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The winners of the 2020 Nobel Prize in Physiology or Medicine are (from left to right): Harvey J. Alter (born 1935), Michael Houghton (born 1949) and Charles M. Rice (born 1952). They were awarded for the discovery of the hepatitis C virus, which was crucial in the fight against blood-borne hepatitis, a major global health problem that causes cirrhosis and liver cancer in people around the world.

Dobitnici ovogodišnje Nobelove nagrade za medicinu su (sleva nadesno): Harvi J. Alter (rođen 1935), Majkl Houton (rođen 1949) i Čarls M. Rajs (rođen 1952). Oni su nagrađeni za otkriće virusa hepatitisa C, što je bilo od presudne važnosti za borbu protiv hepatitisa koji se prenosi krvlju, velikog globalnog zdravstvenog problema koji izaziva cirozu i rak jetre kod ljudi širom sveta.

ORIGINAL ARTICLES

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Chronic rhinitis in glassblowers

Hronični rinitis kod stakloduvača

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Abstract

Background/Aim. Glassworkers, especially glassblowers are in close contact with a variety of chemical and physical harmful agents at their workplace. Upper aerodigestive pathway is predominantly vulnerable to these agents. Breathing of warm volatile substances and dust, and mouth touch with glassblower's pipe are the main ways for chronic respiratory mucosa inflammation. The aim of this study was to estimate effect of workplace environment in a glass manufacturer plant, as a causative factor, on the prevalence of chronic rhinitis in glassblowers. **Methods.** Studied groups, one hundred glassblowers and 100 nonglassblowers in a same factory, were examined for diagnosis of chronic rhinitis. **Results.** This investigation confirmed that chronic rhinitis prevalence among glassblowers was significantly higher than that in non-glassblowers. The duration of exposure to harmful factors was not a significant factor for chronic rhinitis development. **Conclusion.** On their workplace, glassblowers are exposed to greater influence of noxious factors, and they have statistically greater risk for getting chronic rhinitis than nonglassblowers who work in the same work environment. Glass production by glassblowing is highly significant risk factor for getting chronic rhinitis, but the exposure period is not.

Key words:
glass; occupational exposure; prevalence; rhinitis; workplace; risk assessment.

Apstrakt

Uvod/Cilj. Stakloduvači su na radnom mestu izloženi različitim fizičkim i hemijskim štetnim agensima. Sluznica gornjeg aerodigestivnog trakta (nosa, usne šupljine, ždrela i larinksa) je naročito izložena ovim faktorima. Udisanje toplog vazduha, gasova, čestica prašine i oralni kontakt sa stakloduvačkom lulom su najvažniji faktori koji mogu uzrokovati hronično zapaljenje sluznice gornjeg respiratornog trakta. Cilj ove studije je bio da ispita da li je i u kojoj meri radna okolina u fabrici stakla uzročni faktor za visoku prevalenciju hroničnog rinitisa kod stakloduvača. **Metode.** Eksperimentalna grupa se sastojala od 100 slučajno odabranih stakloduvača muškog pola, dok je kontrolnu grupu činilo 100 muškaraca, zaposlenih u istom pogonu za proizvodnju stakla, koji nisu bili stakloduvači. **Rezultati.** Ovo istraživanje je potvrdilo da je prevalencija hroničnog rinitisa kod stakloduvača bila značajno veća nego kod radnika kontrolne grupe. Dužina ekspozicije štetnim faktorima nije bila značajan faktor u nastanku hroničnog rinitisa. **Zaključak.** Stakloduvači su na radnom mestu izloženi većem uticaju štetnih faktora i imaju značajno viši rizik od dobijanja hroničnog rinitisa od radnika drugih zanimanja u istom radnom okruženju. Proizvodnja stakla je visokorizičan faktor za dobijanje hroničnog rinitisa, ali period ekspozicije štetnim agensima nije.

Ključne reči:
staklo; profesionalna izloženost; prevalenca; rinitis; radno mesto; rizik, procena.

Introduction

Glass production is an essential aspect of the economy, especially due to wide use of different glass types in human everyday life. Glassblowing is one of the main ways of glass manufacturing. Glassblower's employment is very difficult and associated with diverse serious health threats. Severe infrared emission from glass furnaces, warm gases, evaporations and dust and glassblower's pipe are the main forms of exposure to harmful agents in glassblowers.

Chronic rhinitis is nonspecific inflammation of the nasal mucosa in duration of more than 12 weeks. According to the histopathological changes of the mucosal layer, chronic rhinitis can be divided into hypertrophic and atrophic and based on main causative factors, chronic rhinitis can be divided into allergic, infective and nonallergic noninfective rhinitis[1]. Occupational rhinitis ("work-related rhinitis") could be defined as chronic inflammation of the nasal mucosa, characterized by intermittent or persistent nasal congestion, sneezing, rhinorrhea, itching, and/or hypersecretion, which

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are consequences attributable to a workplace setting, but not to factors outside the workplace [1, 2]. This form of rhinitis may be allergic, consequent to exposure to a sensitizing factors through an immunological mechanism, and nonallergic, mediated by nonimmunological mechanism [1]. The most severe form of occupational rhinitis is corrosive rhinitis, which is characterized by permanent inflammation of the nasal mucosa sometimes associated with ulceration and perforation of the nasal septum [1].

Yoruk et al. [3] have found that denim sandblasters exposed to crystalline silica had considerable upper airway complaints in addition to pulmonary ones. The findings on the upper airway of the patients were: higher rate of rhinitis and adenoid vegetation, increased pH value in the nasal secretions and increased time of mucociliary clearance.

Irritation and inflammatory responses, epithelial changes, nasal host defense effects, systemic immune response, and nasal airflow resistance changes are sinonasal responses to various inhaled chemicals. Earliest physiologic response mediated by trigeminal nerve are irritative effects, which include a nasal and eyes burning sensation, nasal congestion, sneezing, headaches, cough, and reflex apnea. The initial nonspecific nasal inflammatory responses on inhaled pollutants are dependent on irritation response via the mechanism of neurogenic inflammation [4], and later through cytotoxic damage of mucosa, which cause recruitment of inflammatory cells. Impaired mucociliary clearance due to exposure to harmful chemicals in air could result in retention of secretions and consequent infection. Immunotoxic effect to nasal mucosa exerted by many airborne chemicals and compromised phagocytic and killing ability could lead to impaired host resistance and clinical infection [5]. Epithelial changes are result of increased epithelial permeability and consequent hyperresponsiveness to inhaled stimuli. Chronic decrease in nasal mucus flow caused by constant or repeated exposures to various air pollutants has been concerned as an etiologic factor in chronic rhinitis [6].

Moreover, intensive infrared radiation and high air temperature from glass furnaces and low humidity cause irritation of nasal mucosa. These factors lead to significant increase of nasal glands secretion and vasodilatation via trigeminal reflex. Nasal mucosa becomes wet, edematous and hyperemic, that is initial stage of chronic rhinitis. Longer exposure leads to hypertrophy and finally to atrophy of nasal glands, decreasing of their secretion and blood perfusion, and dryness of nasal mucosa. Final point is generalized atrophy of whole nasal mucosa. Nasal mucosa becomes pale, dry, atrophic, while mucociliary defense considerably decreases [4–6].

A diversity of chemicals like metal oxides (aluminum, antimony, arsenic, cadmium, chromium copper, manganese, and nickel), silica, sulfur dioxide, acrolein and asbestos have important role for melting and coloring of glass. Fumes and dust that include these substances have irritant and noxious influence to upper respiratory tract, particularly to the nasal mucosa [7].

Inhalation of fumes, gases and dust and primarily blowing glassworker's pipe are essential forms of contact to harmful influences in glassblowers. Red-hot glass in furnaces and on the end of glassblower's pipe is on temperature of 1,100°C. Therefore, high temperature and different volatile substances and fumes arise from molten glass to the glassblower's mouth and other parts of upper aerodigestive pathways via blow-pipe.

In four German glass factories, Raithel et al.[8] have found significant higher air concentration of nickel. Concentration of this metal was significantly higher in glassblower's urine than in an unexposed control group, too. Correlation of nickel compounds with upper respiratory malignancies is well known (IARC, 2018)[9].

Occupational exposition to hexavalent chromium compounds is confirmed to be causative factor for paranasal sinuses, laryngeal and lung cancer, which prevalence is 15–20 times higher than in unexposed population [10].

Szmeja et al. [11] reported high incidence of the chronic inflammation of upper respiratory pathways in workers employed in glass industry. They claimed that this was probably related to silica dust exposition.

The aims of this study were to determine the prevalence of chronic rhinitis in glassblowers and nonglassblowers, to check whether or not glassblowers have significantly higher prevalence of chronic rhinitis than the control group, as well as to establish which etiologic factors have most significant influence on prevalence of chronic rhinitis in glassblowers.

Methods

The investigation was conducted in the Serbian Glass Factory, Paraćin, Serbia. One hunderd randomly selected male glassblowers made the experimental (exposed) group, while the control group was made of 100 male nonglassblowers workers from the same factory, which worked near glassblowers. All procedures were conducted in accordance with the Helsinki Declaration. All participants provided written informed consent for participating in this analytical cross sectional study.

For this study specific questionnaire was prepared, with participant's general data (age, workplace, years of employment), hazardous life-style behavior and anamnesis of earlier illness, injuries, surgery of upper aerodigestive tract and nasal related symptoms.

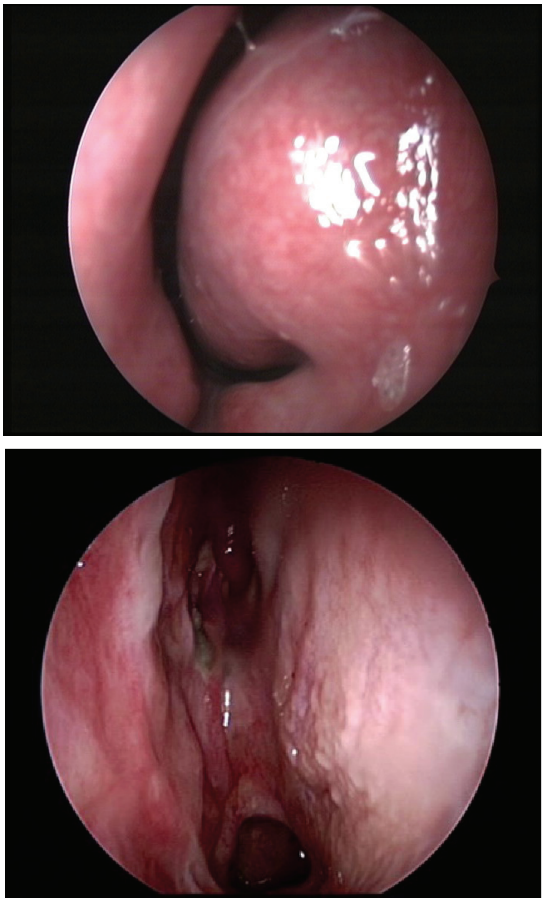
In view of smoking practice, participants were divided in the groups of current smokers and non-smokers (never smoked). In the smokers group, number of cigarettes per day was noted.

Regarding alcohol abuse, three groups according to the daily intake of alcohol were created: up to one beverage per day, drinking one to two beverages a day and serious drinkers – more than two drinks a day, based on guidelines of the National Institute on Alcohol Abuse and Alcoholism [12].

Diagnostic criteria

Only workers with clinically confirmed nonallergic, nonpolypoid and noninfectious inflammation of the nasal mucosa in duration for more than 12 weeks were considered

for this study. Main symptoms of chronic rhinitis were nasal congestion, rhinorrhea, sneezing and itching in the nose. A routine ear, nose, throat examination including anterior and posterior rhinoscopy and nasal endoscopy was performed in all participants. Endoscopical signs of nasal chronic inflammation were long lasting edema, mucosal hyperrhemia and hypertrophy, viscous nasal secretions (Figure 1A), or, rarely, atrophy and dryness of the nasal mucosa (Figure 1B), particularly in the region of the inferior turbinates. Negative X-rays of paranasal sinuses and absence of nasal polyps by endoscopy were made for differentiation from chronic rhinosinusitis with nasal polyps.



