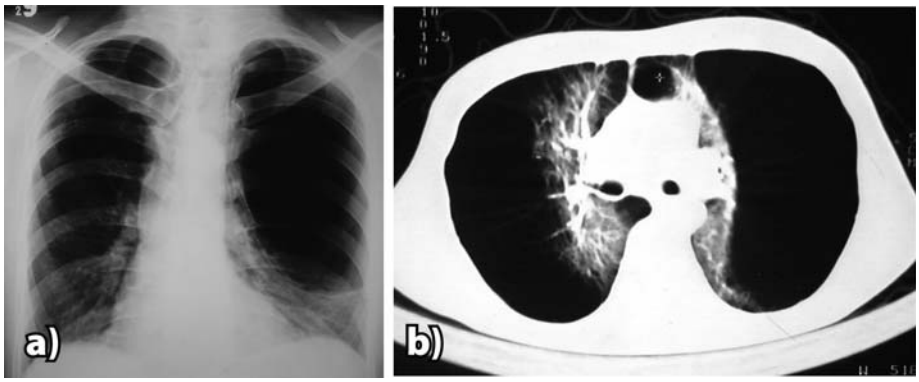

Fig. 1 − Bilateral diffuse massive pneumatoceles with minimal normal surrounding lung parenchyma shown by a) chest radiography, and b) computed tomography (CT) scan.

right-sided pneumonia. *Klebsiella spp.-Enterobacter spp.* has been isolated from sputum. Lung function test results were similar to previous ones. After combined antibiotic therapy, the patient was dissmised fully recovered.

The patient came to see a doctor next time after two years, at the age of 36, when the signs and symtoms of pulmonary infection appeared again. Standard chest x-ray showed bronchopulmonary infiltrates in the basal part of the left lung and the absence of the previously diagnosed bulla in the right lung – the status known as autobullectomy.

Control radiograph showed liquid formation in the bulla. CT scan showed large bulla in the left lung, 15 × 9 cm in diameter, with the liquid collection density of 9 Hounsfield unit (HU) (Figure 2).

After 5 weeks of antibiotic treatment, liquid collection disapeared.

Lung function tests showed moderate restriction pattern with decreased transfer factor and transfer coefficient (Lung transfer factor − TL 37%; transfer factor *per* unit alveolar volume − TL/VA 52%). His stabile condition has been maintaining for the next five years up to the patient's age of 41, when deterioration of his health started slowely. Hemoptysis appeared occasionally, together with dyspnea, purulent sputum production, and finger clubbing. Due to deterioration of pulmonary function with respiratory gases disturbation to severe degree of hypoxemia, he was hospitalized again at the age 42. This time, chest radiography and CT scan showed infiltration in the midle part of the right lung (Figure 3).

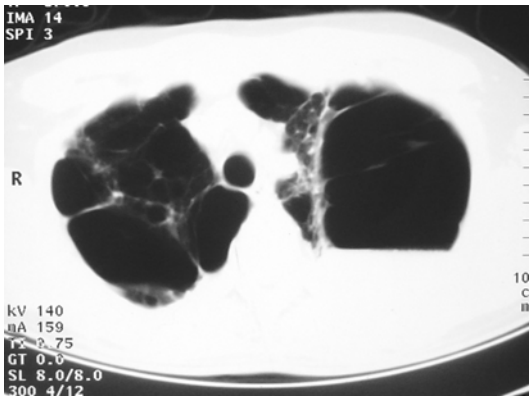

Fig. 2 – Computed tomography (CT) scan showed large bulla in the left lung, 15 × 9 cm in size, with the liquid collection density of 9 Hounsfeld unit (HU).

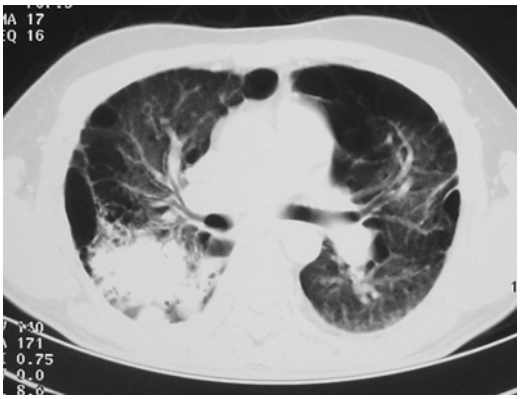

Fig. 3 – Chest radiography and computed tomography (CT) scan showed infiltration in the middle part of the right lung.

The diagnosis of lung adenocarcinoma was confirmed by bronchoscopic biopsy. The patient died at the end stage of lung cancer after a 12-year follow-up of the previously diagnosed bullous lung diseases.

Discussion

We presented a 12-year history of the patient with gigantic bullous disease that is also known as vanishing lung syndrome (VLS), a primary bullous disease of the lung, or type I bullous disease, defined as giant bulla in one or both upper lobes, occupying at least one third of the hemithorax and compressing surrounding normal lung parenchyma [9]. VLS in the presented patient was discovered by chance during an onset of respiratory infection. Clinical manifestations of bulla depend on the presentation of signs and symptoms of obstructive pulmonary disease. Usually, it is asympthomatic with periods of exacerbation with complications, and recovery, even when spontaneous rupture of the bulla with increase lung function occurs. It is shown that compressed lung parenchyma is functional and that surgical intervention would improve lung function and sustain progression of the disease. It would also decrease the risk of complications such as pneumothorax, infections of bulla, malignant alteracy and hemoptysis. While surgery in patients with isolated bulla should be recommended, bullous lung disease especially with complicated respiratory failure is extremely difficult to select for surgery. Although the presented patient was smoker at younger age, he had bullous lung disease with respiratory failure, so it was possible to discuss surgery [8, 10, 11].

Risk of cancer in patients with bullous disease is 36 times higher than in the normal lung parenchyma [10]. The majority of these lung cancers are non-small-cell tumors, and this was the case with the reported patient, who had lung adenocarcinoma [4]. The frequency of lung cancer associated with bullous emphysema has been estimated at 2−6% [12].

The potential carcinogenic mechanisms of bullous disease are still unrevealed. One of explanations is that carcinogens may inhibit antielastase enzymes, resulting in intervalveolar-septal destruction with consequent bulla formation. There is another opinion that constitutional or congenital factors may cause bullous disease and sinchronously may also contribute to lung cancer predisposition [4]. Related to this, the inner lining of a bulla may be more sensitive and susceptible to metaplastic alteration or disturbed aeration of bullae may allow easier carcinogens deposition. Another opinion suggests that the association of bullous lung disease and lung cancer is not only the consequence of shared tobacco smoking exposure, but, more probably, partial reflexion of a shared genetic predisposition to chronic smoking-induced inflammation [11].

Recent studies have put new light to the susceptibility to this disease. A genome-wide association study has revealed genetic variants in the nicotinic acetylcholine receptor (nAChR). The variants located on the chromosome 15q24/25 have been found to be associated with a risk for nicotine dependence, lung cncer, and chronic obstructive pulmonary disease (COPD) [12, 13]. When it comes to emphysema, the 15q24/25 locus in nAChR was associated with both the presence and severity of the disease independently of total exposure to tobacco smoke (expressed in pack/years). The finding suggests that nAChR is causally involved in alveolar destruction process as a potentially common pathogenic mechanism in chronic obstructive pulmonary disease (COPD) and lung cancer. Even, if the increased air space is not managed by removal, as in our reported patient, air trapping with the bulla may contribute to generating of lung cancer [10].

The results of the studies on the association between COPD and lung cancer showed that disturbed lung function based on reduced forced expiratory volume in 1s (FEV_1) is more important than the overall tobacco smoking exposure for lung cancer appearence [11, 14]. In a chest CT screening study on 23 patients, the vast majority of subjects with lung cancer had either spirometric confirmation of COPD or radiological proof of variable intensity emphysema [15]. Mortality studies show that 20−30% subjects with COPD die from lung cancer [16]. Due to such a strong association COPD should be considered a major risk factor for lung cancer, greater than duration or tobacco smoke exposure [11]. There is an opinion that lung function should be checked and followed up for assesing the risk of lung cancer just as it is reasonable to measure blood pressure in prediction of stroke, bone mineral density for eventual future bone fractures or body mass index as a risk factor in diabetes mellitus [14, 17, 18]. Measuring lung function should be very important for evaluation of lung cancer risk. This procedure of lung function testing for evaluation of lung cancer risk might be valuable in smoking cessation and targeted CT screening [15, 18].

Although it is suggested that airway obstruction and emphysema have been recognized as potential risk factors for development of lung cancer, the knowledge on clinical factors that contribute to lung cancer occurrence in subjects with COPD is still insufficient. A research showed that lung cancer can be expected in outpatients with COPD, more frequently in older ones with milder airflow obstruction (COPD stages I and II) and lower body mass index. Lung diffusion capacity of carbon monoxide less than 80% is associated with cancer diagnosis. The study showed squamous cell carcinoma as the most common histological type [19]. Nowadays, clinicians knowing these factors make efforts in early detection of lung cancer and its treatment. The presence of emphysema affected disease outcome in patients with non-small cell lung cancer. Found common company of dominant emphysema and COPD in patients with lung cancer (70%) led to recommendation to consider these facts in prognostic studes on comorbidity [20].

Tobacco smoking is considered major risk factor for lung cancer. The presented patient was a heavy smoker. Chronic inflammation secondary to tobacco nicotine exposure is one of possible mechanisms between COPD and lung cancer. It holds up thinking that cancer develops at sites of fortified chronic inflammation [21]. In smokers with bullous lung disease, tobacco smoke ingrediens might have additional synergistic action with other factors that lead to cancerogenesis. Tobacco smoking influences macrophages and neutrophils that relax metalloproteinases and reactive oxygen species. Such actions cause physiological modifications detected in COPD and /or lung cancer [22]. Epithelial-mesenchymal transition, disturbed repair,

oxidative stress and cell proliferation probably take parts in the common elementary process, which binds the two diseases and causes extensive remodelling.

Smokers with genetic predisposition to excessive response to tobacco smoke contents have increased potential for lung tumor growth. Extensive airway remodelling may lead to epithelial-mesenchymal modification and malignant changes of respiratory epithelium. Still is unknown why some smokers develop pulmonary and not cardiovascular disease, and why some of those with pulmonary disease get COPD, some other cancer and some evolve both the diseases [23].

Literature data suggest chromosomal loci and several candidate genes associated with COPD and lung cancer such as 1q21-23 (C-reactive protein – CRP and interleukin – IL 6R), 4q22 (family – FAM 13A), 4q24 (glutathione S-transferase, C-terminal domain chonaining – GSTCD) and 4q31 (hedgehog interacting protein – HHIP and glucophorin A – GYPA) [24]. Research is needed to explaine genetic correlation between COPD and lung cancer.

Conslusion

In clinical practice, even asymptomatic smokers with pulmonary bullae should be carefully checked up annually. High-resolution chest CT to discover possible lung cancer is especially recommended.

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CASE REPORT

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Severe vaso-occlusive retinopathy associated with systemic lupus erythematosus

Teška vazookluzivna retinopatija udružena sa sistemskim eritematoznim lupusom

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Abstract

Introduction. Systemic lupus erythematosus (SLE) is a systemic idiopathic autoimmune inflammatory disease, with multiple organ involvement. Severe vaso-occlusive retinopathy is a rare, sight threatening lupus-related manifestation of the disease, which is more common in patients with coexisting antiphospholipid syndrome. **Case report.** We reported a 36-year-old female with severe vaso-occlusive retinopathy that manifested in the absence of antiphospholipid syndrome. In a 4-year follow-up, despite aggressive systemic corticosteroid and immunosuppressive therapy and panretinal laserphotocoagulation treatment, the disease progressed to retinal neovascularisation, neovascular vitreoretinopathy, neovascular glaucoma and, consecutively, severe visual loss. As the final option for preservation of visual function, *pars plana* vitrectomy with laserphotocoagulation was performed and had good results. Progression of ophthalmological findings indicated the progression of the systemic disease, as well as neurolupus. **Conclusion.** Severe vaso-occlusive retinopathy occurred as the ophthalmological manifestation of SLE in the absence of antiphospholipid syndrome, but correlated with neurolupus and led to visual deterioration despite the treatment.

Key words:
lupus erythematosus, systemic; retinal diseases; retinal neovascularization; comorbidity; vitrectomy.

Apstrakt

Uvod. Sistemski *lupus erythematosus* (SLE) predstavlja sistemsko idiopatsko autoimunsko inflamatorno oboljenje, koje može zahvatiti brojne organe. Teška forma vazookluzivne retinopatije je retka oftalmološka manifestacija ove bolesti koja može ugroziti vid, a koja se češće javlja kod bolesnika sa udruženim antifosfolipidnim sindromom. **Prikaz bolesnika.** U radu je prikazana 36-godišnja bolesnica sa teškom formom vazookluzivne retinopatije koja je nastala u odsustvu antifosfolipidnog sindroma. Tokom 4-godišnjeg praćenja, uprkos agresivnoj sistemskoj kortikosteroidnoj i imunosupresivnoj terapiji i panretinalnoj laserfotokoagulaciji, došlo je do progresije bolesti i nastanka retinalne neovaskularizacije, proliferativne vitreoretinopatije i neovaskularnog glaukoma i, posledično, do slabljenja vida. *Pars plana* vitrektomija sa laserfotokoagulacijom bila je poslednja mera u očuvanju vidne funkcije i imala je dobre rezultate. Progresija očne bolesti ukazivala je na progresiju sistemske bolesti i neuroloških manifestacija lupusa. **Zaključak.** Teška forma vazookluzivne retinopatije nastala je kao oftalmološka manifestacija SLE u odsustvu antifosfolipidnog sindroma, a njena progresija bila je povezana sa pogoršanjem neurolupusa i dovela je do teškog slabljenja vida uprkos tretmanu.

Ključne reči:
lupus, eritematozni, sistemski; mrežnjača, bolesti; mrežnjača, neovaskularizacija; komorbiditet; vitrektomija.

Introduction

Systemic lupus erythematosus (SLE) is an idiopathic autoimmune inflammatory disease that can affect multiple organs. The main features of the disease include the presence of circulating autoantibodies to one or more components of cell nuclei, generation of circulating immune complexes and activation of complement system resulting in tissue destruction [1]. Severe vaso-occlusive retinopathy is a rare lupus-related manifestation with poor visual prognosis, more common in patients with coexisting antiphospholipid syndrome [1–3].

We reported a 36-year-old female with SLE and severe vaso-occlusive retinopathy that occurred in the absence of antiphospholipid syndrome.

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Case report

In April 2008, a 36-year old female was admitted to the Clinic due to a sudden loss of vision in her right eye. Visual acuity in the right eye was 0.5/60 and in the left 0.3. The patient had been diagnosed with SLE 6 years before according to the international criteria proposed by the American College of Rheumatology [4], and was treated with corticosteroids and antimalarial agents (chloroquine 250 mg *per* day). Ophthalmological examination revealed perivascular sheathing, extensive cotton-wool spots, intraretinal haemorrhages, retinal oedema and wide areas of vascular occlusion on the periphery of both eyes (Figure 1). Fluorescein angiography revealed bilateral complete obliteration of macular capillaries and widespread occlusion of small retinal vessels (Figure 2). According to the advice of the rheumatologist, three-day-course pulse dose of methylprednisolone (1000 mg *iv*), followed by oral prednisolone 80 mg *per* day were introduced, as well as intravenous cyclophosphamide 500 mg administered in two-week regimen during the first two months and then continued once a month. After the treatment, visual acuity improved to 2/60 in the right eye and 0.5 in the left.

Further investigations were performed at the Institute of Rheumatology. Laboratory analyses showed elevated erythrocyte sedimentation rate, leucopenia, thrombocytopenia and normal erythrocyte count, haemoglobin rate and biochemical analyses. In immunoserology, elevated titres of antinuclear antibodies and circulating immune complexes were present, while other analyses (anticardiolipin antibodies and lupus anticoagulant) were negative. Renal function was normal. Objectively, the patient had iatrogenic Cushing syndrome with corticosteroid-induced obesity, hirsutism, osteoporosis and hypertension. On chest radiography, small pleural adhesion was detected in the right side. Due to the risk of development of neurolupus, magnetic resonance imaging (MRI) of endocranium was performed and punctiform ischemic lesions were detected in the *corona radiata*.

One year later, large areas of retinal ischemia persisted in both eyes with high risk of retinal neovascularization. Laser photocoagulation was performed in order to impede formation of new vessels. However, despite the aggressive treatment, one year later disc neovascularization with preretinal haemorrhage in the right eye and neovascularization elsewhere (temporal and nasal periphery) in the left eye developed. Panretinal photocoagulation continued (Figure 3) and after the treatment, visual acuity was 1/60 in the right eye and 0.3 in the left one and intraocular pressure was within normal limits with no neovascularisation in iridocorneal angle.

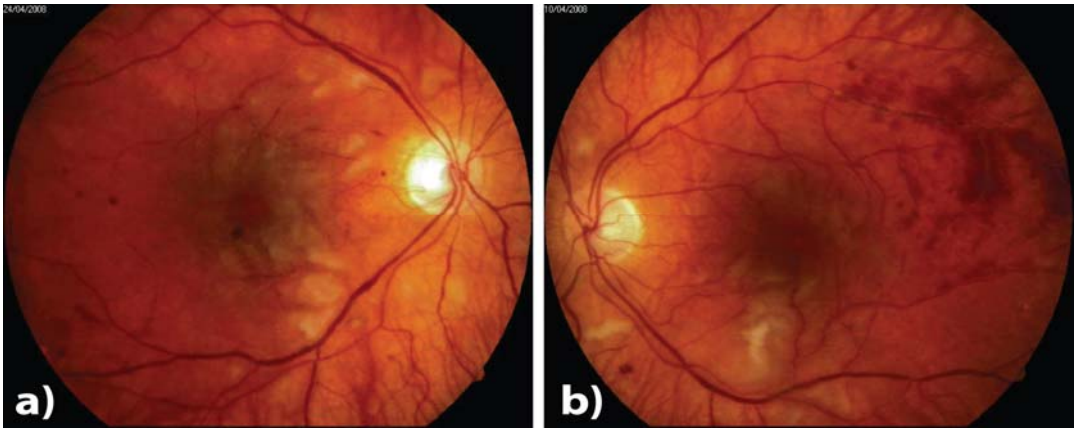

Fig. 1– a) Fundus colour photograph of the right eye showing vaso-occlusive retinopathy with cotton-wool spots and retinal oedema (April 2008), and b) Fundus colour photograph of the left eye showing vaso-occlusive retinopathy with intraretinal haemorrhages, cotton-wool spots and retinal oedema (April 2008).

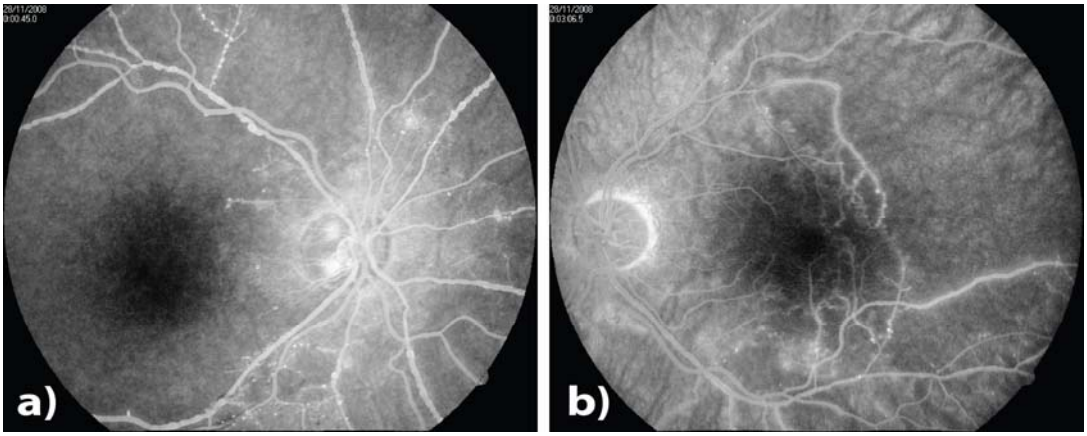

Fig. 2 – a) Fluorescein angiography findings of the right eye with diffuse avascular zones on the posterior pole and periphery of the retina (November 2008), and b) Fluorescein angiography findings of the left eye with wide avascular zones above and temporally of the macula (November 2008).

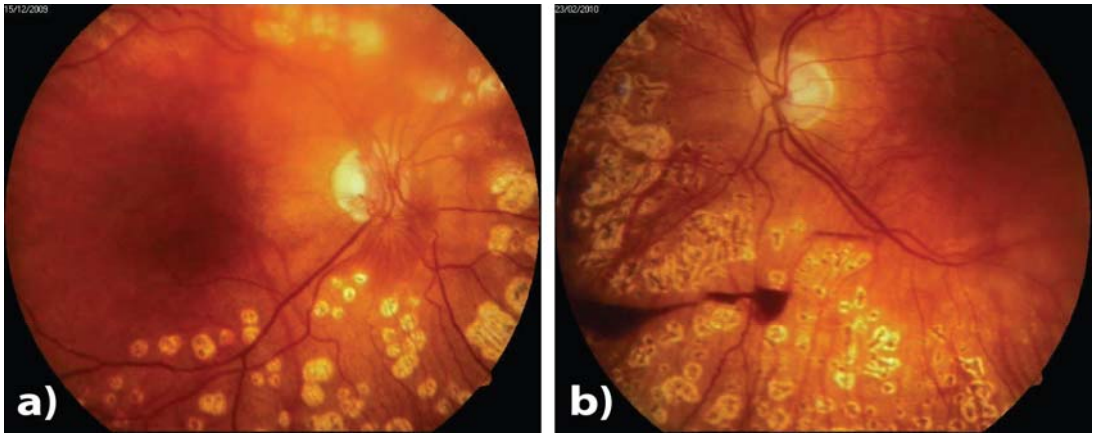

Fig. 3 – a) Fundus colour photograph of the right eye showing neovascularization at disc, intravitreal haemorrhage and scars after panretinal laserphotocoagulation (February 2010), and b) Fundus colour photograph of the left eye showing neovascularization elsewhere, preretinal haemorrhage and scars after laserphotocoagulation nasally and below the optic disc (February 2010).

Progression of the eye disease was noted three years later, with sudden visual loss in the single functional left eye. Visual acuity deteriorated to light perception bilaterally, due to dense vitreous haemorrhages. Also, neovascularisation in iridocorneal angle was noted in the right eye with intraocular pressure in 40 + mmHg range, which could not be controlled even with maximum medicamentous treatment. Vitreoretinal surgery (*pars plana* vitrectomy, endolaser and phaco) was performed in the left eye, because it was estimated that it has more visual potential. After the surgery visual function improved to 0.1 and was stabile for 18 months. During the follow up, repeated analyses for antiphospholipid antibodies were negative.

Discussion

SLE is an autoimmune inflammatory disease characterized by the numerous autoimmune phenomena and lesions in multiple organ systems [5]. Although eye findings are not listed as the criteria for SLE diagnosis, the thrombotic and inflammatory processes associated with SLE, can affect any part of the eye and result in manifestations such as *keratoconjunctivitis sicca*, scleritis, uveitis and retinal involvement [6]. Retinopathy in SLE is the second most common (after *keratoconjunctivitis sicca*) ophthalmological manifestation and usually is presented with cotton-wool spots, with or without intraretinal haemorrhages [2, 5, 6]. Depending on the study group, SLE retinopathy was reported to be present in 3.3–28.1% of all SLE patients, with the incidence rising at the advanced stages of the systemic disease [7, 8]. A severe variant of retinal vaso-occlusive disease is less common and may include central or branch retinal vascular occlusion or diffuse vaso-occlusive retinal disease [9–11] and the latter occurred in the presented case. Visual impairment is usually secondary to ischemic retinopathy and higher incidences were reported in association with antiphospholipid syndrome (APC) [1, 5]. However in the presented case, there were no evidences of this state.