A)

B)

Fig. 1 – Endoscopic view on nasal cavity of a patient with: A) hypertrophic, and B) atrophic form of occupational rhinitis.

The diagnosis of nonallergic noninfectious rhinitis was based on exclusion criteria, i.e. the absence of clinical signs of infection and sensitization to inhalant allergens, demonstrated by skin-prick test (SPT) results or serological analysis for immunoglobulin E (IgE) [13–15].

Subjects with perennial allergic rhinitis, infectious rhinitis, non-allergic rhinitis with eosinophilia syndrome (NARES), medicamentous rhinitis, hormonal rhinitis, etc. were excluded using appropriate diagnostic methods, according to the Diagnostic Tools in Rhinology EAACI Position Paper [14]. The subjects with systemic illness, with positive anamnesis of abuse any of drugs (like cocaine etc.), long-term use of nasal decongestants, previous injuries and surgical procedures on the nasal cavity and paranasal sinuses were excluded too.

Differentiation from perennial allergic rhinitis

SPT was done in all participants with the standard set of respiratory allergens: birch, timothy, mugwort (*Artemisia vulgaris*), dog, cat, horse, mite (*Dermatophagoides farinae, Dermatophagoides pteronyssinus*), moulds (*Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum*), *Olea europaea*, *Parietaria judaica*, *Plantago lanceolata*, *Platanus acerifolia*) [14]. Saline solution (0.9% NaCl) and 1 mg/mL histamine solution were also used in SPT as negative and positive controls, respectively. SPT result was noted as positive if the width of wheal was larger than 3 mm in comparison to the negative control.

ELISA kit (Elitech Diagnostics, France) was used for measurement of total serum IgE level. The level of IgE of more than 100–150 IU/mL considered to be higher than normal [14].

All subjects with positive SPT and/or IgE level above normal were excluded from this study.

Differentiation from infectious rhinitis

Swabs for microbiological evaluation of nasal secretion were provided in all workers with clinical confirmation of chronic rhinitis. Any recognized microbial pathogen existed in more than 1,000 colony per mL was considered as the cause of infectious chronic rhinitis, and these workers were excluded too.

Differentiation from NARES

Profound nasal eosinophilia was revealed by cytology evaluation of scraped nasal mucosa in all participants. Nasal leukocyte counts were determined after fixing of the specimen on plain slide with 95% ethanol and staining with May-Grünwald-Giemsa, by light microscopy (x400) under oil immersion. Twenty percent or more eosinophils in total leukocyte count was considered to be characteristic of NARES [16], and these subjects were excluded from the study.

Statistical analysis

For presentation of numeric variables, descriptive statistics was used as mean values ± standard deviation (SD), while for categorical variables percentages were used. Student *t*-test was used for evaluation of differences in average of age and length of service between evaluated groups. Differences in smoking habits, alcohol abuse, and the prevalence of confirmed chronic rhinitis were evaluated by χ^2 test.

Binary logistic regression model was used to calculate the relative risk for the occasion of chronic rhinitis based on independent predictor variables (age, years of service, smoking, alcohol consumption and group membership). A p value of 0.05 and less was considered to be statistically significant.

For statistical analysis, we used the PASW Statistics 2018 programme.

Results

General characteristics of the investigated cohorts at the moment of the investigation demonstrated no statistically significant differences between the groups in view of the average age, duration of employment, alcohol abuse and smoking practice (Table 1).

Table 1
Main characteristics of the studied population

Parameter	Group		*p*
	exposed	control	
Age (years), mean ± SD	37.5 ± 7.9	39.6 ± 8.9	0.077†
Employment (years), mean ± SD	19.3 ± 8.2	17.5 ± 8.1	0.131†
Smoking habits, n (%)			
nonsmokers	34 (34.0)	26 (26.0)	
up to 10 cigarettes/day	10 (10.0)	12 (12.0)	0.426*
11–20 cigarettes/day	56 (56.0)	62 (62.0)	
Alcohol consumption, n (%)			
rarely or never	23 (23.0)	26 (26.0)	
moderate (1–2 drink/day)	68 (68.0)	61 (61.0)	0.731*
heavy (> 2 drink/day)	9 (9.0)	13 (13.0)	

SD – standard deviation; †Student *t*-test; *Pearson χ^2 test.

Using χ^2 test for assessment of overall chronic rhinitis prevalence in studied groups, we got result: $\chi^2 = 7.498$, DF = 1, $p = 0.006$ (Table 2). We concluded that exposed group had considerably higher prevalence of chronic rhinitis than nonexposed population.

Table 2
Chronic rhinitis prevalence in the exposed group and the control group

Group	Chronic rhinitis		Pearson χ^2	Df	*p*
	yes	no			
Exposed	78	22	1	7.498	0.006
Control	21	97			

Figure 2 presents the prevalence of chronic rhinitis for both studied groups regarding the exposure duration.

By means of binary logistic regression model, we found that only membership to exposed group – glassblowers had statistically significant contribution to the model, with relative risk of 8.387 (Table 3). That means that glassblowers have almost 8.4 times greater risk for occurrence of chronic rhinitis than the control group. Other examined predictor variables (age, years of employment, smoking and alcohol abuse) had not contribution to getting chronic rhinitis.

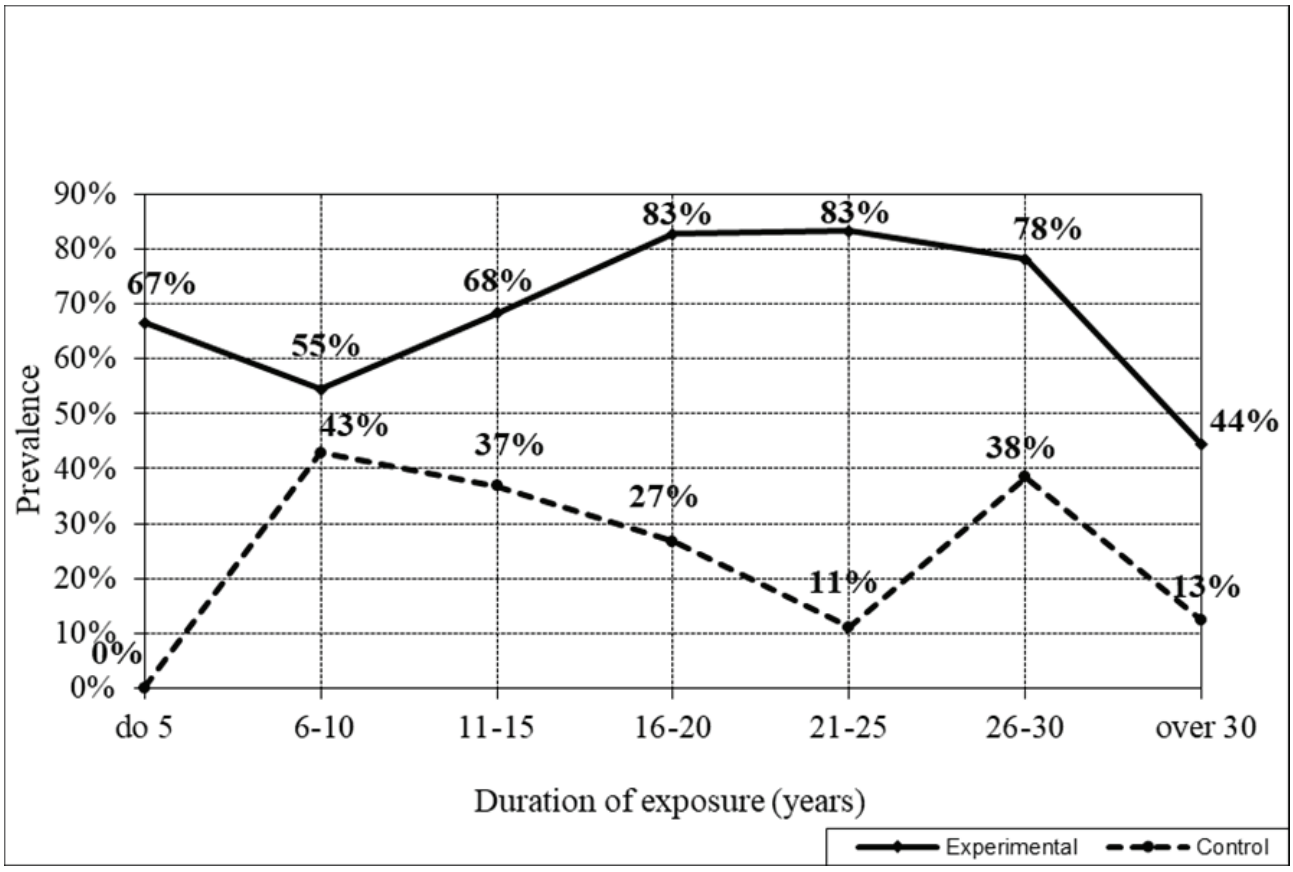


Fig. 2 – Prevalence of chronic rhinitis in the exposed (experimental) group and the control group during exposure period.

Table 3

Relative risk for occurrence of chronic rhinitis in glassblowers and control group

Predictor variables	B	S.E.	Sig.	RR*	95% CI	
					lower	upper
Group						
control				1.000		
exposed	2.127	0.357	0.000	8.387	4.163	16.897
Age	0.056	0.034	0.099	1.057	0.990	1.129
Years of employment	-0.053	0.035	0.124	0.948	0.886	1.015
Smoking	0.039	0.352	0.912	1.040	0.521	2.073
Abuse of alcohol	-0.197	0.376	0.601	0.821	0.393	1.718
Constant	-2.195	0.967	0.023	0.111		

***Relative risk (binary logistic regression); CI – confidence interval.**

Discussion

Among other roles, the nose has the protective function of the lower parts of respiratory system from the ambient harmful influences. More intensive contact of glassblowers with noxious influences could be explained by previous noted closer and more intensive contact with harmful factors in contrast with the control group. This fact could be explanation of more than 8 times higher prevalence of chronic rhinitis in the exposed than in the control group. Additionally, we found that the years of service was not a statistically significant factor for occurrence of chronic rhinitis.

Although glassblowers are exposed to several carcinogenic factors, malignant tumors of the nose and upper aerodigestive tract were not found in our investigation. Some other surveys [8, 9] have noted increased occurrence of malignancies of the nose and paranasal sinuses in glassworkers.

The curves of prevalence distribution of chronic rhinitis in studied groups of workers through years of service were interesting in shape (Figure 2).

Unexpectedly high prevalence of chronic rhinitis (67%) in the exposed group was found at the beginning of their work (0–5 years). At that time, in the control group, no one case of chronic rhinitis was diagnosed. This fact could be explained by rapid and intensive exposition of the glassblower's nasal mucosa to harmful occupational environmental factors. Nasal mucosa, at this time, was not adapted to rapidly and intensively changed microclimatic factors. These facts reveal how harmful microclimatic conditions have more significant influence on the glassblower's nasal mucosa than on that of the control group of workers.

In the second exposure period (6–10 years) prevalence of chronic rhinitis among the glassblowers decreased (55%), whereas increased within the control group (43%), but still less than in the exposed group. During years of service many glassblowers probably acquire some adaptation mechanisms to harmful influences of work ambient, and this could be the explanation for decreased prevalence of chronic rhinitis among glassblowers in later period. Mechanisms of this adaptation were not considered in this study. The control group of workers were employed near glassblowers, but they did not blow glass, so they were less exposed to harmful workplace factors. This fact could explain slower increasing of prevalence of chronic rhinitis in the control group.

After this period, we observed smooth rise of chronic rhinitis prevalence in the exposed group, while this prevalence decreased among control nonglassblowers. The difference in frequency of chronic rhinitis between examined groups increased during time too, and raised maximum in exposition interval 21–25 years of service, when prevalence of chronic rhinitis in the exposed group maximized (83%), and in the control group minimized (11%).

The interval of 26–30 years of service in both studied groups was characterized by the decrease of chronic rhinitis prevalence. The retirement of workers who had the most prominent symptoms and signs of chronic rhinitis or other diseases could explain this fact. Therefore, only workers with relatively good health status remained in manufacturing plant.

Conclusion

On their workplace, glassblowers are exposed to greater influence of noxious factors, and they have statistically greater risk for getting chronic rhinitis than nonglassblowers who work in the same glass factory.

The prevalence of chronic rhinitis increased in both groups of workers during exposure time (years of service), but difference between them was not statistically significant. Therefore, we can conclude that the glass production by glassblowing is highly significant risk factor for getting chronic rhinitis, but the exposure period is not.

We noted the decrease of chronic rhinitis prevalence among glassblowers after 5 to 10 years of service that can be explained by the possible adaptation of the laryngeal mucosa to harmful influences.

On the basis of our results, it is imperative to insist on using adequate standard protective devices on the working place, as well as an adequate ventilation of the workspace. We consider that it is necessary to include at least periodic otorhinolaryngology examination in the regular systematic examinations of glassblowers.

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Remote ischemic preconditioning in patients undergoing coronary bypass grafting following acute coronary syndrome without ST elevation

Kardioprotektivni efekat udaljenog ishemijskog prekondicioniranja tokom hirurške revaskularizacije miokarda kod bolesnika sa akutnim koronarnim sindromom bez elevacije ST segmenta

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Abstract

Background/Aim. A protection of heart and other organs from ischemic-reperfusion injuries can be provided by remote ischemic preconditioning (RIPC) by brief episodes of ischemia and reperfusion in distant tissues. The aim of this study was to assess effects of RIPC on early outcomes in patients underwent coronary bypass surgery (CABG) following acute coronary syndrome without persistent ST segment elevation (NSTEMI ACS). **Methods.** This trial included 42 patients randomized into two groups: the group 1 received RIPC and the group 2 was without RIPC (control group). Pre-, intra- and postoperative parameters were compared but primary endpoint was myocardial injury reflected as value of troponin I measured preoperatively and 1, 6, 12, 24, 48 and 72 h postoperatively. The secondary endpoints were hemodynamic parameters, blood loss, intensive care unit stay, mortality etc. **Results.** The groups 1 and 2 were similar in preoperative characteristics including age, New York Heart Association (NYHA) class, EuroSCORE II, left ventricular ejection fraction. The only significant difference between groups was for triple vessel coronary disease with dominance in the RIPC group [20 (100%) vs. 17 (77.3%), $p = 0.049$]. Cardiopulmonary bypass time [mean (± standard deviation): 83.0 (22.9) vs. 67.0 (17.4) minutes, $p = 0.015$], cross clamp time [57.9 (15.4) vs. 44.3 (14.3) minutes, $p = 0.005$] and number of conduits [median (25–75th percentile): 23.5(3–4) vs. 3(2–3), $p = 0.002$] were different. Other intra- and postoperative variables did not differ between groups. There were no differences in C reactive protein levels and postoperative hemodynamic parameters. Average troponin values in all time points revealed no significant differences between groups ($p_{0h} = 0.740$, $p_{1h} = 0.212$, $p_{6h} = 0.504$, $p_{12h} = 0.597$, $p_{24h} = 0.562$, $p_{48h} = 0.465$ and $p_{72h} = 0.715$, respectively). Furthermore, there were no significant differences in adverse events, hospital stay and mortality between groups. **Conclusion.** Treatment with RIPC during CABG following NSTEMI ACS did not provide better myocardial protection and hemodynamics characteristics but further larger randomized studies are needed t. prove its real value.

Key words:
coronary artery bypass; ischemic preconditioning, myocardial; myocardial revascularization; non-st elevated myocardial infarction; troponon i; treatment outcome.

Apstrakt

Uvod/Cilj. Zaštita srca i drugih organa od ishemijsko-reperfuzonih oštećenja može biti obezbeđena udaljenim ishemijskim prekondicioniranjem (*remote ischemic preconditioning* – RIPC) sa kratkim epizodama ishemije i reperfuzije u udaljenim tkivima. Cilj rada bio je da se utvrdi efekat RIPC na rane rezultate hirurške revaskularizacije miokarda kod bolesnika sa akutnim koronarnim sindromom bez elevacije ST segmenta. **Metode.** Studijom su bila obuhvaćena 42 bolesnika koji su bili randomizovani u dve grupe: grupu 1 koja je bila tretirana sa RIPC i grupu 2 bez RIPC (kontrolna grupa). Poređeni su pre-, intra- i postoperativni parametri, ali je glavni cilj bio miokardna lezija koja se odražava kroz vrednosti koncentracije troponina I merenih preoperativno i 1, 6, 12, 24, 48 i 72 sata postoperativno. Analizirani su vredno-

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sti hemodinamskih parametara, krvarenje, vreme lečenja u jedinici intenzivne nege, mortalitet i ostalo. **Rezultati.** Grupe 1 i 2 bile su slične po preoperativnim karakteristikama, kao što su životna dob, *New York Heart Association* (NYHA) klasa, EuroSCORE II i ejekciona frakcija leve komore. Jedina razlika među grupama bila je u zastupljenosti trosudovne koronarne bolesti sa dominacijom u RIPC grupi [20 (100%) vs. 17 (77,3%), p = 0,049]. Vreme kardiopulmonalnog bajpasa [srednja vrednost (± standardna devijacija): 83,0 (22,9) vs. 67,0 (17,4) minuta, p = 0,015], vreme kleme na aorti [57,9 (15,4) vs. 44,3 (14,3) minuta, p = 0,005] i broj graftova [medijan (25–75. percentil): 3,5 (3–4) vs. 3 (2–3), p = 0,002] bili su različiti. Ostale intra- i postoperativne varijable se nisu razlikovale među grupama. Nije bilo razlike u vrednostima C reaktivnog proteina i postoperativnih hemodinamskih parametara. Srednje vrednosti troponina u svim ispitivanim vremenskim intervalima nisu pokazale značajnu razliku među grupama (p_{0h} = 0,740, p_{1h} = 0,212, p_{6h} = 0,504, p_{12h} = 0,597, p_{24h} = 0,562, p_{48h} = 0,465 i p_{72h} = 0,715). Takođe, nije bilo značajne razlike u pojavi neželjenih događaja, dužini trajanja bolničkog lečenja i mortalitetu između grupa. **Zaključak.** Primena RIPC tokom hirurške revaskularizacije miokarda kod bolesnika sa akutnim korornarnim sindromom bez elevacije ST segmenta ne obezbeđuje bolju miokardnu zaštitu i hemodinamske kararkteristike, ali su neophodne veće randomizovane studije da bi se dokazao pravi efekat RIPC.

Ključne reči:
aortokoronarno premošćavanje; miokard, prekondicioniranje, ishemijsko; miokard, revaskularizacija; infarkt miokarda bez st elevacije; troponin i; lečenje, ishod.

Introduction

Remote ischemic preconditioning (RIPC) by brief episodes of ischemia and reperfusion in distant tissues can provide protection of heart and other organs from ischemic-reperfusion injuries [1–3]. Perioperative myocardial necrosis during cardiac surgery is predominantly caused by myocardial protection failure and is associated with increased morbidity and mortality [4]. It has been shown that RIPC can attenuate cardiomyocyte injury [4]. Cardioprotection methods are important to avoid post-ischemic myocardial dysfunctions which appeared during coronary artery bypass grafting (CABG) and are reflected by cardiac troponin (cTn) release. Previous studies have proved that RIPC has cardioprotective effect with reduced release of cTn levels during cardiac surgery and can improve better clinical outcomes [4]. Acute coronary syndrome (ACS) is clinical presentation of coronary artery disease and includes unstable angina (UA), non ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI) [5]. Majority of patients with NSTE-ACS are treated with percutaneous coronary interventions (PCI) but about 10% of them require surgical revascularization (CABG) [6]. In this group of high risk patients is difficult to balance between ischemic-reperfusion injuries and bleeding complications in relation to the timing of surgery [5]. In addition to an urgent revascularization which carries a certain risk, acute myocardial ischemia is an additional risk factor for adverse events in postoperative course. Previous investigations have shown the effect of RIPC on myocardial protection during CABG [1, 3, 7] and adult valve surgery [8, 9], abdominal aortic surgery [10] and congenital heart surgery [2].

The aim of the present randomized, prospective, feasibility study was to reveal whether or not RIPC provides additional myocardial protection to standard cardioplegic techniques during CABG following NSTEMI ACS. For these reasons we analyzed cTn levels, hemodynamic parameters and compared outcome of surgical procedure by analyzing postoperative major complications.

Methods

This prospective, randomized, pilot, single-center study was conducted between June 2016 and November 2017. The study protocol was approved by the local Ethics Committee. All patients provided written informed consent for participation in the trial. Eligible patients were adults with ACS NSTEMI with chest pain and electrocardiogram (ECG) abnormalities required urgent CABG. Patients were randomized using previously generated randomization list in computer software PASS 11.0. Efron`s biased coin algorithm with 1:1 allocation ratio was used for randomization list generation. Forty-four eligible patients were included and randomized but two patients from the RIPC group were excluded from trial. In the first case, iatrogenic aortic dissection occurred during CABG and procedure was extended into ascending aortic reconstruction concomitant with CABG. In the second case, the patient was hemodynamic unstable after induction of general anesthesia and intra aortic balloon pump was inserted preoperatively. A total of 42 patients were divided into two groups: the RIPC group (3 cycles of 5 min right upper arm ischemia by inflation of a blood pressure cuff to 200 mmHg and 5 min of reperfusion) and the control group (cuff was uninflated around right upper arm for 30 min) after induction of anesthesia [11]. Our protocol was modified and cuff was inflated on right upper arm because we harvested left radial artery as conduit in some cases. Anesthesiologists who applied the RIPC protocol were not blinded but they were not involved in data collection and interpretation. All other participants in trial, including patients, were blinded.

Patient selection

Patients were included in the study if they had ACS NSTEMI unsuitable for percutaneous treatment but required CABG on the current admission. All of them were designated for urgent isolated CABG according to clinical and coronary angiographic findings recruited with cardiospecific enzymes levels. Exclusion criteria were: elective CABG, ad-

ditional valve surgery, poor left ventricular function (left ventriculkar ejection fraction < 25%), redo surgery, peripheral upper limbs occlusive vascular disease, off pump surgery, simultaneous carotid endarterectomy, acute or chronic infections, autoimmune diseases, hepatic dysfunction and recent pulmonary embolism or myocardial infarction or PCI or any other reasons for increased preoperative cTnI concentration.

Perioperative management

Premedication consisted of atropine (0.5 mg), midazolam (0.1 mg/kg) and morphine (4 mg) intramuscularly. Anesthesia was induced with midazolam (0.3–0.4 mg/kg), fentanyl (10–15 μg/kg) and rocuronium (0.6 mg/kg), and maintained with sevoflurane (minimum alveolar concentration 0.5–1.2 % atm) or with propofol and continuous infusion of fentanyl (1.5 μg/kg/h). After induction of anesthesia pulmonary artery catheter (HANDS-OFF Thermodilution Catheter, Arrow International Inc, Reading, PA) was inserted. Moderate hypothermic cardiopulmonary bypass (32°C) was established through cannulation of the ascending aorta and right atrium. Then RIPC was applied as aforementioned above or blood pressure cuff was remained uninflated. Surgical revascularization was performed through median sternotomy. Both internal thoracic arteries, radial artery and saphenous veins were used as conduits. Heparin was administered to achieve an activated coagulation time above 400 seconds. Membrane oxygenation (INSPIRE™ SORIN, Sorin Group Italia) and roller pump (Stockert SORIN S5, Sorin Group Italia) were used. Nonpulsatile flow on cardiopulmonary bypass (CPB) was maintained at 2.2–2.4 L/ (min/m^2), and perfusion pressure between 50 mmHg and 70 mmHg. Cardioplegic arrest was achieved by anterograde administration of cold blood cardioplegia (4 °C). Proximal graft anastomoses were performed with total single clamp or partial side clamping of the ascending aorta. After rewarming to 36.7–37 °C, weaning from CPB was supported by inotropic drugs. Systemic anticoagulation was reversed by protamine sulphate according to a standard protocol (1 mg/300 IU of heparin). A standard protocol for early postoperative care in an intensive care unit (ICU) was followed.