Similar to the presented case, Mendrinos et al. [9] reported a case of bilateral combined central retinal artery and vein occlusion associated with normal levels of antiphospholipid antibodies and magnetic resonance imaging (MRI) confirmed central nervous system (CNS) vasculitis. The patient was treated with intravenous, followed by oral prednisone and cyclophosphamide, but unfortunately, despite all interventions, extensive vaso-obliteration led to permanent vision impairment. Also, Ho et al. [11] reported a case of bilateral severe SLE vaso-occlusive retinopathy in the absence of antihpospholipid syndrome that led to devastating vision deterioration. In addition, Zou et al. [10] reported a case of bilateral central retinal artery occlusion (CRAO) as the primary manifestation of with normal levels of antiphospholipid antibodies. Visual acuity deteriorated despite the systemic corticosteroid and immunosuppressive treatment [11].

Severe diffuse bilateral vaso-occlusive retinopathy as a complication of SLE, with the normal concentration of antiphospholipid antibodies is a rare complication in SLE. This type of retinopathy may be explained by the immune complex deposition, microthrombosis and endothelial damage in small blood vessels in the retina and choroid [2]. In the presented case, permanent loss of vision developed despite the treatment (systemic corticosteroid, immunosuppressive therapy and panretinal laserphotocoagulation). The patient's poor visual acuity along with bilateral optic nerve pallor could be attributed to posterior ischaemic optic neuropathy. Vitreoretinal surgery was the final option that impeded the retinal proliferative disease and improved the visual function. Furthermore, patient had vaso-occlusive retinopathy associated with similar changes in CNS and therefore had to receive the most aggressive systemic treatment, since it was shown that patients with neurolupus have the poorest prognosis in respect to survival rates [6].

Conclusion

Vaso-occlusive retinopathy is a rare lupus-associated complication and its presence is highly suggestive for SLE activity. It is usually, although not exclusively, a manifestation of antiphospholipid syndrome. Treatment should be

prompt with close communication with rheumatologists and aimed to prevent further thrombosis and complications arising from neovascularisation.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Pseudomesotheliomatous carcinoma of the lung

Pseudomezoteliomatozni karcinom pluća

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Abstract

Introduction. Pseudomesotheliomatous lung carcinoma is a special, rare entity characterized by large pleural growth and minor invasion of lung tissue. Clinically, radiologically, macroscopically and even histologically this tumor can be misdiagnosed as malignant pleural carcinoma. **Case report.** We represent a 64-year-old male patient, former smoker. Due to difficulties in the form of dry cough, feeling of discomfort and pain in the right hemithorax, fatigue, heavy breathing, sweating, fever up to 39.6°C the patient was treated as with combined antibiotic therapy (macrolides, cephalosporins and penicillin), but without improving of his condition. Chest radiography showed a shadow of pleural effusion by the height of the front end of the third right rib. Chest MSCT showed the extremely thickened pleura apically and to the posterior along the upper right lobe in addition to existence of massive pleural effusion. Subpleural condensation of parenchyma ranging about 30 mm was described in the upper right lobe. Cytological analysis of the pleural effusion showed the presence of malignant cells impossible to differentiate whether they were metastasis of adenocarcinoma or malignant pleural mesothelioma. By histochemical and immunohistohemical analyses of a pleural sample, pseudomesotheliomataus lung adenocarcinoma was diagnosed. **Conclusion.** Pseudomesotheliomataus carcinoma of the lungs can be a diagnostic problem. Its diagnosis is based on recognition of histopathological characteristics which enable its discernment from the epithelial variant of malignant pleural mesothelioma.

Key words:
lung neoplasms; adenocarcinoma; diagnosis; diagnosis differential; mesothelioma, malignant; prognosis; treatment outcome.

Apstrakt

Uvod. Pseudomezoteliomatozni karcinom pluća je poseban, redak entitet koji se karakteriše obimnim pleuralnim rastom i manjim zahvatanjem plućnog parenhima. Klinički, radiološki, makroskopski, pa i histološki ovaj tumor može biti pogrešno dijagnostikovan kao maligni mezoteliom pleure. **Prikaz bolesnika.** U radu je prikazan bolesnik, star 64 godine, bivši pušač. Zbog tegoba u vidu suvog kašlja, osećaja nelagodnosti i bolova u desnoj polovini grudnog koša, malaksalosti, otežanog disanja, pojačanog znojenja, povišene telesne temperature i do 39,6°C lečen je kombinovanom antibiotskom terapijom (makrolidima, cefalosporinima i penicilinom) bez poboljšanja. Na radiografiji grudnog koša uočena je senka pleuralnog izliva do visine prednjeg okrajka trećeg rebra sa desne strane. Multislajsni skener (MSCT) grudnog koša pokazao je izrazito zadebljalu pleuru apikalno i posteriorno uz gornji desni režanj, uz postojanje masivnog pleuralnog izliva. U gornjem desnom režnju opisana je subpleuralna kondenzacija parenhima promera oko 30 mm. Citološkom analizom pleuralnog izliva viđene su maligne ćelije za koje nije bilo moguće odrediti da li se radi o metastazi adenokarcinoma ili malignom mezoteliomu pleure. Histohemijskom i imunohistohemijskom analizom uzorka plućne maramice utvrđeno je postojanje pseudomezoteliomatoznog adenokarcinoma pluća. **Zaključak.** Pseudomezoteliomatozni karcinom pluća može da predstavlja dijagnostički problem. Njegova dijagnoza bazira se na prepoznavanju patohistoloških karakteristika koje omogućavaju njegovo razlikovanje od epitelne varijante malignog mezotelioma pleure.

Ključne reči:
pluća, neoplazme; adenokarcinom; dijagnoza; dijagnoza, diferencijalna; mesoteliom, maligni; prognoza; lečenje, ishod.

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Introduction

Pseudomesotheliomatous carcinoma (PMC) of the lungs is a special variant of peripheral lung cancer characterized by extensive pleural growth and minimal invasion of lung parenchyma. Clinically, radiologically and macroscopically it cannot be discerned from malignant epithelial pleural mesothelioma [1–7]. The diagnose is made with immunohistochemical staining (IHC). The term, pseudomesotheliomatous lung cancer, was first introduced by Harwood et al. [1] in 1976 showing that it was a special variant of lung cancer. The frequency of this carcinoma is not known due to limited number of case reports in the literature.

Case report

A 64-year-old male patient, former smoker, had difficulties in the form of dry cough, feeling of discomfort in the right hemithorax that had appeared one year before coming to specialist examination. Due to intensifying of difficulties along with appearing of malaise, fatigue, heavy breathing, sweating, fever up to 39.6°C, pain in the right hemithorax that lasted a month, the patient was treated as an outpatient with a combined antibiotic therapy of macrolides, cephalosporins and penicillin, but without improving of his condition.

In his personal anamnesis the patient reported diabetes and hypertension because of which he had been taking oral hypoglycemic drugs and antihypertensives.

In clinical findings upon admission he was eupneic, acyanotic, hemodinamically compensated. On auscultation, over the bottom half of the right lung there was a weakened to silent breathing sound, with a shortened percussion sound.

Laboratory analysis showed upon admission increased sedimentation of erythrocytes [98 mm/h (normal < 15 mm/h)], increased number of white blood cells (Leu), 11.49×10^9/L (normal range – NR 4–11 × 10^9/L), decreased number of erythrocytes (Er) 3.81 × 10^{12} (NR 4.5–6.5 × 10^{12}/L), hemoglobin (Hgb) 103 g/L (NR 130–180 g/L), hematocrit (Hct) 0.32 L/L (NR 0.40–0.54 L/L), glycemia 7.6 mmol/L (NR 4.1–5.9 mmol/L), urea 9.5 mmol/L (NR 2.5–7.7 mmol/L), lactate dehydrogenase (LDH) 783 U/L (NR 208–378 U/L), alkaline phosphatase (ALP) 36 U/L (NR 90–360 U/L). Other parameters of biochemical analyses and transaminases were inside the normal values. Spirogram and flow/volume curves were normal. Chest radiography showed a shadow of pleural effusion by the height of the front end of the third right rib (Figure 1). Chest multislice computed tomography (MSCT) showed the extremely thickened pleura apically and to the posterior along the upper right lobe in addition to the existence of massive pleural effusion. Subpleural condensation of parenchyma ranging about 30 mm was described in the upper right lobe. Precarinal lymph nodes were ranging up to 15 mm (Figure 2) Abdominal ultrasound examination was normal.

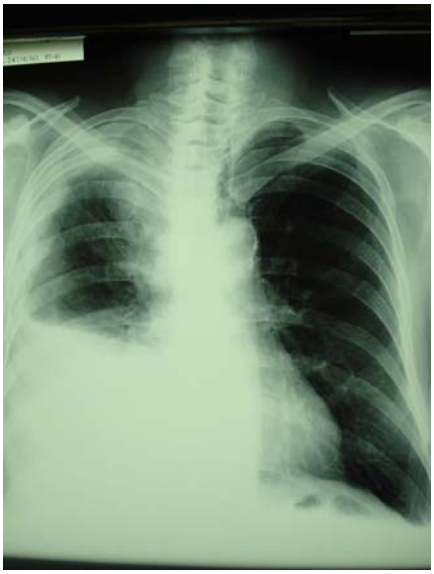
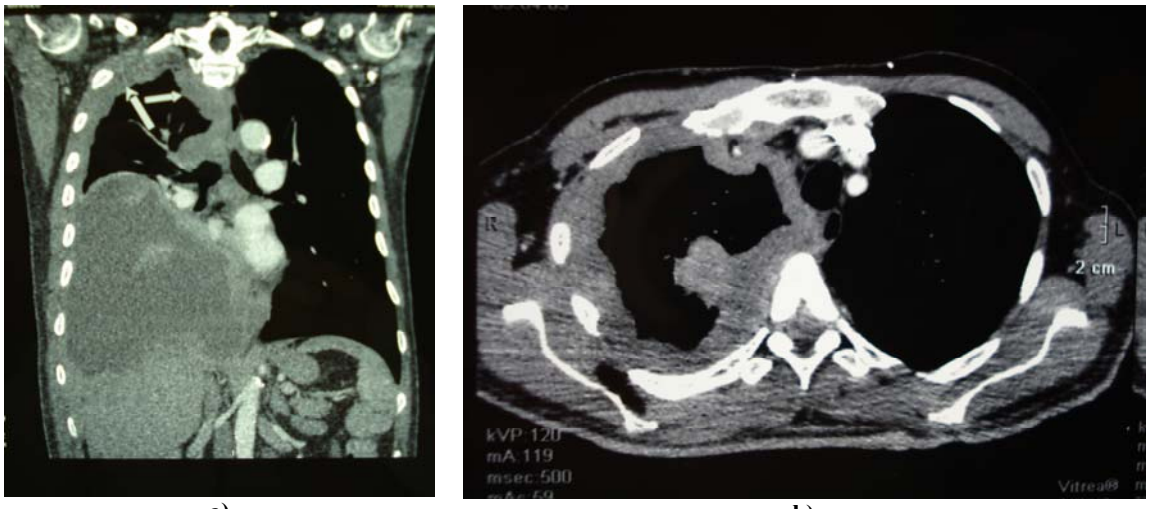


Fig. 1 – Chest X-ray showing pleural effusion shadow up to the height of the front end of the third right rib.



a) **b)**

Fig. 2 – a) Axial and b) Coronal section of computed tomography scan showing the thickened pleura apically and to the posterior along the upper right lobe in addition to the existance of massive pleural effusion. In the upper right lobe there is a subpleural condensation about 30 mm in diameter. Precarinal lymph nodes with 15 mm of size.

Bronchoscopically, hyperemia of the mucosa of the end portion of the right main bronchus was detected as well as in the apical segment of the upper lobe with mild extramural compression.