Data collection

The primary endpoint was to assess the perioperative myocardial injury reflected as cTnI concentration levels during first 72 h after CABG. Venous blood samples for measurement of cTnI concentrations were collected prior to surgery and 1, 6, 12, 24, 48 and 72 h after surgery. Concentrations of cTnI were measured using a specific two-side immunoassay (Access 2® Backman Coulter, USA). The reference range was 0–0.04 ng/mL. cTnI values above 0.1 ng/mL were considered as abnormal. Venous blood samples for measurement of creatine kinase (CK) and its muscle-brain (MB) isoform (CK-MB), C-reactive protein (CRP) and white blood cells (Le) count were collected prior to the surgery and 24 h after surgery. Serum CRP and CK-MB concentrations were determined by turbidometry with the UniCelD × C600® analyzer (Beckman Coulter USA). CRP concentrations below 5.0 mg/L and CK-MB concentrations 0–25 IU/L were considered to be within the reference range. Hemodynamic measurements in the form of cardiac output (CO), mean arterial pressure (ARP), pulmonary capillary wedge pressure (PCWP) and cardiac index (CI) were performed prior to surgery and 1, 6, 12, 24 h after surgery and in minority of cases 48h and 72 h after surgery. Arterial blood samples for pH, lactate and glucose concentrations measurements were obtained prior to surgery and 24 h postoperatively. Perioperative myocardial infarction, new onset of atrial fibrillation, cerebrovascular adverse events, infections, renal functions, mechanical ventilation time, ICU and hospital stay and intrahospital mortality were recorded.

Statistical analysis

Results are presented as count (percent), mean (± standard deviation) or median (25–75th percentile), depending on data type and distribution. The *t*-test, Mann-Whitney *U* test, Pearson χ^2 test and Fisher's Exact test were used to assess significant differences between groups. The Linear mix model and General linear model were used to evaluate differences between groups regarding blood parameters in follow-up period. All *p*-values less than 0.05 were considered significant. All data were analyzed using the SPSS 20.0 (IBM corp.) and R for Windows 3.3.0 (CRAN project).

Results

Study included 42 patients, 20 in the RIPC group (47.62%) and 22 in the control group (52.38%). Average age of all participants was 64.8 ± 9.2 years, minimum 43 and maximum 79 years. Majority of participants were males, n = 38 (84.4%). Comparisons between examined groups regarding basic characteristics of participants are presented in Table 1.

As shown in Table 1, patients had similar basic characteristics including age, gender, body mass index (BMI), NYHA class and EuroSCORE. Distribution of risk factors was almost identical across groups. Cardiovascular characteristics of patients were also very similar in both groups. The only significant difference between groups was for triple vessel coronary disease with its dominance in the RIPC group. Additionally, from the patients treated with dual antiplatelet therapy (DAPT) preoperatively, 3 (25%) of the patients from 12 (60%) in the RIPC group were operated within 24 h and 2 (13.3%) of 15 (68.2%) of the patients from the control group were operated within 24 h ($p = 0.628$).

We performed coronary angiography in the RIPC group within 24 h in 8 (40%) of the patients and in 12 (60%) of the patients more than 72 h before surgery. In the control group coronary angiography was done within 24 h in 5 (22.7%) of the patients, in 1 (4.5%) patient between 1 and 3 days and in 16 (72.7%) of the patients more than 72 h before surgery ($p = 0.326$). Preoperatively, elevated cTnI concentrations in the RIPC group was present in 11 (55.0%) of the patients and in 11 (50.0%) of the patients in the control group ($p = 0.746$).

Table 1

Basic characteristics of patients in examined groups

Paremeter	RIPC (n = 20)	Control (n = 22)	*p*-value
Age (years), mean (± SD)	64.3 (11.0)	65.4 (7.6)	0.728[a]
Gender (male), n (%)	17 (85)	18 (81.8)	1.000[c]
BMI, mean (± SD)	27.1 (3.2)	28.6 (3.8)	0.193[a]
NYHA class III–IV, n (%)	10 (50)	12 (54.5)	0.768[b]
Euroscore II, median (25–75th percentile)	3.36 (1.40–5.30)	2.1 (1.34–3.73)	0.413[d]
Ever smoker, n (%)	15 (75)	16 (72.7)	0.867[b]
Hypertension, n (%)	17 (85)	20 (90.9)	0.656[c]
Hypercholesterolaemia, n (%)	18 (90)	20 (90.9)	1.000[c]
Diabetes mellitus, n (%)	10 (50)	13 (59.1)	0.554[b]
Peripheral vascular disease, n (%)	3 (15)	2 (9.1)	0.656[c]
Carotid artery stenosis (> 75%), n (%)	4 (20)	1 (4.5)	0.174[c]
Previous myocardial infarction, n (%)	8 (40)	12 (54.5)	0.346[b]
Triple vessel coronary disease, n (%)	20 (100)	17 (77.3)	0.049[c]
Left main coronary disease (> 50%), n (%)	10 (50)	11 (50)	1.000[b]
Left ventricular EF (%), mean (± SD)	42.5 (6.6)	40.9 (8.1)	0.488[a]
Hospital stay before CABG (days), median (25–75th percentile)	4.5 (1–6.5)	5 (0–8)	0.484[d]
Medication, n (%)			
DAPT	12 (60)	15 (68.2)	0.580[a]
beta blockers	19 (95.5)	21 (95.5)	1.000[c]
ACE inhibitors	14 (70.0)	18 (81.8)	0.477[c]
oral nitrates	6 (30.0)	9 (40.9)	0.461[a]

RIPC – remote ischemic preconditioning: BMI – body mass index; NYHA – New York Heart Association; EF – ejection fraction; CABG – coronary artery bypass graft; DAPT – dual antiplatelet therapy; ACE – angiotensin converting enzyme; SD – standard deviation.
[a]*t* test; [b]Pearson χ^2 test; [c]Fisher's Exact test; [d]Mann-Whitney *U* test.

Operative and postoperative parameters in examined groups are presented in Table 2. As shown in Table 2, the RIPC group had significantly higher CPB time, aortic cross clamp time and number of conduits. Other operative parameters showed no significant differences between groups. It was obvious that some parameters had higher percentages in the RIPC group than in the control one, however with no statistical significance, probably due to small sample size. None of the participants had infection and only one patient died after surgery.

All patients were examined regarding CK-MB and CRP values before and 24 h after surgery. Median values with 25–75th percentiles in both examined groups are presented in Table 3. Delta values represent differences between 24 h postoperative and preoperative values. There were no significant differences between groups in preoperative, postoperative or delta CK-MB and CRP values. Median values were very similar in both groups with rather higher values in the control group, except for CRP postoperative levels.

Table 2

Operative and postoperative parameters in examined groups

Parameter	RIPC (n = 20)	Control (n = 22)	*p*-value
CPB time (min), mean (± SD)	83.0 (22.9)	67.0 (17.4)	0.015[a]
Aortic cross clamp time (min), mean (± SD)	57.9 (15.4)	44.3 (14.3)	0.005[a]
Number of conduits, median (25–75th percentile)	3.5 (3–4)	3 (2–3)	0.002[d]
Inotropes > 12 h, n (%)	13 (65)	9 (40.9)	0.118[b]
MV time (min), median (25–75th percentile)	12.5 (10–15)	13.5 (11–16)	0.503[d]
Atrial fibrillation, n (%)	7 (35)	3 (13.6)	0.152[c]
Drainage (mL), median (25–75th percentile)	650 (400–1375)	600 (450–700)	0.313[d]
Reintervention, n (%)	2 (10)	1 (4.5)	0.598[c]
Respiratory insufficiency, n (%)	3 (15)	2 (9.1)	0.656[c]
Infection, n (%)	0	0	–
ICU stay (days), median (25–75th percentile)	2 (1–5)	2 (2–4)	0.767[d]
Postoperative hospital stay (days), median (25–75th percentile)	7 (6.5–16.5)	7 (6–8)	0.405[d]
Mortality, n (%)	1 (5)	0	0.476[c]

RIPC – remote ischemic preconditioning; CPB – cardiopulmonary bypass; MV – mechanical ventilation; ICU – intensive care unit; SD – standard deviation.
[a]*t* test; [b]Pearson χ^2 test; [c]Fisher's Exact test; [d]Mann-Whitney U test.

Table 3

CK-MB and CRP in examined groups before and after the surgery

Parameter	RIPC (n = 20)	Control (n = 22)	*p*-value*
CK-MB (IU/L)			
preoperative	11.1 (8.8–17.1)	13.2 (10.4–16.8)	0.242
24 h postoperative	24.5 (19.0–58.9)	38.8 (25.1–57.4)	0.314
delta CK-MB	13.5 (3.6–36.6)	17.3 (10.5–40.5)	0.465
CRP (mg/L)			
preoperative	4.25 (3.10–9.75)	5.25 (1.30–10.80)	0.870
24 h postoperative	139.5 (111.8–178.5)	134.1 (89.9–175.3)	0.724
delta CRP	127.4 (107.4–168.5)	131.2 (88.9–159.3)	0.782

***Note*: Results are presented as median (25–75th percentile).**
RIPC – remote ischemic preconditioning; CK-MB – creatin kinase-muscle, brain isoform; CRP – C reactive protein.
***Mann-Whitney *U* test.**

cTn values were examined in seven time points. Average values in all time points revealed no significant differences between groups (p_0 = 0.740, p_1 = 0.212, p_6 = 0.504, p_{12} = 0.597, p_{24} = 0.562, p_{48} = 0.465 and p_{72} = 0.715, respectively). Delta troponin was calculated as difference between 72 h cTn and baseline cTn. Comparing results between groups, we did not obtain significant differences regarding delta cTn (0.46 ± 1.96 vs. 0.08 ± 2.59 ng/mL; p = 0.696). Using linear mixed model, we also obtained no significant differences between groups regarding mean troponin values during follow up (p = 0.816). Troponin mean values with 95% confidence intervals are presented in Figure 1. The cTnI reached a peak level at 6 h in the RIPC group while the maximum cTnT level was reached at 24 h in the control group; this trend did not reach statistical significance.

Beside troponin, following parameters were examined during follow- up in five time points (baseline, 1 h, 6 h, 12 h and 24 h after surgery): CO, ART, PCWP, CI and mixed venous oxygen saturation (SVO2). In time points 48 h and 72 h, only several patients have measurements and therefore we excluded these measurements from further analysis. Using general linear model, we did not obtain significant influence of RIPC vs. control on CO, ART, PCWP, CI and SVO2 parameters (p = 0.490, p = 0.943, p = 0.208, p = 0.422 and p = 0.594, respectively). When comparing differences between groups in each time point, we did not obtain any significant difference regarding examined parameters except PCWP in baseline (RIPC vs. baseline, p = 0.027). Listed parameters except SVO2 are presented in Figure 2.