Right sided diagnostic thoracocentesis showed effusion with biochemical characteristics of exudate. Cytological analysis of the pleural effusion showed the presence of malignant cells impossible to differentiate whether they were metastasis of adenocarcinoma or malignant pleural mesothelioma (Figures 3–5).

Histopathological examination of pleural sample from blind biopsy presented the tissue made of large groups of neoplastic glands of medium size surrounded by thick cellular stroma lined in fascicular order (Figure 6).

The presence of intracellular neutral mucine and the periodic acid-Schiff (PAS+) consistence was histochemically proven within tumor cells alongside with intracytoplasmatic intensive PAS diastase (PAS-D) positivity (Figure 7).

The immunophenotype of the analyzed tumor indicated the profile of adenocarcinoma – pan CK (cytokeratin), CK 5/6, CK 7 (Figure 8), which were positive in more than 50% of the tumor cells, thyroid transcription factor (TTF) in around 30% of cells, CD 15 in around 15% of cells, with extreme positivity of carcinoembryonic antigen (CEA) (Figure 9). Calretinin reactions (Figure 10), thrombomoduline and anti-mesothelioma antibody (HBME) gave non-specific reaction in cytoplasm of few cells where pan cytokeratin antibody (panCK) was detected in the same time.

This microscopic image alongside with data of tumor localization, histochemical and immunohistochemical analysis confirmed that the tumor was pseudomesotheliomatous adenocarcinoma of the lung.

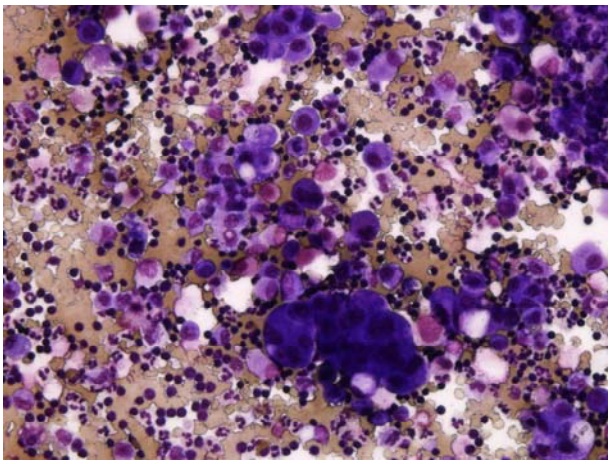


Fig. 3 – Cytospin specimen of pleural fluid showing admixture of dominating lymphocytes, neutrophils, eosinophils, macrophages, mesothelial cells, and clusters of reactive mesothelial cells in the background of erithrocytes. Clusters of cells similar to mestothelial ones by morfology are also seen, but their chromatine is coarse, with visible nucleolus, and cytoplasmic vacuoles [May-Grunwald Giemsa (GMM), ×20].

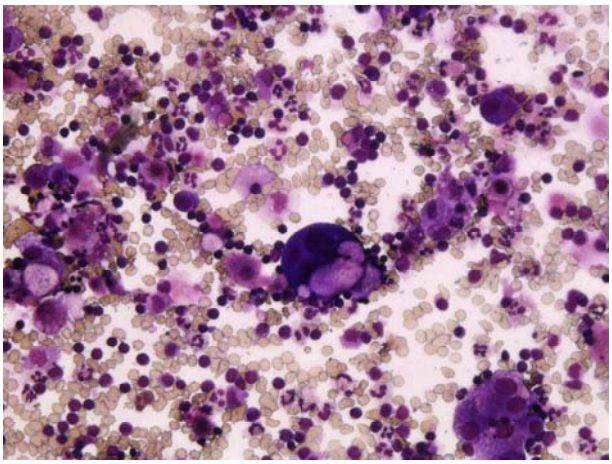


Fig. 4 – Cytospin of the same specimen of pleural fluid that emphises one of the numerous single cells that have irregular, often multiple nuclei, prominent nucleoili, and abundant cytoplasmic vacuoles [May-Grunwald Giemsa (GMM), ×20].

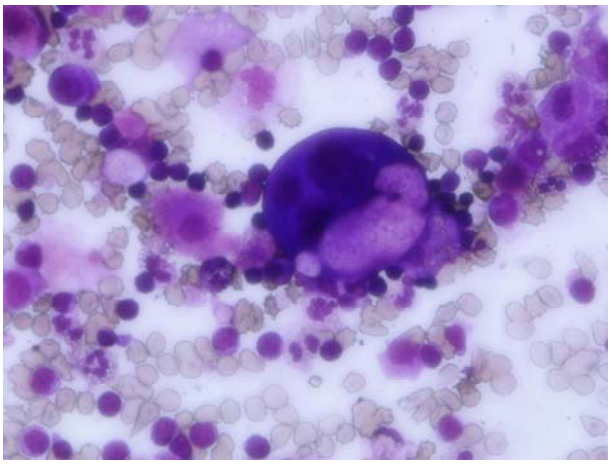


Fig. 5 – Single cell with cytomorfology of malignant cell [May-Grunwald Giemsa (GMM), ×100].

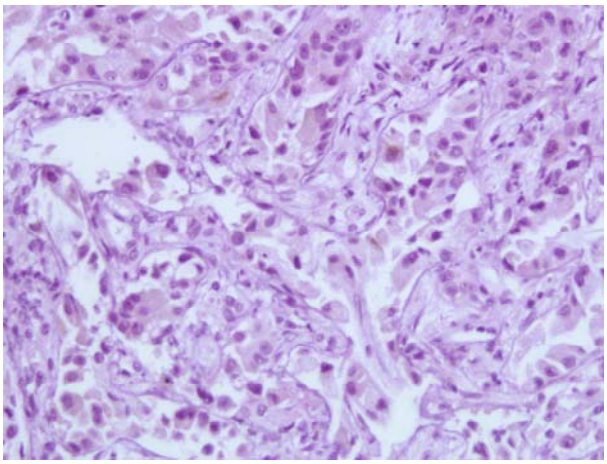


Fig. 6 – Infiltrated parietal pleura with acinar and tubular formations of epitheloid cells with abundant cytoplasm [hematoxillin and eosin (HE), ×200].

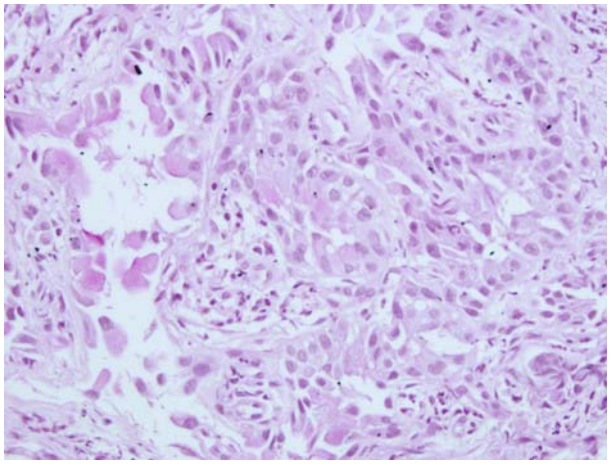


Fig. 7 – Anti-mesothelium antibody (PAS-D) resistant content in tumor cells (×200).

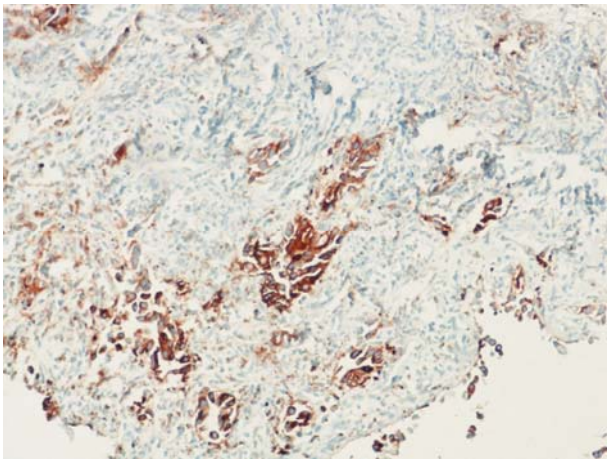


Fig. 8 – Intensive immunohistochemical reaction with carcioembrionic antigen (CEA, ×100).

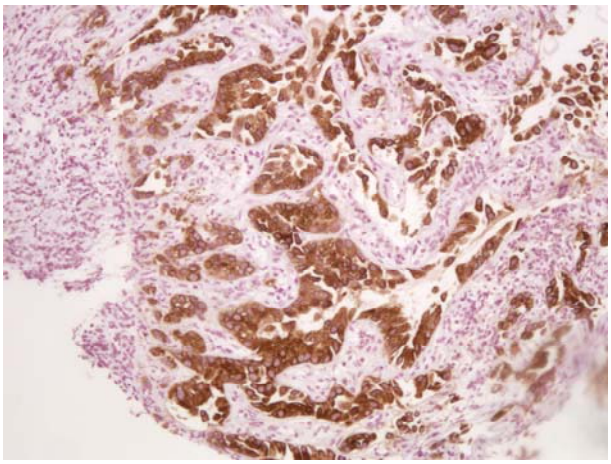


Fig. 9 – Malignant glandular forms with acinar pattern of tumor cells (cytokeratin 7 – CK7, ×100).

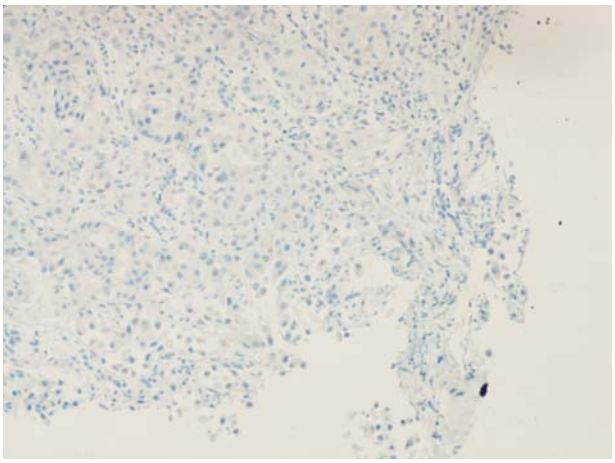


Fig. 10 – Negative immunohistochemical reaction of tumor cells (Calretinin, ×100).

According to the decision of the Oncological Consilium the treatment was thoracal drainage with pleurodesis and chemotherapy by the protocol with gemcitabine and cisplatin. After two cycles of chemotherapy by previously given protocol progression of disease was determined. Because of a poor performance status Eeastern Cooperative Oncology Group 3 (ECOG 3) the treatment was continued by application of only symptomatic therapy.

Discussion

Pseudomesotheliomatous tumors are a heterogenous group of tumors. Most often these are adenocarcinomas [8], but there are reports on pleomorph carcinomas, small-cell carcinomas, basaloid carcinomas, carcinosarcomas [8], neuroendocrine carcinoma [9], large cell carcinoma [10]. Also, there are reports on squamous carcinoma, paget carcinoma [11] and atypical carcinoid [12].

According to former knowledge, unlike mesothelioma, pseudomesotheliomatous cancer cannot be clearly connected to the exposition to asbestos or other environment carcinogens [1]. Newer researches show the connection between increased asbestos concentration in the lung tissue and the appearance of PMC [13].

The commonest histopathological diagnostic problem in pleural biopsy is the distinction between mesothelioma and adenocarcinoma, mostly pulmonary adenocarcinoma. There are different histological patterns of epitheloid mesothelioma, including acinar pattern, the commonest subtype of pulmonary adenocarcinoma.

Different histochemical and immunohistochemical methods were suggested to aid a differencial diagnosis of pleural mesothelial cancer and pleural metastasis of adenocarcinoma [1–7, 14–17].

The presence of neutral mucins, PAS-D, CEA, CK7 and thyroid transcription factor-1 (TTF-1) positive reaction with "carcinoma markers" and negative with "mesothelioma markers" (trombomodulin, calretinin, CK5/6 and HBME) lead to the diagnosis of adenocarcinoma.

Extremely positive reactions to Lu-M1 and B, 72.3, are useful in discerning pseudomesotheliomatous lung cancer and malignant mesothelioma of the pleura. Also, the results of some studies suggest that immunohistochemical demonstration of surfactant apoprotein inside tumor cells [18] invading the pleura, may be helpful to differentiate between metastasis of lung cancer and metastasis of extrapulmonary malignant tumors.

Despite similarities with type II and Clara cells, one cannot conclude that tumor derives from these cells. During the development of cancer, repressed genes can be activated, ones that regulate differentiation, which in the end leads to transformation of epithelial cells to highly specialized cells as type II and Clara cells are. Because of its significant desmoplastic reaction, the recommended approach is like to that in scar adenocarcinoma.

Despite its resemblance to invasive adenocarcinoma [World Health Organization (WHO) classification 2015] [19–21] and other peripheral lung cancers constituted of type II and Clara cells, pseudomesotheliomatous carcinoma has a distinctly different biological behaviour. Because of its extensive invasion of the pleura, patients have bad prognosis. Average period of survival is 8 months, similar to IV stadium non-small cell lung cancer (NSCLC) [8, 22] which is the consequence of tumor aggessiveness and characteristic localisation of tumor – near the rich lymphatic pleural network.

Conclusion

Pseudomesotheliomatous carcinoma of the lungs is a rare entity that can represent a diagnostic problem. The diagnosis of pseudomesotheliomatous lung cancer is based on recognition of the specific histopathological characteristics, that enable its discerning from epithelial variant of pleural malignant mesothelioma.

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CASE REPORT

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Apical root-end filling with tricalcium silicate-based cement in a patient with diabetes mellitus: A case report

Punjenje kanala korena cementom na bazi trikalcijum-silikata kod bolesnika sa dijabetesom melitusom

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Abstract

Introduction. The material used for root-end filling has to be biocompatible with adjacent periapical tissue and to stimulate its regenerative processes. Tricalcium silicate cement (TSC), as a new dental material, shows good sealing properties with dentin, high compression strengths and better marginal adaptation than commonly used root-end filling materials. Although optimal postoperative healing of periapical tissues is mainly influenced by characteristics of end-root material used, it could sometimes be affected by the influence of systemic diseases, such as diabetes mellitus (DM). **Case report.** We presented apical healing of the upper central incisor, retrofilled with TSC, in a diabetic patient (type 2 DM) with peripheral neuropathy. Standard root-end resection of upper central incisor was accompanied by retropreparation using ultrasonic retrotips to the depth of 3 mm and retrofilling with TSC. Postoperatively, the surgical wound healed uneventfully. However, the patient reported undefined dull pain in the operated area that could possibly be attributed to undiagnosed intraoral diabetic peripheral neuropathy, what was evaluated clinically. **Conclusion.** Although TSC presents a suitable material for apical root-end filling in the treatment of chronic periradicular lesions a possible presence of systemic diseases, like type 2 DM, has to be considered in the treatment outcome estimation.

Key words:
periapical diseases; oral surgical procedures; dental cements; silicates; diabetes mellitus, type 2; comorbidity; diabetic neuropathies.

Apstrakt

Uvod. Materijal koji se koristi za retrogradnu opturaciju kanala korena trebalo bi da bude biokompatibilan sa okolnim periapeksnim tkivom i da stimuliše procese njegove regeneracije. Trikalcijum silikatni cement (TSC), kao novi dentalni materijal, pokazuje dobro zaptivanje, visoku kompresivnu snagu i bolju ivičnu adaptaciju u odnosu na standardno korišćene materijale za retroopturaciju. Iako postoperativno zarastanje periapikalnog tkiva najviše zavisi od karakteristika materijala za retroopturaciju, ponekad na uspeh zarastanja može uticati i prisustvo neke sistemske bolesti kao što je dijabetes melitus (DM). **Prikaz bolesnika.** Prikazali smo apikalno zarastanje u predelu gornjeg centralnog sekutića, nakon retroopturacije sa TSC, kod bolesnika sa DM tipa 2 i prisutnom perifernom neuropatijom. Standardna resekcija korena gornjeg centralnog sekutića bila je urađena retropreparacijom ultrazvučnim nastavcima do dubine od 3 mm i retrogradnom opturacijom sa TSC. Zarastanje postoperativne regije bilo je u fiziološkim granicama. Bolesnik se, međutim, žalio na nedefinisan, tup bol u predelu operisane regije koji je verovatno bio povezan sa nedijagnostikovanom intraoralnom dijabetičnom perifernom neuropatijom, što je potvrđeno kliničkim nalazom. **Zaključak.** Iako TSC predstavlja pogodan materijal za retrogradnu opturaciju kanala korena zuba u lečenju hroničnih periradikularnih lezija, u proceni ishoda lečenja treba imati u vidu i moguće prisustvo perifernih manfestacija sistemskih bolesti kao što je DM tipa 2.

Ključne reči:
periapeksne bolesti; hirurgija, oralna, procedure; zub, cement; silikati; dijabetes melitus, insulin-nezavisni; komorbiditet; dijabetesne neuropatije.

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Introduction

The primary goal of periradicular surgery is to seal the apex of the root canal hermetically, preventing the passage of microorganisms or their products into adjacent periapical tissues. Traditionally, root-end filling is obtained by amalgam or different types of cement [1]. However, modern apical surgery is still seeking for the material with superb long-time mechanical properties and excellent apical obturation together with biostimulation of regenerative processes of apical tissues [2]. Beside the mentioned properties of the material used, it has to be biocompatible to the neighbouring periapical tissues [3]. On the other hand, there are still some possible complications of periradicular surgery related to disadvantages of materials used for root-end filling [4, 1].

Tricalcium silicate cement (TSC), as a new dental material, shows mechanical and safety profile which could improve the quality of apical obturation [5, 6]. It was also shown that TSC possesses good sealing properties with dentin and high compression strengths [7, 6]. TSC provides better marginal adaptation than commonly used root-end filling materials [8].

Although optimal postoperative healing of periapical tissues is mainly influenced by the characteristics of root-end material used, it could sometimes be affected by the peripheral appearance of systemic diseases [4]. Diabetes mellitus (DM) results in delayed wound healing and associated complications in dental treatments [9, 10]. It was already shown that DM decreased osteoblasts function in the rat model [11]. In addition, microvascular changes found in DM may decrease the reparatory processes of soft and hard tissue and, gradually, could lead to postoperative complications, such as diabetic neuropathy [12–15].

The aim of this report was to present apical healing of the upper central incisor, retrofilled with TSC, in a diabetic patient with possible peripheral neuropathy, as a complication associated with type 2 DM.

Case report

A 53-year-old man, suffering from type 2 DM, with peripheral neuropathy and cardiovascular complications (ASA III), was referred by his general dental practitioner to the Clinic of Oral Surgery, Faculty of Dental Medicine, University of Belgrade, for root apical surgery of the right central incisor. Clinical examination showed the presence of a fistula in the region of the root apex of the tooth. There were no signs and symptoms of acute dental infection, although the patient indicated unpleasant discomfort and unmarked chronic pain of the alveolar ridge on the right side. In addition, retroalveolar radiogram was done and short canal filling with well demarcated slight periapical radiolucency were seen around the root apex of tooth (Figure 1). It was decided to perform root-end surgery, implying resection of the root-end and retrofilling with tricalcium silicate cement (TSC) (Biodentine®, Septodont, Saint des Fausses, France) under high magnifying glass.

Standard root resection included sectioning the root-end with fissure bar for approximately 2 mm; retro-preparation was done using ultrasonic retro-tips, to the depth of 3 mm (Figure 2). TSC was placed in the 3 mm deep retrograde cavity of the root-end (Figure 3). After the setting time was finished, the wound was debrided and closed primarily.

One month after the operation, the patient complained to constant, undefined, dull pain and discomfort in the operated region, which lasted for the next 3 months. Clinical examination and control retroalveolar radiogram did not show any signs of pathological lesion (Figure 4). There was no fistula in the region of oral mucosa or attached gingiva. Also, there were no periodontal pockets around the tooth 11. Regarding that, it was necessary to distinguish the possible presence of vertical root fracture, which usually cannot be diagnosed radiographically. For that reason the re-entry was done. When a full-thickness trapezoid mucoperiostal flap was elevated, almost complete bone healing in the operated area was present (Figure 5). There were no signs of vertical root fracture. At the end, the operated area was copiously irrigated with saline and interrupted sutures were placed. Follow-up was done 3 and 6 months and 2 years after re-entry and there were no changes both clinically and radiographicaly. During these observation periods the mentioned disturbances at the operated region were recorded occasionally, with different intensity and usually lasted for several weeks.

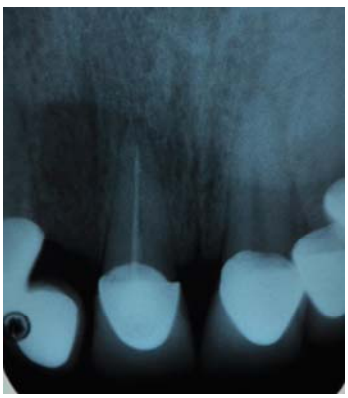

Fig. 1 – Incorrect root canal 11 filling and slight apical radiolucency.

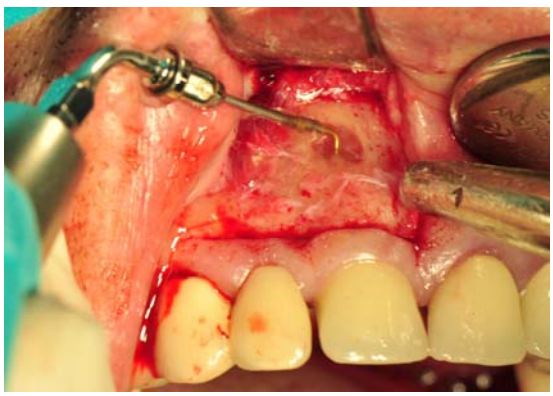

Fig. 2 – Retropreparation with ultrasonic retrotip.

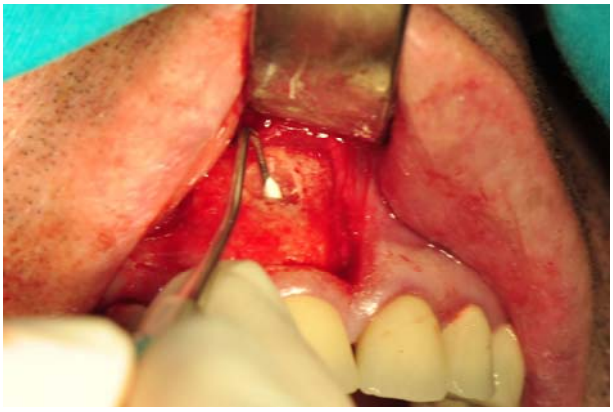

Fig. 3 – Root end filling with tricalcium silicate cement (TSC).

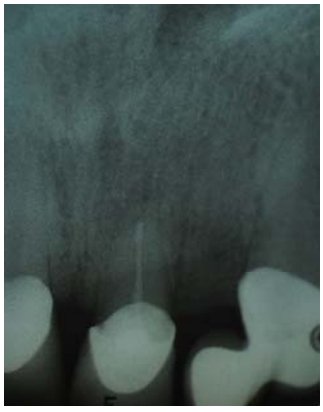

Fig. 4 – Control retroalveolar radiogram 3 months after the surgery.

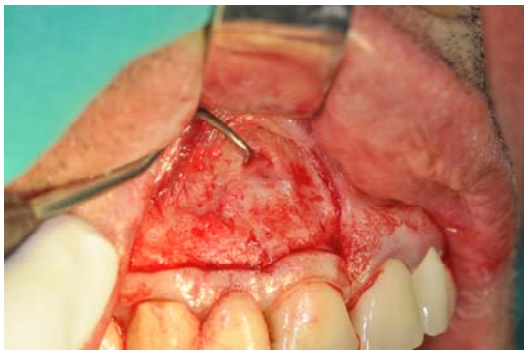

Fig. 5 – Complete bone healing after re-entry.