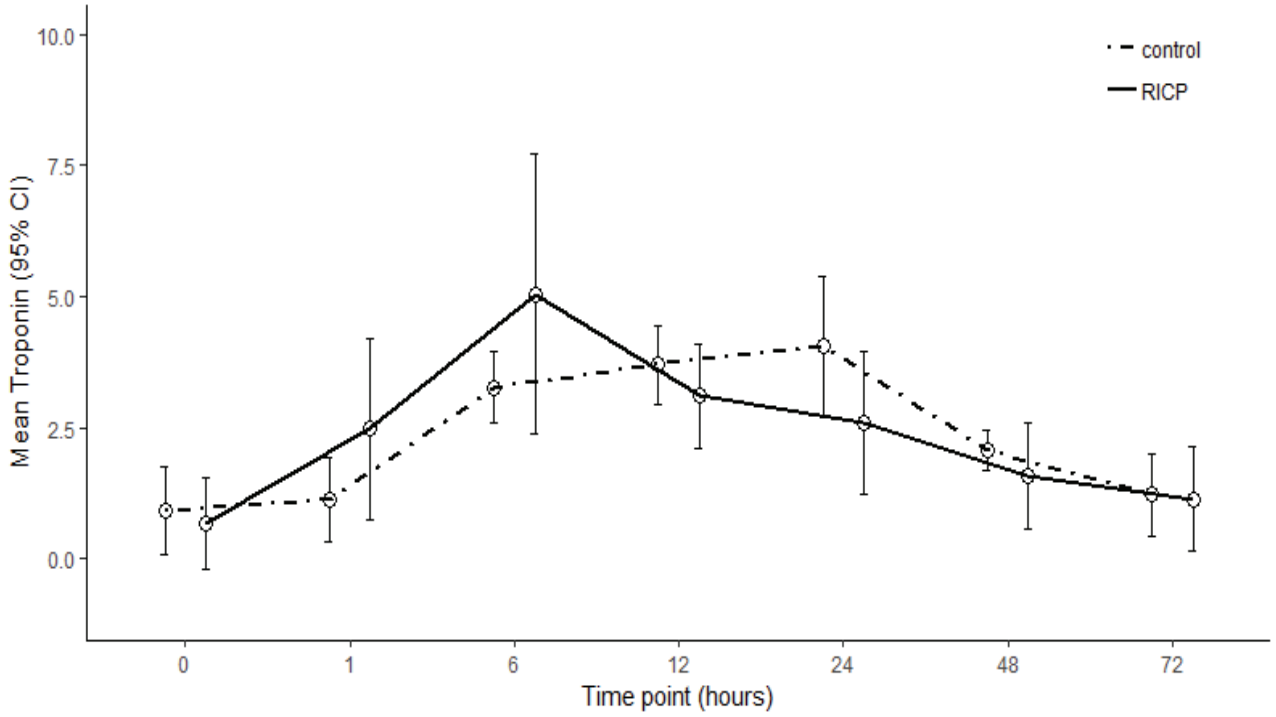

Fig. 1 – Troponin values (in ng/mL) during follow-up in examined groups.
RIPC – remote ischemic preconditioning; CI – confidence interval.

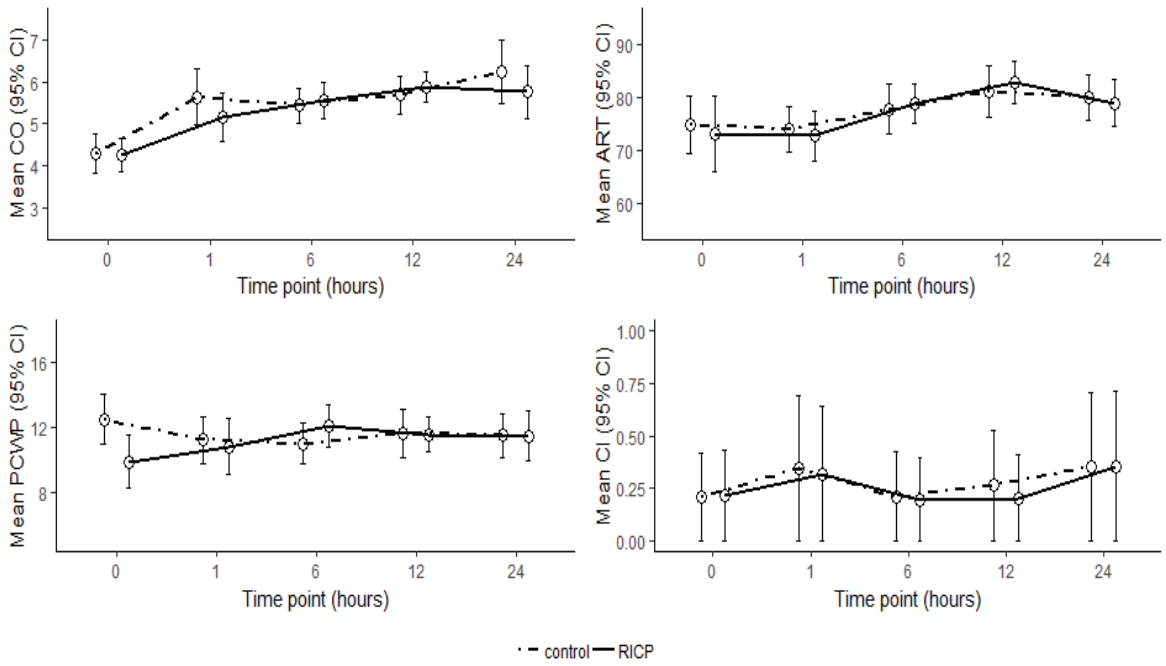

Fig. 2 – CO (L/min), ART (mmHg), PCWP (mmHg) and CI values in examined groups during follow-up. CO – cardiac output; ART – mean arterial pressure; PCWP – pulmonary capillary wedge pressure; CI – cardiac index. RIPC – remote ischemic preconditioning; CI – confidence interval.

Discussion

Our study was, to our knowledge, the first randomized prospective trial that assessed cardioprotective effect of RIPC in high risk patients undergoing CABG in NSTEMI ACS. In recent years, there has been an increasing interest in cardioprotective effect of RIPC during cardiac surgery, however results still remain controversial. Two large prospective, randomized trials that included patients with high EuroSCORE and combined procedures (CABG with valve or ascending aorta replacement) showed that RIPC did not reduce perioperative major adverse cardiac and cerebral events [12, 13]. Majority of previous investigations excluded urgent patients, thus proved that RIPC enhanced myocardial protection during elective CABG [1–3]. Our trial involved patients with NSTEMI ACS, with high risk of perioperative major adverse events and did not reveal beneficial cardioprotective effect of RIPC. Rahman et all. [14] included elective and urgent (NSTEMI ACS within 30 days) adult patients undergoing CABG but without patients who had angina within 48 h of surgery. All our patients were operated in next 24 h of NSTEMI ACS onset on the current admission. Only few studies included high risk cardiac surgery patients but did not prove that RIPC reduced cTnT, acute kidney injury or ICU support requirements [14–16]. In our trial, preoperative data were different between groups only in total amount of triple vessel coronary disease (all patients in the RIPC group) and it reflects on significant difference in CPB time, aortic clamp time and number of conduits. We performed coronary angiography within 24 h at the day of admission and before surgery in 8 patients in the RIPC group and in 5 patients in the control group, without statistical difference. All other patients in both groups were examined more than 3 days before surgery (except 1 patient from the control group who was examined between 1 and 3 days) and were admitted at hospital for elective CABG, carotid or abdominal aortic surgery but developed ACS NSTEMI while waiting for surgery. Ghosh and Galiñanes [17] investigated RIPC effects in CABG with or without CPB and revealed that RIPC had additional cardioprotective effect in beating heart surgery but not in "on pump" surgery because CPB induces preconditioning by itself [17]. We also believe that this difference in preoperative data had no impact on cTnI release. Furthermore, we observed the peak cTnI level at 6 h in the RIPC group while the maximum cTnI level in the control group was reached at 24 h. This observation suggest that RIPC may play a role in faster recovery from reperfusion injury after on-pump CABG. In line with our findings, several studies showed that RIPC reduced myocardial injury in patients undergoing CABG with cold blood cardioplegia [8] and crystalloid cardioplegic arrest [18]. In our trial, cardioplegic arrest was achieved by anterograde administration of cold blood cardioplegia in all cases, however we did not reveal additional cardioprotective effect. Results from our study demonstrated that postoperative hemodynamics characteristics and ICU inotropic support requirements did not differ between groups. Also, RIPC did not reduce mechanical ventilation time, ICU or postoperative hospital stay, mortality remained lower in the control group but without significant difference. Finally, only one death occurred in the RIPC group due to an acute kidney injury but small number of major adverse events could induce wrong conclusion.

These data were achieved on limited number of patients. Small sample size was main obstacle to extract any strong conclusion. Our study did not include patients with triple vessel coronary disease because we tried to establish whether all consecutive patients in our tertiary healthcare center undergoing urgent CABG surgery could profit from RIPC. We focused on surgical findings but disregarded preoperative anesthetic medication standardization. Although, it is hard to achieve homogeneous patient cohort, we hope our further investigation will give some firm conclusions.

Conclusion

Although limited by a small sample size, our results showed that RIPC in urgent high risk patients with NSTEMI ACS undergoing CABG did not reduce cTnI release, did not improve hemodynamics characteristics and did not improve early postoperative clinical outcomes. However, further multicenter, randomized trials are mandatory before assessing the real value of RIPC cardioprotective effects.

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Psychometric evaluation of the short version of the Defense Style Questionnaire on Serbian healthy adult men

Psihometrijska procena kratke verzije Upitnika mehanizma odbrane na uzorku zdravih muškaraca u Srbiji

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Abstract

Background/Aim. The most famous instrument for measuring psychological mechanisms of defense is the Defense Style Questionnaire (DSQ-40), which has been increasingly used in our country. In Serbia empirical norms for male adolescent population and for elderly male adults were established. The aim of this study was psychometric evaluation of the short version of the DSQ-40 on a Serbian male middle-age non-clinical sample. **Methods.** Internal consistency, factor structure and discriminant and concurrent validity of the DSQ-40 were studied in 284 high selected male professional military personnel aged 23 to 53 years (35.09 ± 7.21 years). **Results.** The Cronbach alpha coefficient revealed a high internal consistency for the entire scale, which confirms the compactness and high reliability of this questionnaire [Intraclass correlation coefficient (ICC) = 0.724; $p < 0.01$]. **Conclusion.** Evaluation of Serbian version of DSQ-40 confirms intraclass highly significant coefficients of correlation. The present findings support the applicability of the Serbian version of DSQ-40.

Key words:
defense mehanisms; military personnel; male; adult; surveys and questionnaires; psychological tests; serbia.

Apstrakt

Uvod/Cilj. Najpoznatiji instrument za merenje psiholoških mehanizama odbrane je Upitnik za procenu mehanizama odbrane (DSQ-40), koji se sve više koristi u našoj zemlji. U Srbiji su ustanovljene empirijske norme za muško adolescentno stanovništvo i za starije muške odrasle osobe. Cilj ove studije bio je psihometrijska procena kratke verzije upitnika za mehanizme odbrane (DSQ-40) na nekliničkom uzorku muškaraca srednjeg životnog doba u Srbiji. **Metode.** Interna konzistentnost, struktura faktora i diskriminacija i istovremena validnost DSQ-40 su proučavani kod 284 visoko selektovanih profesionalnih vojnih lica muškog pola, starosti od 23 do 53 godine (35,09 ± 7,21 godina). **Rezultati.** Cronbach-ovi alfa koeficijenti ukazali su na veliku unutrašnju konzistenciju za celu skalu, što potvrđuje kompaktnost i visoku pouzdanost ovog upitnika [*Intraclass correlation coefficient* (ICC) = 0.724; $p < 0.01$]. **Zaključak.** Evaluacija srpske verzije DSQ-40 potvrdila je veoma značajne međuklasne koeficijente korelacije. Sadašnji nalazi podržavaju primenjivost srpske verzije DSQ-40.

Ključne reči:
odbrambeni mehanizmi; vojna lica; muškarci; odrasle osobe; ankete i upitnici; psihološki testovi; srbija.

Introduction

A psychoanalytic concept of defence mechanisms originated by Sigmund Freud, has been recognized as one of the most important contributions in bringing together the psychoanalytic theory and empirical research. Ego defence mechanisms are defined by Anna Freud as "the ways and means by which the ego wards off unpleasure and anxiety, and exercises control over impulsive behaviour, affects and instinctive urges" [1].

Ego defenses are regarded to function at an unconscious level to preserve homeostasis and prevent excessive anxiety forcing its way into consciousness, whether the anxiety occurs from conflict between the person and the outside environment or within the person, guarding personal self-esteem and affecting on the whole way of acting personality in relation to the environment. Defence mechanisms represent a relatively steady aspect of personality, so that a set of defence mechanisms that one person uses points to the psychological profile of the personality. After the age of 25 years a

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set of defence mechanisms that a person uses becomes relatively stable [2, 3].

Measuring psychological defence mechanisms is very difficult and quite unreliable, so only a few instruments for evaluating these characteristics have been developed.