Discussion

Different materials have been used for filling root-ends. *In vitro* and *in vivo* studies have shown that mineral trioxide aggregate (MTA), as a gold standard, has considerable sealing ability and better marginal adaptation to dentin [16–18], compared to amalgam, super-EBA (ethoxy-benzoic acid) and IRM (intermediate restorative material) cement [19]. However, many drawbacks of MTA, such as difficulties with handling, long setting time and high cost, restricts its use as a root-end filling material. In addition, it was found that in higher concentrations, MTA was toxic to cementoblasts [20].

Tricalcium silicate-based cement – Biodentine®, was introduced as a bioactive material, with the idea of overcoming disadvantages of MTA. Its bioactivity was shown on pulp cells by stimulation biomineralisation [21]. Likewise, new calcium silicate-based cement induced odontoblast stimulation and the production of tertiary dentin in the rat pulp injury model [22]. Applied directly onto human pulp, TSC induced stimulation, biomineralization and odontoblast differentiation [23, 21]. It was also shown that TSC induced osteoblast differentiation in mesenchimal stem cells [24]. When used as root-end filling material, TSC showed the least microleakage compared to other cements [25]. TSC-based cement produced more prominent Ca^{2+} and Si^{2+} uptake into the root canal dentine than MTA when used as a root canal obturation material in bovine incisors [2]. Butt et al. [26] suggested that Biodentine possesses better sealing ability, higher compressive strength and better handling consistency than MTA. The most recent study of Bhavana et al. [27] revealed that TSC had higher antibacterial and antifungal activity than MTA.

Having in mind all the mentioned advantages and direct biological effect of TSC on bone healing, it was expected to have a successful surgical result after being used for retrofilling in the presented patient, showing clinical and radiographic evidence of complete healing. However, the patient reported undefined dull pain in the operated area a month after surgery, which lasted for next 3 months. Pain in the operated area could possibly be attributed to undiagnosed intraoral diabetic peripheral neuropathy, concerning the fact that the patient suffered from type 2 DM for more than 10 years. It was also documented that signs of intraoral peripheral neuropathy, such as the loss of intraoral sensation, hyperesthesia, dysesthesia and temporomandibular dysfunction could be related with clinically evident peripheral diabetic neuropathy [28]. The possible detrimental influence of diabetic neuropathy in progression of chronic orofacial pain could be also corroborated with unpleasant burning mouth syndrome and nonspecific soreness that affect intraoral structures [29]. Furthermore, it was proposed that prolonged effect of local anaesthetic solution could provoke pain in the operating area. Namely, it was already shown that the incidence of diabetic neuropathy increased after spinal and neuraxial block anesthesia [30, 31]. Dull pain that the patient described could possibly be explained by microangiopathy of peripheral dental nerves associated with DM and adjunct prolong ischemic effect of vasoconstrictors from local anaesthetic that was administered in the close proximity to peripheral nerves. Atypical facial pain

(AFP) could be considered in differential diagnosis, having in mind its chronic character. Idiopatic or AFP could be described as deep or superficial, poorly localised, and sometimes bilateral pain, predominantly in middle-aged and older women [32, 33]. On the other hand, dull character of pain localised only in the operated area, could possibly be attributed to peripheral diabetic neuropathy, distinguishing it clinically from AFP.

Conclusion

Tricalcium silicate cement presents a suitable material for apical root-end filling with good mechanical and biological properties. However, there are still little data concerning long-term results of using TSC as a root-end filling material in clinical trials, especially in the risk group of patients such as patients with diabetes mellitus, with changed peripheral healing capacity. Further long-term clinical studies are needed to precisely determine clinical and biological behaviour of TSC as a root-end filling material and to confirm the direct evidence of regeneration of periapical tissues in humans.

Acknowledgements

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CASE REPORT

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Case report of gross hematuria in the nutcracker syndrome resolved by renocaval reimplantation

Prikaz izlečenja bolesnika sa obilnom hematurijom kod sindroma *nutcracker* primenom renokavalne reimplantacije

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Abstract

Introduction. Nutcracker syndrome is defined as a set of signs and symptoms secondary to compression of the left renal vein (LRV) in the acute anatomic angle between the aorta and its superior mesenteric branch. **Case report.** A 38-year-old woman with asymptomatic and "idiopathic" gross hematuria came to the Clinic for Vascular and Endovascular Surgery, Clinical Center of Serbia in Belgrade. Hematuria was documented by cystoscopy and was found to be unilateral, located to the left urethral orifice. The contrast-enhanced multidetector computed tomography (MDCT) scan showed a stenotic LRV due to the extrinsic compression in the angle formed by the ventral aorta and superior mesenteric artery (MSA), with a jet of contrast through the lumen. Considering the negative investigations for more common causes of hematuria, its incapacitating nature, and above mentioned imaging findings suggestive of the nutcracker syndrome, an indication for the open surgical correction of the LRV entrapment was established. The patient underwent reimplantation of the LRV into the more distal inferior vena cava (IVC), to relocate it out of the constrictive aortomesenteric space. Intraoperative findings were notable for blood flow turbulence in the LRV and hypertrophy of its tributaries, which were ligated. We presented the first published case in the Serbian literature on nutcracker syndrome with hematuria resolved by renocaval reimplantation. **Conclusion.** This case report demonstrates that renocaval reimplantation, as the open surgery technique, could be the adequate method for resolving gross hematuria in patients with nutcracker syndrome.

Key words:
renal nutcracker syndrome; diagnosis; vascular surgical procedures; renal veins; replantation; treatment outcome; serbia.

Apstrakt

Uvod. *Nutcracker* sindrom se definiše kao skup znakova i simptoma nastalih usled kompresije leve renalne vene u oštrom uglu koji formiraju aorta i gornja mezenterična arterija. **Prikaz bolesnika.** Bolesnica, stara 38 godina, sa obilnom, asimptomatskom i idiopatskom hematurijom primljena je na lečenje u Kliniku za vaskularnu i endovaskularnu hirurgiju Kliničkog centra Srbije u Beogradu. Hematurija je potvrđena cistoskopijom. Nađeno je da se radi o unilateralnoj hematuriji lokalizovanoj na levom ureteralnom orificijumu. Multidetektorskom kompjuterizovanom tomografijom (MDCT) angiografijom utvrđena je stenoza leve renalne vene, kao posledica kompresije, u uglu koji formiraju gornja mezenterična arterija i ventralna strana aorte. Uzimajući u obzir negativne nalaze o uzrocima hematurije, njen onesposabljavajući karakter i prethodno pomenuti nalaz MSCT angiografije, postavljena je sumnja na *nutcracker* sindrom kao i odluka o operativnom lečenju. Kako bi se leva renalana vena dislocirala iz aortomezenteričnih klešta, učinjena je njena reimplantacija udaljenije na venu kavu. Intraoperativni nalaz potvrdio je turbulentan tok u levoj renalnoj veni, torturozitet i našikanost njenih povezanih pritoka. Ovo je prvi primer *nutcracker* sindroma u Srbiji koji je izlečen renokavalnom reimplanatcijom. **Zaključak.** Rad ukazuje na adekvatnu vrednost renokavalne reimplantacije, kao otvorene hirurške tehnike, u lečenju obilne hematurije kod bolesnika sa *nutcraker* sindromom.

Ključne reči:
nutcracker sindrom, renalni; dijagnoza; hirurgija, vaskularna, procedure; vv. renales; replantacija; lečenje, ishod; srbija.

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Introduction

The nutcracker syndrome is defined as a set of signs and symptoms secondary to compression of the left renal vein (LRV) in the acute anatomic angle between the aorta and its superior mesenteric branch. Extrinsic compression of the left kidney main venous drainage leads to local venous hypertension, with the development of collaterals, pelvic varicose, and gonadal tributary reflux, resulting in hematuria, left flank pain and symptoms of pelvic congestion [1–3].

We presented the first published case in the Serbian literature of the nutcracker syndrome with hematuria resolved by renocaval reimplantation.

Case Report

A 38-year-old women of two was referred to our clinic because of the chronic "idiopathic" gross hematuria, which occurred intermittently for the last ten years, but recently became persistent and demanded blood transfusions for the correction of anemia, prompting more extensive diagnostic investigation. Besides hematuria, the patient complained of no other symptoms, and her past medical, surgical and family history was noncontributory. Except for anemia (laboratory findings: Er 3.2 × 10^{12}/L; Hb 104 g/L; Ht 0,32l/L; MCV 80 fl; MCH26 pg; MCHC 300 g/L) and asthenic constitution, physical examination was unremarkable, with soft and no tender abdomen, without any signs of peripheral vascular lesions, and novulvar varicosities. The patient underwent repetitive urine cultures and excretory urography with negative results. Hematuria was documented by cystoscopy and was found to be unilateral, located to the left urethral orifice. Urine cytology was negative for malignancy. After we excluded urological, nephrology, gynecological and oncological reasons for hematuria, contrast-enhanced multidetector computed tomography (MDCT) revealed a hyperacute aortomesenteric angle with impingement of the left renal vein, findings that were corroborated by the contrast inferior cava venography with selective catheterization of the left renal vein. Dilated and tortuous adrenal and gonadal venous tributaries were also noted.

The contrast-enhanced MDCT scan showed a stenotic LRV due to the extrinsic compression in the angle formed by the ventral aorta and superior mesenteric artery (MSA), with a jet of contrast through the lumen (Figures 1 and 2). Color Doppler ultrasonography and selective retrograde left renal venography showed dilatation of the distal part of the LRV and gonadal vein with pelvic varicosities (Figure 3).

Considering the negative investigations for more common causes of hematuria, its incapacitating nature, and abovementioned imaging findings suggestive of the nutcracker syndrome, an indication for the open surgical correction of

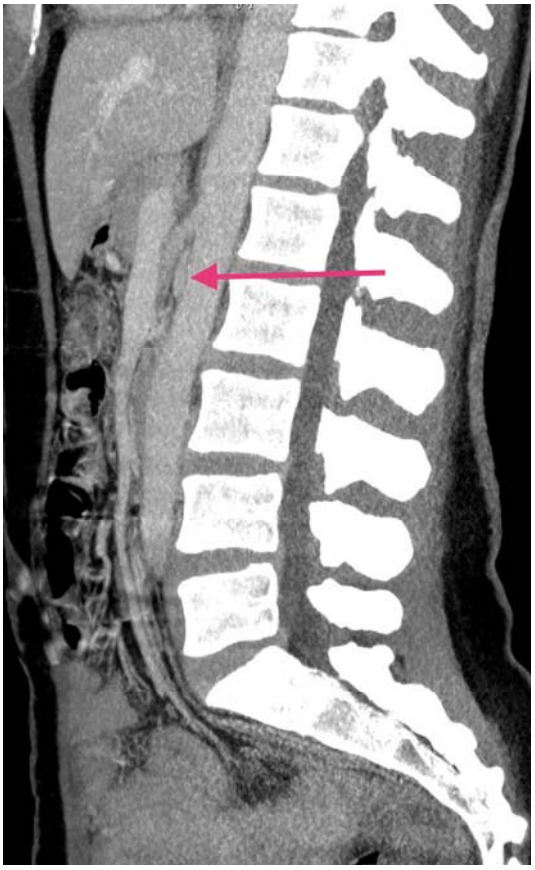

Fig. 1 – Compression of the left renal vein between the aorta and the superior mesenteric artery, multidetector computed tomography (MDCT) longitudinal section

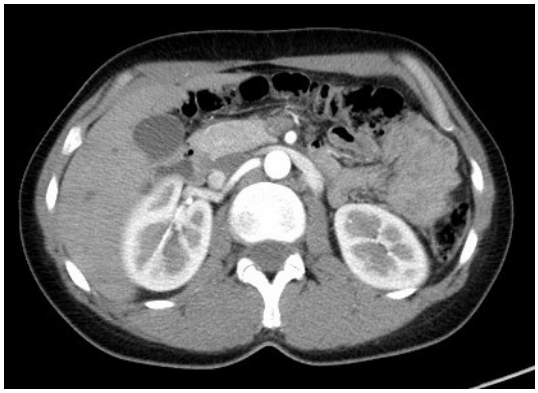

Fig. 2 – Compression of the left renal vein between the aorta and the superior mesenteric artery, multidetector computed tomography (MDCT) transversesection

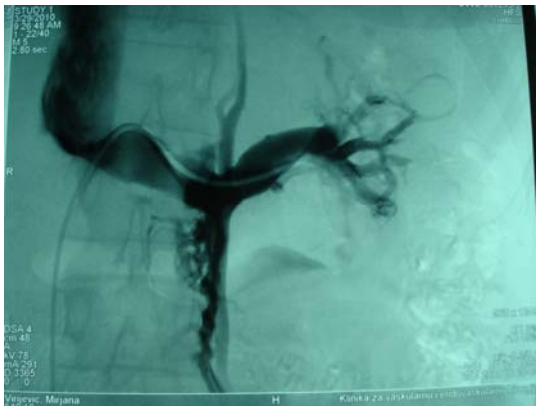

Fig. 3 – Selective retrograde left renal venography showing dilatation of the distal part of the left renal vein (LRV) with pelvic varicosities

the LRV entrapment was established. Under general anesthesia, using midline laparotomy approach, the patient underwent reimplanation of the LRV into the more distal inferior vena cava (IVC), to relocate it out of the constrictive aortomesenteric space. Intraoperative ultrasound findings were notable for blood flow turbulence in the LRV and hypertrophy of its tributaries, which were ligated.

Discussion

The true incidence of the symptomatic nutcracker syndrome in a general population is unknown. It seems that this syndrome is most common in women in the third and fourth decades of life [4]. In their pathologic study, Macmahon and Latorraca [5] elucidated an association between the nutcracker syndrome and hematuria.

According to the current international experience, there are several ways of solving this problem – by nephrectomy, nephropexy, renocaval reimplantation of the LRV or LRV stenting [4, 6–16]. We opted for the renocaval reimplantation as the most commonly used method, and because of our experience with open surgical procedures and lack of endovascular material at that moment. The LRV was excised from the entry site at the IVC and reanastomosed with the IVC 4 cm below the original anatomic site. There were no intraoperative or early postoperative complications (< 30 days). During the postoperative follow-up of one and a half year macrohematuria despaired. According to the previously published reports, in most similarly treated cases of the nutcracker syndrome hematuria resolved in up to 5 months of follow-up. Control duplex-sonography, CT venography, and conventional contrast venography confirmed correct hemodynamics and morphology of the reconstructed LRV. Laboratory findings restored to the normal range (Er 4.28×10^{12}/L; Hb 130 g/L; Ht 0.420 L/L; MCV 85 fl; MCH 31.1 pg; MCHC 330 g/L).

Conclusion

This case report demonstrates that renocaval reimplantation, as the open surgery technique, could be the adequate method for resolving gross hematuria in patients with nutcracker syndrome.

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Hippocrates – The Father of Modern Medicine

Hipokrat – otac moderne medicine

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Introduction

There is hardly any area of medicine, preventive or clinical, and personality psychology, basic or developmental, unmarked by Hippocrates. Modern ethic principles are also based on the guidelines left behind by this great physician more than two and a half millenniums ago. With his extensive work, he built the foundations of modern medicine and medical ethics. Hippocrates' era was the time of general enlightenment and individualism [1]. Critical analysis, being the imperative in those times, created a new prospective on the world based on empirical cognition. Hippocrates synthesized previous knowledge and his own observations creating his own biology-medical theory and a system of treatment (hippocratism). Hippocrates' medicine is clinical and individualistic. He became renown during his lifetime and his fame further grew to the status of a legend. For Plato, he represents a prototype of a physician in the same way Phidias is a prototype of a sculptor. Aristotle calls him the "great" and Galen the "divine". He remains, to this very day, known as "the Father of Medicine" [1, 2].

Biographical information

The Father of Modern Medicine was born on the island of Cos in the family of the Asclepiads, physicians and priests, followers of the god Asclepius. He learned medicine in his family which was a common practice in those times. His ancestor Apollonides was a personal physician of the Persian king Artaxerxes. His other ancestor Nebrus, born around 580 BC, had two sons Gnosidicus and Krizos. According to the available resources, name Hippocrates appears for the first time around 500 BC with the birth of Krizos' son. He was known as Hippocrates I and he was the grandfather of Hippocrates II, the founder of modern medicine. The sons of Hippocrates II, Thessalus and Draco, were physicians too, as well as his son-in-law Polybus. This tradition continued for generations to come. The physicians of the island Cos created an agreeable professional Asclepian community where they shared their knowledge and skills with each other and kept that knowledge within their Cosian community. Even his first biographer Soranus of Ephesus, renown obstetrician, pediatrician and writer who lived during the second century BC, belonged to the 20th generation of Cosian Asclepiad. Besides the learned knowledge which was based on observations of a sick organism as a whole, he strived to determine the principles of pathological processes in order to determine the diagnosis. He further expended his knowledge by travelling as a traveling physician, periodeut. His biographers have determined that he was practicing in Egypt, Libya, Asia Minor, but primarily in his home country Hellas, especially Athens. At the time, Athens was at the peak of its political power and cultural creativity (the golden age of Pericles) during which Plato, Phidias, Aeschylus, Euripides, Sophocles, Aristophanes and Socrates were also active. There are written records which indicate that he was travelling around Abdera, Thasos, Thrace, and Larissa in Thessaly where he died in 377 BC [3–6].

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There are little descriptions of his appearance. According to Aristotle, he was small in height, and depicted on coins as a bald man with preserved ring of hair, thin beard and sizable nose. It is believed that the statue dated from the fourth century, discovered on Cos, and the bust with encryption of Markios Demetrios, discovered in Ostia, represents Hippocrates.

According to the legend, he was buried near Larissa and, until the second century, his gravesite was visited by many. The grave was inhabited by bees whose honey had healing properties. There is a spring and an ancient plane tree on Cos. According to the legend, it was under this tree where Hippocrates used to lecture his students and followers [5, 7].

Hippocrates' work

Collective medical knowledge of his times, Hippocrates encyclopedically documented in the first ever written textbook of western medicine titled "Corpus Hippocraticum" (Hippocratic Corpus). This work consists of 72 books divided in 53 chapters. It is believed that the first 28 books contain his original work and that the remaining books were added by other authors. Approximately 100 years later, in the 3rd century BC, these works were entrusted to the Alexandrian library by the Egyptian diadochi (heirs of Alexander the Great). The oldest preserved transcripts of these works, dated from the 10th and 11th century, are located in the libraries in Paris, Vienna, Vatican [5]. Additions to the Hippocratic Corpus, contributed by his students, still cause controversies. Many scholars analyzed the style and contents of the collection. They determined that the collection is heterogeneous, and thus not written by one person since styles and opinions vary. The majority of those authors are from the Asclepiad of Cos. Their views are: the wholeness of the organism, the importance of the diagnosis, and introduction of general roborant therapy. Certain parts are marked by the opposing opinions of the Cnidian school which emphasizes the importance of locating the illness and local therapy. Parts of the collection are focused on the Sicilian school which stresses the importance of pneuma in the etiology of illness. Even though the entire collection was written using the Ionic dialect of Greek language, there are minor differences within the collection. Differences are present even in regard to the audience of the writings: scientific circles and colleagues, students and broader audience. There are parts which served as personal notes to various authors of the collection [1, 3, 5].

Hippocrates' beliefs

There are only fractions of the written records about Hippocrates' beliefs which originate in the pre-Alexandrian period. According to one such record, contained in Plato's "Phaedrus", Hippocrates believes that the temper (nature) of the body and the temper of the soul cannot be understood without understanding the temper of the wholeness. Everything needs to be regarded as a part of a unique cosmos which renders his approach holistic and which is documented with the following thoughts:

"Everything is divine and everything is human. Everything is one and one is everything. Everything is similar and everything is different." He believes that the same principles govern organism and cosmos. "Nothing vanishes entirely and nothing is being created that hasn't existed before." He talks of life which is everywhere and if there is life then death is impossible, unless that contradicts the wholeness of things. "To be born and to die is the same, to mix and to separate is the same, to grow and to decline is the same" [8].

In Hippocrates's times it was implied that nothing takes place without divine intervention. That is exemplified with his words: "I also believe that those illnesses are divine in nature, as all others are, none of them being more divine or human in nature than the other, but that all are similar and come from God. Each one having unique nature and none appear without natural cause [8]."

Maturing as a physician, Hippocrates managed to "separate wheat from the chaff" and not to look for the causes of illness on a whim or in capriciousness of gods but strictly in natural causes. In such way, he separated medicine from magic and occultism and established scientific foundations what is his greatest achievement [1]. Denouncement of the divine is exemplified in the following words:

"...wealthy, with their significant wealth, offer gods great sacrifices and many sworn gifts, showing thereby their respect, which poor due to poverty do to the lesser extent. Aside from that the poor curse them for not giving them wealth, so the punishment for such sins should be expiated by the poor rather than the wealthy [8]".

Hippocrates' school was founded on the principles of Empedocles and Pythagoras [1]. Teachings of Empedocles are concerned with four elements (water, fire, earth, air) and four body qualities (cold, hot, dry, wet) [9]. He believes that the organism naturally strives to establish balance and to heal. Pythagoras principal of illness is based on imbalance of the harmony of constituting parts of organism. According to Hippocrates, body consists of four essential fluids ("humors"): blood – *sanguis*, phlegm – *flegma*, yellow bile – *hole*, black bile – *melaina*. One of these fluids dominates in the organism and thereby determines the constitution of the organism. Health is the result of harmony of these fluids and illness is the sign of occurring imbalance. This is the so-called humoral approach or humoral medicine [6]. A parallel with the modern approach, in terms of imbalance of the organism's homeostasis which causes the illness, can be recognized.

Based on this belief, he developed the first known classification of kinds and types of temperaments. Depending on the kind of the fluid that dominates in the organism, there are four types of temperament. In choleric type yellow bile is predominant. Their feelings are strong and expressed easily. They confront others readily. In sanguine type blood is predominant, and they are generally cheerful and optimistic. When black bile is predominant, the temperament is melancholic, and the person is gloomy and introverted. Phlegm is predominant in phlegmatic type, and individuals are optimistic and cheerful, but have suppressed emotions and rarely react emotionally [10, 11].

Hippocrates' medicine in aphorisms and on airs, waters and places

Physicians of that era did not dissect humans, but did so with animals. This was the only way to become acquainted with anatomy. Treating the wounded provided opportunity to

learn about the human body. Descriptions of skeleton and muscular system, heart, liver and spleen are good, but descriptions of bloodstream, nerves, brain and internal reproductive organs are confusing. Bone and muscle surgery was practiced, but internal organ surgery was not performed [4]. It was believed they were not important because gases and fluids were the cause of internal illnesses [7]. The knowledge of anatomy and pathology were limited, as expected of the times in which he lived [1]. As mentioned above, pathology was based on the humors principles, *ie* in the harmony of the four body fluids ("humors"). Beside these fluids, there were solid components and those were bones, membranes, blood vessel walls and flesh.

Hippocrates' teachings were dialectic and in accordance with Heraclitus' * views: This world is an ever-living fire, in measures being kindled and in measures going out*.

His dialectic is evident in his thoughts that the nature is a matter which is continually moving and constantly mixing. It is without beginning and end and it strives to endless and perfect eurythmic harmony. This natural flow encompasses the health of every organism and it is based on the unison of the opposites.

Functioning of living beings is based on nature (*physis*) and life force (*dinamis*). "Vital heat" which lies in the heart supports life. This warmth is created by the supply of air and blood. It allows transformation of food to body fluids and organ tissue. The center of thoughts, emotions and desires is in the brain, feelings and movement commands are carried out *via* pneuma.