There are several versions of the instrument: Defense Style Questionnaire-88, (DSQ-88), Defence Style Questionnaire-60 (DSQ-60), and Defence Style Questionnaire-40 (DSQ-40). The most famous instrument of this kind in the world is the DSQ-40, which in recent years is increasingly used [4].

The DSQ-40 is the 40-item version derived from the original questionnaire developed in 1983 by Bond et al. [5].

Bond et al. [5] proposed the DSQ, a self-administered questionnaire developed to assess defence styles, which relies on the subject's self-report of conscious derivatives of defence (coping) and it is vulnerable to the subject's response distortions [4, 6]. Bond et al. [5] stated, although "it would be impossible to conclude anything about isolated defence mechanisms, we hoped that we could approximate the measure of groups of defence mechanisms that we call defence styles" [5, 6].

The DSQ is a widely used self-report, simple and economical (cost-effective) instrument that estimates groups of defences called defensive styles according to Vaillant's [7–9] continuum ranging from immature (maladaptive) defences to mature (adaptive) defences [10]. The DSQ measures defensive styles rather than defence mechanisms separately, because measuring defences is not reliable.

It is used in many studies including different mental disorders like psychosis [11], anxiety, depression [12], addictions [13], etc. or to test whether specific defence styles could predict psychopathology in adolescents [14–16].

The DSQ has been translated in numerous languages in different countries including Brazil [17, 18], Turkey [19], Japan [20], Romania [21], Pakistan [22], France [23, 24], Ireland [25], Finland [26].

Based on statistical analysis and comparison of results with appropriate norms obtained in foreign studies, it has been found that the defensive style and structure of defense psychological mechanisms influence certain socio-demographic and cultural characteristics of the respondents, so that the DSQ-40 must be adapted to a particular population, which does not diminish its practical value and applicability for diagnostic and selection purposes.

In our country, Čabarkapa and Dedić [27] presented development of the DSQ-40, its basic characteristics and empirical norms obtained on our population. Examination included two examined groups in military population of male sex only: soldiers of the adolescent age (n = 400) and officers of the adult age (n = 165). In their investigation, psychometric evaluation of the DSQ-40 was not done.

The aim of this study was psychometric evaluation of the DSQ-40 on a Serbian male non-clinical sample.

Methods

Participants and procedure

Study was conducted in September 2016 in the three barracks of infantry units of the Serbian Armed Forces, in which all professional military personnel (PMP) were exposed to approximately the same professional burden.

A total of 284 randomly selected male PMP (officers, non-commissioned officers and professional soldiers) were included in this study. Obviously, this group involved PMP who were highly selected, psychophysically healthy persons and homogenized by gender (exclusively male), age (23 to 53 years; 35.09 ± 7.21 years on average) and education (60% with 12 years of schooling).

The sample size was determined based on the formula for determining sample size. This number was added 10%, because of the possibility that the questionnaire will not be fully filled and, in this way, we received a sample size of 284 respondents, with a previous decision that the alpha error level is 0.05, and the beta level at the limit of 0.01 giving a 90% strength of the study [28].

The participants gave their informed consent to participate in the study. The questionnaire was completed during class time: It was anonymous and no compensation was offered.

This study was conducted with approval by the Ethics Committee of the Faculty of Medal Sciences, University of Kragujevac. The General Staff of the Serbian Armed Forces approved this study. The Ministry of Defence gave a special permit for the research in the Serbian Armed Forces units.

Instruments

The DSQ-40 is a psychological test consisting of 40 claims about personal attitudes related to 20 defense mechanisms, where each defence mechanism is represented with two questions. Eight questions are related to mature, as well as neurotic mechanisms, while 24 questions are addressed to immature mechanisms. Using a scale of 9 degrees, each respondent is asked to indicate how much he/she agrees with the particular one with the assertion where the degree of agreement increases with the number (1 = I disagree, 9 = I completely agree). A result related to Defensive style represents an average score obtained from the simple.

Answers to all questions of the same set are gathered, while the score for each the individual defence mechanism calculates as the average response to the questions that make up this defense mechanism [9].

The DSQ-40 measures three factors (styles) of defense mechanisms: 1) Mature defense style (humor, anticipation sublimation and suppression), 2) Neurotic defense style (pseudoaltruism, reactive formation undoing and idealization), and 3) Immature defense style (autistic fantasy, projection, dissociation, somatization, rationalization, displacement, isolation, acting-out, devaluation denial, passive aggression, splitting).

Statistical analyses

Statistical analysis was carried out using Statistical Package for the Social Sciences (IBM SPSS) software version 20.0.

For each examined variable the mean value and the standard deviation are calculated (SD).

An exploratory factor analysis (EFA) was performed on 40 items (principal components extraction with Varimax rotation). A solution was selected on the basis of the scree test. For analysis of the DSQ-40 scale reliability, the value of the Cronbach alpha coefficient was used. Analysis of the connection between all segments of the defenses mechanisms mutually and their correlations with total score was done.

Results

An analysis of the DSQ-40 scale reliability [Intraclass correlation coefficient (ICC)], with the value of the Cronbach alpha coefficient being high, 0.795, and analysis of changes in the coefficient by eliminating individual issues showed that this part of the questionnaire proved to be very consistent and reliable, and that there were no issues whose elimination would significantly increase the reliability of whole scale coefficient. Also, the value of ICC was highly significant confirming the compactness and high reliability of this questionnaire (ICC = 0.724; $p < 0.01$) (Table 1).

Scree plot is shown on Figure 1.

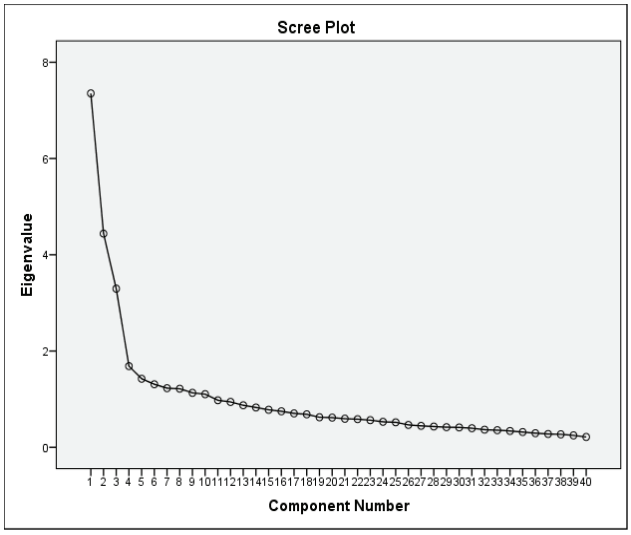

Fig. 1 – A line plot of eignvalues of principle components in an analysis of the Serbian version of the Defense Style Questionnaire (DSQ-40).

An exploratory factor analysis performed on 40 items (principal components extraction with Varimax rotation) of the DSQ-40, identified 10 significant factors explaining approximately 61% of the variability of the entire model (Table 2).

The first factor consisted of 8 DSQ questions: 3, 5, 59, 61, 68, 81 and 84, which built four mature defenses: humor, anticipation, sublimation and suppression. The first factor was called mature defense style.

The second factor consisted of 5 DSQ questions: 13, 37, 38, 58 and 63, which built four neurotic defenses: pseudo-altruism, reactive formation, undoing and idealization. The second factor was called neurotic defense style.

Factors from 3 to 10 consisted of questions that built immature defenses mechanisms. They were called common immature defense style.

Table 1
Cronbach's alfa coefficients of the Defense Style Questionnaire (DSQ-40)

Questions (number)	Cronbach's alpha
DSQ1	0.792
DSQ3	0.805
DSQ5	0.803
DSQ6	0.794
DSQ8	0.796
DSQ12	0.790
DSQ13	0.797
DSQ16	0.790
DSQ23	0.784
DSQ24	0.783
DSQ27	0.788
DSQ28	0.789
DSQ29	0.792
DSQ31	0.786
DSQ37	0.787
DSQ38	0.786
DSQ40	0.789
DSQ42	0.786
DSQ43	0.786
DSQ45	0.787
DSQ51	0.784
DSQ53	0.789
DSQ54	0.788
DSQ58	0.795
DSQ59	0.799
DSQ61	0.799
DSQ62	0.785
DSQ63	0.780
DSQ66	0.787
DSQ68	0.798
DSQ69	0.791
DSQ71	0.790
DSQ73	0.791
DSQ76	0.785
DSQ81	0.805
DSQ82	0.784
DSQ83	0.786
DSQ84	0.803
DSQ86	0.793
DSQ88	0.787

The third factor consisted of 4 DSQ questions: 12, 54, 66 and 69 (projection, passive aggression, displacement), the fourth factor consisted of 3 DSQ questions: 16, 23 and 24 (denial, dissociation, devaluation), the fifth factor consisted of 2 DSQ questions: 31 and 40 (fantasy), the sixth factor consisted of 3 DSQ questions: 42, 53 and 73 (splitting); the seventh factor consisted of 2 DSQ questions: 27 and 28 (acting out), the eighth factor consisted of 2 DSQ questions: 43 and 45 (splitting); the ninth factor consisted of 2 DSQ questions: 51 and 62 (somatization) and the tenth factor consisted of 3 DSQ questions: 1, 6 and 8 (rationalization).

DSQ questions 29, 71 and 76 did not include any of 10 components of our model, which can be seen in Table 2.

An analysis of connection between questions regarding the mature defense style showed a positive and statistically significant correlation ($p < 0.001$) between all segments of the mature defense with each other and with total score. That means that an increase in any segment of the mature defenses is accompanied by the increase in other segments and total score of this part of the DSQ-40, and vice versa, a reduction of any segment of the Mature defenses is accompanied by the reduction of all others.

The most important component of the total score of the mature defenses was sublimation, and the smallest one that contributed was humor, both being statistically significant ($p < 0.001$) (Table 3).