It can be concluded that "*physis*" and "*dinamis*" represent wholeness and that human nature belongs to nature's wholeness. Body and soul are closely related and the sickness of one dictates the sickness of the other. Causes of illnesses are different and they can be internal or external, and they cause the imbalance. Disease is a biological occurrence and it proceeds according to the laws of nature, and opposite to the laws of harmony. Disease is essentially *dyscrasia*, the imbalance of body fluids [1]. Hippocrates believes that diseases are caused by atmospheric influences, improper nutrition, poisoning, prevented elimination of body excrements, mental excitement and hereditary factors. When *dyscrasia* occurs, the organism tends to establish appropriate balance and in order for that to happen the nature uses a unique process of cooking which transforms unhealthy matter into harmless state and excretes it from the organism. Cooking is manifested as fever of inflammation. Excretion is performed on specific days of the illness and if any harmful parts are retained they cause chronic disease, local build-up and periodic disease re-occurrence [1, 12, 13]. Analogy with modern understandings is obvious. Physician's craft is based in assisting nature:

Natura sanat, medicus curat – Physician treats, nature heals.

Quo natura vergit, eo ducendum – Where nature intends, there where it will act [8].

Basic principals are exemplified in the following versus:

Ne quid nimis – Nothing in excess.

Primum non nocere – Firstly, do no harm [8].

Contraria contrariis curantur – Opposite is treated with opposite [9].

*Heraclitus (535–475 BC), Greek pre-Socrates philosopher.

"For the most difficult disease prompt and conscious treatment is the best" [8].

Clinical practice

Hippocrates was the first to develop a plan of examination, determination of the diagnosis, prediction of disease progress and outcome, and treatment. It is all being performed at the bedside (*kline* – Greek language) [6]. xamination begins with gathering of information about the patient and the illness *anamnesis*. The approach to the patient was especially important:

"Physicians must readily perform not only what the profession dictates but to care about the approach to the patient, assisting the patient and so forth" [8].

Physical examination

The examination continues with bedside examination and determination of "*status preasens*" – current condition. It is necessary to "rely on facts and conclude wisely". He emphasized that information are gathered using eyes (*visitatio*), ears (*auscultatio*), nose (*olfato*), taste (*degustatio*), hand (*palpatio*) and other means available: observing, touching, smelling and tasting. Inspection, palpitation, succession, percussion and auscultation are the basis of every physical examination even today [4]. He determines body temperature by placing his hand on the patient's chest. One of his well-known descriptions is still in practice, and that is "*facies* Hippocratica" description of the facial expression near the collapse and death:

"Pointy nose, indented eyes, sunken temples, ears cold and shriveled, firm, stretched and dry skin on the forehead, skin on the face yellow or dark, blue or lead-like" [7].

Hippocrates was familiar with pulmonary murmurs, pleural friction and sounds produced by succession *ie succussio Hippocratis*. That is how he determined the spot for incision in order to remove the empyema. Palpation was used to determine the size and consistency of the spleen which was important since malaria was very common [4, 7].

Excrement examination

Beside patient examination, attention was also given to excrement examination. In this way, he was able to diagnose albuminuria:

"Thick, oily foam on the surface of urine indicates sever kidney disease" [8].

He experimented with the behavior of saliva in salt water and what it smells like when thrown on the hot coal. In the modern sense of the term diagnosis, symptom analysis was not aimed at determining the disease diagnosis. He treated the patient, not the disease. The disease prognosis was equally important for each case individually [7].

Disease prognosis

Some examples of disease diagnosis and prognosis can be found in the Canon of medicine in the 5th chapter, aphorism 61, 37, 31, 13, and in the 5th chapter of Aphorisms, aphorisms 8, 2:

"If a woman is not menstruating and in the meantime there are no tremors or fever, but she is nauseous, you can hope she is pregnant" [8].

"When an expecting woman's breasts suddenly weaken, she will miscarry" [8].

"When an expecting woman bleeds, she is miscarrying, the older the embryo, the greater the risk" [8].

"When a sick person expectorates foamy blood, that blood originates in the lungs" [8].

"If the chest of individuals suffering from pleuritis are not cleansed by expectoration, within fourteen days, abscess is being formed" [5].

"Spasms which occur after an injury indicate a fatal outcome" [5].

He provided remarkable descriptions of an abnormal respiration pattern known today as Cheyne-Stokes respiration, and nail clubbing associated with chronic heart and lungs diseases – *digiti Hippocratici*.

Most often mentioned are fevers due to pneumonia, pleuritis, malaria, typhus, tuberculosis, puerperal sepsis. There are good descriptions of ileus, tetanus, apoplexy, joints diseases, sciatica, epilepsy, cystitis, kidney disease [1].

The biological nature of illness determines the therapy, *ie* "nature is the healer". His methods of treatment were based on natural processes and in accordance with the aforementioned principle "*Primum non nocere*" [7].

Conservative therapy

Conservative therapy is primarily based on hygienic-dietary methods and traditional symptomatic treatments using ointments and herbs, mixtures and dressings and suppositories [1]. Mixtures are herbal and they can be infusers, decoctions and powders [7].

Healthy lifestyle based on moderation is often advocated:

"When limits are exceeded, both sleep and insomnia are a bad sign" [5].

"It is not good to overindulge, nor to starve, nor anything else that is unnatural" [8].

"It is better to consume smaller and more tasteful amounts of food and fluids than bigger and tasteless [8]."

He respected moderation even in physician's involvement in the treatment of the illness and was therefore criticized by the Roman physicians four centuries later. They regarded his therapy as a mere observation of death since it was not an active participation. Hippocrates relied on moderation, gradualness and gradation in his therapeutic involvement in the following manner:

"Excessive purging or indulging in food, sudden temperature change, as well as any other change in the body is dangerous, as is any other excessiveness. It is wiser to gradually move from one state to the other" [5].

Cleansing and bloodletting

Often used therapy was cleansing which was believed to have the ability to restore the balance in the organism:

"During purging remove everything that organism will benefit from theirs excretion, and retain the matter with opposite properties" [8].

"If that what is supposed to be eliminated was removed properly, it will be beneficial to the patient and easy for patient to cope with. If that is not performed, the effect is opposite" [8].

Bloodletting was also a common treatment:

"Bloodletting alleviates the difficulties associated with urination, internal vein should be let" [8].

"Individuals who benefit from bloodletting and purging, should cleanse and let blood in the spring" [8].

Implementation of warming and cooling

Another method of treatment was the use of water to cool and warm up[†]:

"Inflammation and painful joints, without wounds, whether caused by gout or sprain, in most cases are relieved with copious amounts of cold water which reduces the inflammation and alleviates the pain" [8].

"Aromatic bath induces menstruation, and it would be even more beneficial if it was not causing headache, dizziness" [8].

"Individuals with vision difficulties should consume copious amounts of wine, wash their eyes with plenty of warm water and let blood" [5].

"Temperature which does not originate in the liver should be reduced using warm dressings" [5].

However, even in this form of therapy he does not deviate from moderation and attention to harmful effects:

"Cold is harmful to bones, teeth, nerves, brain, spine, while heat is beneficial" [8].

"Often and plentiful use of heat produces following negative effects: flabby skin, deterioration of nerves, dizziness, hemorrhaging, unconsciousness, and all these can cause death" [5].

The use of wine

Wine holds a special place in Hippocrates's medicine and therapy. It had healing properties, *ie* it was considered a medicine, not food or beverage:

"Restlessness, yawning and fear disappear when one drinks natural wine mixed with equal amount of water" [8].

"Stranguria and dysuria are treated with copious amounts of wine and bloodletting, and it is the veins of the inner side of the arm that should be let" [5].

Surgery

Surgery was already significantly developed and routinely performed procedures were: skull trepanning (*trepanation*), amputation of gangrenous extremities, *paracentesis thoracis* for empyema and draining of kidney and liver abscess.

Especially efficient was the surgery of external illnesses and injuries (fractures and dislocations). Reposition of dislocated shoulder, hip and jaws are still carried out in the same

[†]Today still we cool recent injury and warm chronic injury.

manner. In case of fractures extension and immobilization were employed.

Hemorrhoids and polyps were successfully operated, while in case of more advanced tumors they were not able to stop bleeding.

They insisted on good hygiene and some of the bandages were very similar to the modern, thus one of the most commonly used nowadays is called *mitra Hippocratis*.

Significant attention was given to psychotherapy where they employed knowledge from Asclepiad temples. The therapy algorithm is summarized in the aphorism:

"Illnesses untreatable with medications are treated with surgery. The ones untreatable by surgery are treated with burning. The ones untreatable with burning are considered incurable" [5].

His faith in the power of the nature was without reservation and it is summarized in the following words:

"*Natura sanat, medicus curat"* – Nature heals, physician treats‡ [1, 5, 7, 13].

Medical geography: quotes from descriptions of *De aere, aquis et locis* – On airs, water and places

It represents the earliest example of medical geography, it covers two topics. One of them is the influence of environment on medical conditions, and the other deals with the influence of environment on anthropological and ethnic characteristics of the people of Europe and Asia. The first part covers the importance of physician's knowledge of local climate factors, quality of water, air, food, and winds required for successful treatment:

"One who strives to truly investigate science, is required to proceed in the following manner: Firstly, attention should be given to effects seasons can produce… Then there is the question of warm and cold winds, especially the ones present everywhere, as well as those present only in certain parts. Aside from that, one should think about the properties of water… One is required to pay attention to whether the ground is bare and dry, or covered with trees and damp… Attention should be given to which way of life is pleasant to the natives, whether they are drunks, overeaters and idle, or are they devoted to exercises, hard work and small amounts of alcohol" [8].

Ars medica versus scientia medica

In Hippocrates' time there were no technique, technology, laboratory and other auxiliary diagnostic servi ces, yet the treatment could be successful. Even the skill of curing was developed as an art. The question is – in spite or because of it? One could get the impression that the computerized diagnostics and already prepared algorithms of the differential diagnosis blur the essence. Sometimes the problem in today's organization of health services is that medical specialists and subspecialists cure or only exclude the diseases of their domain. The general practice is overloaded, tertiary services do their "exclusion" work and thus report "which diseases are not present" is not rare. Medical consideration about patient and his individuality is somewhat lacking. Are these controversies inevitable price of civilization and technical-technological advancement, as for example the alienation? Or is it possible to join *scientia medica* with *ars medica*? The answer lies in the return to the essence of the relationship between the doctor and a patient. The postulates of Hippocrates could be successfully implemented into modern scientific achievements and for the common good of man and mankind in general. This is the only way to avoid conversion of modern technologically strong medicine into frozen (inhuman) relationship between the doctor and a patient. Man is not just a live organic machine, he has something sublime and majestic – emotions and soul, he has personality.

Conclusion

There is almost no area of medicine, psychology and ethics on which Hippocrates did not leave his trace. He laid the foundations of what we now call the individualistic and holistic medicine. Around the world, medical students take the Hippocratic oath upon graduation.

Corpus Hippocraticum contains principles that are becoming more actual with the development of holistic medicine, the individual patient approach and medical geography. The greatest contribution of Hippocrates to medicine is separation from demonic, magical and religious medicine. He is the founder of anamnesis and physical examination, which are the basis of modern propedeutics. Today's clinicians use the procedures, concepts and terms introduced by Hippocrates. Psychology of personality today also studies the temperament based on the division male by Hippocrates'. One might say that the ethics of modern human society is based on the Hippocratic Oath. Although the works of Hippocrates originate two and a half millennia ago, they are still relevant because human remained the same.

The pace of modern life and functions of society lead to general neglect of the human wholeness. If we would adhere to the basic principles of Hippocrates we inherited, the world would be a much nicer and safer place to live in.

‡It is still considered that medicine assists nature when we are young and fights it in the older age.

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OBAVEŠTENJE

U drugoj štampanoj knjizi Udžbenika interne medicine (SESIL), u poglavljima 514 – Bolesti usta i pljuvačnih žlezda i 518 – Karcinom glave i vrata, došlo je do greške u navođenju imena prevodioca. Umesto imena Božidara Jakovljevića koje je navedeno, treba da piše ime prof. dr Milovana Dimitrijevića.

Suizdavač, Medija centar „Odbrana“, izvinjava se zbog nastale greške.

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

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Tabele

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