Table 2

Rotated Component Matrix

Question number	Component									
	1	2	3	4	5	6	7	8	9	10
DSQ1	0.002	0.296	-0.108	0.089	0.058	0.120	0.079	-0.059	0.141	**0.668**
DSQ3	**0.664**	-0.381	0.028	-0.179	-0.025	0.031	0.145	0.008	0.148	0.092
DSQ5	**0.724**	-0.037	-0.045	0.065	-0.146	0.210	-0.163	0.045	-0.014	-0.038
DSQ6	-0.106	0.416	-0.038	0.155	0.019	-0.223	-0.131	-0.055	-0.024	**0.586**
DSQ8	0.391	-0.109	-0.035	0.192	-0.098	-0.120	-0.133	-0.076	-0.088	**0.508**
DSQ12	0.215	0.035	**0.580**	0.187	0.061	-0.075	0.243	-0.175	0.088	-0.276
DSQ13	-0.107	**0.423**	0.007	0.279	-0.328	0.408	-0.103	-0.204	0.121	0.008
DSQ16	-0.002	0.112	0.106	**0.674**	-0.055	0.088	-0.075	-0.030	0.071	0.197
DSQ23	-0.008	0.151	0.052	**0.772**	0.173	0.036	0.056	0.116	0.137	0.012
DSQ24	-0.082	0.253	0.174	**0.515**	0.078	-0.191	0.383	0.141	-0.103	0.118
DSQ27	-0.061	-0.037	0.208	0.036	0.057	0.026	**0.799**	0.183	0.044	0.067
DSQ28	0.052	0.032	0.245	0.016	0.180	0.053	**0.614**	-0.054	0.266	-0.242
DSQ29	*-0.361*	*0.225*	*0.333*	*0.122*	*0.141*	*-0.316*	*0.109*	*-0.148*	*-0.001*	*0.293*
DSQ31	-0.051	0.002	0.205	0.145	**0.772**	-0.074	0.114	0.104	0.085	0.045
DSQ37	-0.054	**0.685**	0.113	0.181	-0.113	-0.098	-0.018	0.070	0.049	0.153
DSQ38	-0.037	**0.722**	-0.001	0.312	0.083	-0.126	0.010	0.073	-0.022	-0.135
DSQ40	-0.090	0.020	0.199	0.022	**0.748**	0.043	0.055	0.087	0.166	-0.064
DSQ42	-0.193	0.296	0.131	0.412	0.296	**-0.513**	0.139	0.027	0.110	-0.039
DSQ43	0.101	0.171	0.097	0.145	0.101	0.041	-0.025	**0.768**	0.205	-0.048
DSQ45	0.080	-0.037	0.192	-0.019	0.154	-0.017	0.369	**0.691**	0.187	-0.113
DSQ51	-0.013	0.148	0.164	0.222	0.170	-0.089	0.070	0.164	**0.671**	0.047
DSQ53	-0.252	0.188	0.372	0.160	0.180	**-0.527**	0.057	0.106	0.144	0.028
DSQ54	-0.061	0.142	**0.560**	-0.018	0.159	-0.021	-0.064	0.032	0.430	-0.107
DSQ58	-0.071	**0.517**	-0.238	0.187	0.081	-0.056	0.060	-0.292	0.211	0.104
DSQ59	**0.624**	0.124	-0.283	-0.011	-0.072	0.011	0.156	-0.016	-0.177	0.040
DSQ61	**0.747**	0.062	-0.161	0.179	-0.021	-0.115	-0.229	-0.074	-0.014	-0.084
DSQ62	-0.093	0.023	0.269	0.052	0.063	-0.038	0.345	0.279	**0.591**	0.044
DSQ63	-0.082	**0.611**	0.345	0.163	0.136	-0.061	0.123	0.063	0.025	0.132
DSQ66	-0.113	0.047	**0.699**	0.134	0.177	-0.099	0.159	0.004	0.093	-0.073
DSQ68	**0.492**	0.098	-0.036	-0.014	-0.327	0.039	0.057	0.221	-0.148	0.272
DSQ69	-0.152	-0.100	**0.579**	-0.016	-0.055	0.232	0.054	0.152	0.197	0.085
DSQ71	*-0.124*	*0.031*	*0.263*	*-0.071*	*0.330*	*0.222*	*0.236*	*-0.009*	*0.307*	*0.112*
DSQ73	0.222	-0.071	0.100	0.264	0.188	**0.642**	0.146	0.110	-0.018	-0.050
DSQ76	*-0.083*	*0.251*	*0.249*	*0.218*	*0.372*	*0.235*	*0.074*	*0.351*	*-0.239*	*0.124*
DSQ81	**0.651**	-0.260	-0.092	-0.159	0.163	0.377	0.010	0.149	-0.039	-0.080
DSQ82	-0.089	0.179	**0.654**	0.026	0.181	-0.103	0.171	0.182	0.061	-0.042
DSQ83	-0.119	-0.044	**0.573**	0.112	0.308	0.057	0.247	0.248	-0.123	0.009
DSQ84	**0.626**	-0.343	0.116	-0.288	0.081	0.194	0.118	0.031	0.066	0.029
DSQ86	-0.152	**0.677**	0.014	-0.156	-0.080	0.005	-0.024	0.096	0.086	0.148
DSQ88	0.182	**0.550**	0.117	-0.103	0.258	0.424	0.036	0.136	-0.035	0.025

DSQ – Defence Style Questionnaire.

Table 3

Correlations of mature defense mechanisms

Defense mechanisms		Humor	Anticipation	Suppression	Sublimation
Anticipation	r	0.458			
	p	0.001			
	N	284			
Suppression	r	0.473	0.592		
	p	0.001	0.001		
	N	284	284		
Sublimation	r	0.440	0.662	0.655	
	p	0.001	0.001	0.001	
	N	284	284	284	
Total	r	0.669	0.842	0.846	0.880
	p	0.001	0.001	0.001	0.001
	N	284	284	284	284

r – correlation coefficient; N – total number; *p* – significance.

Table 4

Correlations of neurotic defense mechanisms

Defense mechanisms		Pseudo-altruism	Reaction formation	Undoing	Idealization
Reaction formation	r	0.406			
	p	0.001			
	N	284			
Undoing	r	0.242	0.272		
	p	0.001	0.001		
	N	284	284		
Idealization	r	0.336	0.369	0.241	
	p	0.001	0.001	0.001	
	N	284	284	284	
Total	r	0.728	0.746	0.601	0.701
	p	0.001	0.001	0.001	0.001
	N	284	284	284	284

r – correlation coefficient; N – total number; *p* – significance.

An analysis of the connection between questions regarding the neurotic defense segment of the DSQ-40 showed that there was positive and statistically significant correlation ($p < 0.001$) between all segments of the neurotic defense mechanisms with each other and with total score. That means that increase in any segment of the neurotic defenses was accompanied by an increase in other segments and total score of this part of the DSQ-40, and vice versa, a reduction of any segment of the neurotic defense style was accompanied by reduction of all others.

The most important component of the neurotic defense style was the segment of reactive formation, and the least one contributing to the cancellation was undoing, both of them highly statistically significant ($p < 0.001$) (Table 4).

An analysis of the connection between questions regarding immature defense mechanisms of the DSQ-40 showed that there was a positive and statistically significant correlation ($p < 0.001$) between the majority of segments of the immature defenses with each other and with total score. That means that an increase in any segment of the immature defenses was accompanied by an increase in other segments and total score of this part of the DSQ-40, and vice versa, reduction of any segment of the immature defenses is accompanied by the reduction of all others.

The only negative, but significant correlation was recorded between scores of the rationalization and displacement.

Exceptions of this rule were scores of segments that were not significantly correlated ($p > 0.01$), such as splitting, denial, devaluation from one side, and displacement on the other side, as well as acting out and rationalization. Also, there was no significant correlation between scores of rationalization and somatization, scores of rationalization and projection, and scores of displacement and dissociation.

The most important components of the total score of the immature defenses were devaluation, passive aggression and splitting, and the smallest one was displacement; they all were statistically significant ($p < 0.001$) (Table 5).

An analysis of an interconnection of questions of the DSQ-40 segments showed that there was a positive and statistically significant correlation ($p < 0.001$) between segments of immature and neurotic total scores. That means that an increase in the total score of any immature defense mechanisms was accompanied by an increase in the neurotic de-

fense mechanisms scores of the DSQ-40, and, vice versa, a reduction of any of them led to a decrease in other scores.

If the relationships between the mature and other defense mechanisms were observed, they were negative and highly statistically significant ($p < 0.001$). This could be explained by the fact that an increase in scores in e mature defense mechanisms led to a decrease in scores in the immature and neurotic defense mechanisms, and vice versa (Table 6).

We present the basic characteristics and empirical norms obtained on non-clinical population of male professional military personnel. Items reffered to a defense mechanism are done, too (Table 7).

Table 5

Correlations of immature defense mechanisms

Defense mechanisms		Autistic fantasy	Projection	Dissociation	Somatization	Rationalization	Displacement	Isolation	Acting out	Devaluation	Denial	Passive aggression	Splitting
Projection	r	0.344											
	p	0.001											
	N	284											
Dissociation	r	0.153	0.185										
	p	0.010	0.00										
	N	284	284										
Somatization	r	0.365	0.403	0.139									
	p	0.001	0.001	0.019									
	N	284	284	284									
Rationalization	r	0.113	0.069	0.572	-0.008								
	p	0.052	0.246	0.001	0.887								
	N	284	284	284	284								
Displacement	r	0.237	0.259	0.098	0.282	-0.117							
	p	0.001	0.001	0.101	0.001	0.050							
	N	284	284	284	284	284							
Isolation	r	0.411	0.342	0.253	0.301	0.118	0.330						
	p	0.001	0.001	0.001	0.001	0.050	0.001						
	N	284	284	284	284	284	284						
Acting Out	r	0.312	0.368	0.116	0.531	-0.030	0.308	0.383					
	p	0.001	0.001	0.051	0.001	0.618	0.001	0.001					
	N	284	284	284	284	284	284	284					
Devaluation	r	0.287	0.299	0.490	0.275	0.385	0.060	0.345	0.212				
	p	0.001	0.001	0.001	0.001	0.001	0.315	0.001	0.001				
	N	284	284	284	284	284	284	284	284				
Denial	r	0.253	0.270	0.558	0.172	0.464	0013	0247	0.115	0563			
	p	0.001	0.001	.001	0.004	0.001	0828	0001	0051	001			
	N	284	284	284	284	284	284	284	284	284			
Passive aggression	r	0.372	0.528	.247	0.473	0.137	0.241	0461	0360	0309	0302		
	p	0.001	0.001	0.001	0.001	0.021	0.001	0001	0001	0001	.001		
	N	284	284	284	284	284	284	284	284	284	284		
Splitting	r	0.336	0.349	0.313	0.334	0.272	0.091	0368	0387	0406	0396	0453	
	p	0.001	0.001	0.001	0.001	0.001	0.126	0001	0001	0001	0001	0001	
	N	284	284	284	284	284	284	284	284	284	284	284	
Total	r	0582	0604	0600	0590	0444	0371	0641	0564	0678	0632	0678	0675
	p	0001	0001	0001	0001	0001	0001	0001	0001			0001	0001
	N	284	284	284	284	284	284	284	284	284	284	284	284

r – correlation coefficient ; N – total number; *p* – significance.

Table 6

Correlations of total scores of defense mechanisms

Defense mechanisms		Total neurotic	Total immature
Total immature	r	0.513	
	p	0.001	
	N	284	
Total neurotic	r	-0.286	-0.213
	p	0.001	0.001
	N	284	284

r – correlation coefficient; N – total number; *p* – significance.

Table 7

Defense mechanisms in Serbian healthy adult men (n = 284)

Defense mechanism	Item	Mean	SD
Mature		6.48	1.66
humor	8, 61	7.72	1.41
anticipation	68, 81	5.91	2.07
suppression	3, 59	6.43	2.11
sublimation	5, 84	5.87	2.49
Neurotic		4.26	1.36
pseudo-altruism	1, 86	5.70	2.07
reaction formation	13, 63	4.10	2.03
undoing	71, 88	3.09	1.76
idealization	51, 58	4.19	1.95
Immature		3.47	1.12
autistic fantasy	31, 40	2.37	1.81
projection	12, 66	2.54	1.61
dissociation	23, 37	4.16	1.97
somatization	28, 62	2.77	1.80
rationalization	6, 38	5.67	2.00
displacement	69, 73	2.71	1.63
isolation	76, 83	3.33	1.97
acting out	27, 46	3.18	1.96
devaluation	24, 29	4.49	2.21
denial	16, 42	3.73	1.96
passive aggression	54, 82	2.76	1.78
splitting	43, 53	3.95	2.04

SD – standard deviation.

Discussion

In the present study, we provided an evidence for the appropriateness of the DSQ-40 version for use in male middle age population (23 to 53 years old; 35.09 ± 7.21 years on the average). Our evaluation of Serbian version of DSQ-40 confirmed highly significant intraclass correlation coefficients as well as the compactness and high reliability of this questionnaire.

In our country, Čabarkapa and Dedić [27] established the empirical norms for male adolescent population (n = 400, 19–27 years old, 20.40 ± 1.44 years, on the average) and for elderly adults (n = 165) divided into two groups: A-group (n = 80 respondents, 38-45 years old, mean = 42.64 ± 3.16 years) and B-group (n = 85 respondents, 45–56 years old, 49.32 ± 2.39 years, on the average) [27]. In this way, our research completed the gape for empirical norms for the middle age male population, between adolescence and the elderly population. Besides, in mentioned investigation of Čabarkapa and Dedić [27], a psychometric evaluation of the DSQ-40 was not done, which is the advantage of our investigation. On the other hand, we showed that the DSQ-40 could be used in investigations, not only in clinical, but also in nonclinical settings.

Using the DSQ-40 is an easy and economical way to determine defense mechanisms, as well as hierarchically grouping defense mechanisms into defensive styles in a respondent population. The advantages of the DSQ-40 are that it saves time and does not require highly trained professionals to use it, that is, it is easy to process results. In military psychology and psychiatry, the DSQ-40 has the significance of defining a defensive style that describes the behavior of professional military personnel in the unit, as the degree of their adaptation to the military environment.

Deviations from standard values and empirical norms obtained on our sample of respondents can serve as a measure of psychopathology, which can help doctors in assessing psychopathology. Adaptive style measurement using the DSQ-40 can be useful as a measure of psychopathology and can help in risk assessment, treatment planning and assessment of treatment course. Also, it can be used in assessing a disease remission, as well as in assessing vulnerability to possible diseases [11–16].

Limitation

The study was conducted among male professional military personnel. The sample was homogeneous regarding gender, and social, cultural, economic and educational characteristics. However, further investigations require the validation of the DSQ-40 on a female sample, too.

The factor structure of the Serbian version needs further exploration, regarding the immmature defenses factors. Dispersion of immature defense mechanisms, included in 7 factors, was expected, because our respondents were mental and somatic healthy military personnel, mostly with mature personal organization using mature defenses mechanisms.

However, the factor structure of the Serbian version of the DSQ-40 needs further exploration. Further research

should also consider the validation of the DSQ-40 both on a larger non-clinical sample and on a clinical sample.

In further work, a focus should be put on improving psychometric characteristics of the DSQ-40 and additional correlation and factor analyses, an individual and associated defense mechanisms and, more accurately, it should estimate differences that can exist with respect to defense mechanisms in subjects that differ in age, gender and psychological status, which would confirm our standardization of this instrument in the Serbian population.

Conclusion

Psychometric evaluation of the short version of the DSQ-40 performed on the Serbian middle age healthy men supports its applicability in non clinical settings.

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The role of cognitive emotion regulation strategies in health related quality of life of breast cancer patients

Značaj kognitivnih strategija emocionalne regulacije za kvalitet života obolelih od karcinoma dojke

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Abstract

Background/Aim. Breast cancer is often accompanied by patients' unpleasant emotional states, which can significantly affect both the undergoing treatment and the quality of life of patients. The aim of this study was to examine the mediating role of cognitive emotion regulation strategies in relation between emotional distress and various aspects of patients' quality of life, which would further indicate different psychotherapeutic interventions in psycho-oncological practice. **Methods**. The sample consisted of 97 breast cancer patients. Emotional distress was measured by the Depression Anxiety Stress Scale (DASS-21), cognitive emotion regulation strategies were measured using the Cognitive emotion Regulation Questionnaire (CERQ-36), while various aspects of health related quality of life were assessed using the Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire. Multiple simultaneous mediations between variables were established using the process macro INDIRECT for SPSS**. Results.** Positive refocusing had positive effects both on physical [a = -0.83, b = 0.50, ab = -0.42, standard error (SE) = 0.14; 95% confidence interval (CI) = 0.17 – 0.83] and emotional well-being (a = -0.83, b = 0.29, ab = 0.24, SE = 0.13; 95% CI = -0.01 – 0.58) of the patients. Rumination negatively affected emotional well-being (a = -0.75, b = -0.33, ab = -0.25, SE = 0.16; 95% CI = -0.71 – -0.01) of the patients. Catastrophizing had a negative impact on social (a = 0.96, b = 0.12, ab = -0.12, SE = 0.13; 95% CI = -0.33 – -0.13) and functional well-being of the patients (a = 0.96, b = -0.16, ab = -0.15, SE = 0.09; 95% CI = -0.32 – -0.01). **Conclusion.** Positive refocusing, rumination and catastrophizing are significant cognitive coping strategies through which the intensity of emotional distress significantly changes, and this can be subsequently reflected in different aspects of patients' health related quality of life. The above mentioned implies potential benefits of implementation of cognitive-behavioral trainings and interventions directed towards acquiring adaptive cognitive emotion regulation strategies, in order to improve the quality of life of breast cancer patients.

Key words:
breast neoplasms; quality of life; cognitive remediation; treatment outcome.

Apstrakt

Uvod/Cilj. Karcinom dojke je oboljenje koje je često praćeno neprijatnim emocionalnim stanjima bolesnica i emocionalnim distresom, što značajno može da utiče kako na proces lečenja, tako i na kvalitet života obolelih. Stoga je cilj ove studije bio da ispita potencijalnu medijacionu ulogu strategija kognitivne emocionalne regulacije u relaciji između emocionalnog distresa i različitih aspekata kvaliteta života obolelih, što bi dalje indikovalo potencijalne psihoterapijske intervencije u kliničkoj psihoonkološkoj praksi. **Metode.** Istraživanje je dizajnirano kao studija preseka, u kojoj je učestvovalo 97 bolesnica sa dijagnozom karcinoma dojke. Emocionalni distres meren je Skalom depresivnosti, anksioznosti i stresa (DASS-21). Strategije kognitivne emocionalne regulacije merene su Upitnikom kognitivno emocionalne regulacije (CERQ-36), dok su različiti aspekti kvaliteta života procenjeni Upitnikom funkcionalne procene terapije karcinoma (FACT-B). Za utvrđivanje multiple simultane medijacije između varijabli korišćen je program makro INDIRECT za SPSS. **Rezultati.** Pozitivnim refokusiranjem bili su ostvareni pozitivni efekti kako na telesno [a = -0,83, b = 0,50, ab = -0,42, *standard error* (SE) = 0,14; 95% *confidence interval* (CI) = 0,17 – 0.83], tako i na emocionalno blagostanje

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bolesnica (a = -0,83, b = 0,29, ab = 0,24, SE = 0,13; 95% CI = -0,01 – 0,58). Ruminacije su se negativno odražavale na emocionalno blagostanje (a = -0,75, b = -0,33, ab = -0,25, SE = 0,16; 95% CI = -0,71 – -0,01) bolesnica. Katastrofiziranjem je bio ostvaren negativan uticaj na socijalno (a = 0,96, b = 0,12, ab = -0,12, SE = 0,13; 95% CI = -0,33 – -0,13) i funkcionalno blagostanje bolesnica (a = 0,96, b = -0,16, ab = -0,15, SE = 0,09; 95% CI = -0,32 – -0,01). **Zaključak.** Pozitivno refokusiranje, ruminacija i katastrofiziranje predstavljaju značajne kognitivne strategije prevladavanja posredstvom kojih se značajno menja intenzitet emocionalnog distresa, što se potom odražava i na različite aspekte kvaliteta života obolelih. Navedeno implicira potencijalne koristi od uvođenja kognitivno-bihejvioralnih intervencija, usmerenih na usvajanje adaptivnih strategija kognitivne regulacije afekta, a u cilju pospešivanja kvaliteta života obolelih od karcinoma dojke.

Ključne reči:
dojka, neoplazme; kvalitet života; kognitivna terapija; lečenje, ishod.

Introduction

Breast cancer is a disease that is treated increasingly successfully, but at the same time introduces significant changes in a patient's life, provokes different types of loss (physical strength, body integrity, independency, sense of control, sexuality, temporary or permanent reorganization of family roles, etc.), involves demanding and long-term treatments with numerous side effects (e.g. hair loss, nausea, hormonal and body weight changes, difficulties in cognitive functioning) which can all initiate the painful and unpleasant emotional states of patients. The prevalence of emotional distress in patients suffering from malignant diseases in the first year after diagnosis is higher than 30% [1, 2], therefore the distress is often noticed as the "sixth vital sign", and there is an increasing emphasis on its timely screening and adequate treatment [3–5]. Emotional distress includes the continuum of negative affective states, from normal and common feelings such as worry, sorrow, anger and fear, to clinically more significant anxious and depressive symptomatology, and these affective states can interfere with the decision-making process [6] and a patient's compliance during treatment, including greater likelihood of a negative outcome [7, 8]. Thus, emotional distress affects and deteriorates the health related quality of life of patients [9]. Health related quality of life is a subjective perception of patients in terms of their overall state of physical, emotional, social and actual functional well-being, so the assessment covers key aspects of patients' lives [10], and one of the main aims of oncological treatment is to increase as much as possible, each of the stated aspects. As emotions are critical to the functioning and goal-oriented behavior and adaptation to the disease and its treatment, and are directly related to both mental and physical health, i.e. subjective well-being of patients, their regulation can significantly affect the quality of life of patients [11]. Emotion regulation involves processes that influence the emotions we have, when we have them, and how we experience and manifest them [12], so when we are faced with negative emotional states, we can use a number of regulation strategies to minimize or otherwise exacerbate their intensity and duration [13, 14]. Since the concept of emotion regulation is very broad and includes wide range of regulatory processes (e.g. biological, social, behavioral, as well as conscious and unconscious cognitive processes), particularly during the stressful situations when it is specified as a coping mechanism, as well as because of some limitations that earlier well- known models of stress and coping revealed (e.g. lack of distinction between behavioral and cognitive components of coping in the Lazarus and Folkman's model), Garnefski et al. [15] have tried to overcame these conceptual problems and they made a 'conceptually pure' measure of self-regulatory, conscious, cognitive aspects of emotion regulation. Therefore, one of the ways in which the regulation of emotions could be achieved, observing from the cognitive-oriented perspective, is through specific strategies of cognitive-emotion regulation or, in other words, through a specific way of thinking during or after a stressful situation itself [15]. This means that if we feel sadness or fear provoked by some event, we can intensify them by focusing on them, for example, through rumination, or further intensify them through catastrophizing, i.e. emphasizing the negative aspects of the event itself. Similarly, we can redirect thoughts to other contents instead of thinking about the event, specifically, positively refocus to possibly reduce the negative affect [16]. Above mentioned implies that some coping strategies are more adaptive than others, i.e. adaptive strategies decreases emotional distress, and lead to better psychological outcomes, while maladaptive strategies can intensify emotional distress and are associated with greater symptoms of psychopatology [16]. The dynamics of cognition and emotions are further clarified by results of neuroimaging studies indicating that in a neural basis, for example, of "reappraisal", as a cognitive aspect of emotion regulation, is the interaction not only of the prefrontal and cingular regions responsible for the cognitive control, but also the interaction of the amygdala and insula, systems involved in emotional reactions, and so, e.g. when we are thinking in a way that intends to intensify the emotional experience and the activity of the amygdala increases as a result, and *vice versa*, a cognitive strategy that aims to reduce the intensity of the emotions also results in decreased activity of the amygdala [17]. Previous studies, done with healthy population and breast cancer patients, have indicated positive effects of using adaptive strategies in reducing the negative affect, as well as in better functioning in interpersonal relations, and that they are beneficial for general psychological and physical well-being. On the other hand, the use of maladaptive strategies is associated with increased symptoms of anxiety and depression, prolonged and more pronounced distress, intensified pain, increased inflammation, higher blood pressure, and generally reduced quality of life [14, 18–21]. Cognitive emotion regulation

strategies such as greater acceptance, positive refocusing, and positive reappraisal were associated with fewer depressive symptoms one month after initial assessment in the study done with women newly diagnosed with breast cancer [20]. The results of another study showed that compared with healthy women, women newly diagnosed with breast cancer reported more frequent use of catastrophizing, a maladaptive cognitive emotion regulation strategy, and less frequent use of adaptive strategies such as positive refocusing, refocusing on planning, and positive reappraisal [21]. Furthermore, self-blame, rumination, and catastrophizing negatively affected their overall quality of life, while on the contrary, acceptance and positive reappraisal had positive effects on the quality of life of breast cancer patients [21].

Considering the importance of cognitive emotion regulation strategies not only for the mental but also for the physical health, and as the topic is still insufficiently explored, especially in the context of clinical psycho-oncology, the aim of this study was to examine the potential mediating role of cognitive emotion regulation strategies in relation between emotional distress and various aspects of the quality of life of breast cancer patients. The assumption is that through adaptive strategies such as planning, acceptance, positive reappraisal, positive refocusing and putting into perspective [16] it is possible to reduce the symptoms of emotional distress, while maladaptive strategies, i.e., catastrophizing, rumination, self-blame and blaming others [16] intensify the symptoms of emotional distress, which then, positively or negatively, reflects on the patients' health related quality of life in its various domains. The findings of the study could provide a clearer insight into the ways in which breast cancer patients regulate their affect, and possibly offer implications for clinical practice, particularly indicating the potential benefits of implementing different cognitive-behavioral interventions in oncological treatment, because one of the main aims of cognitive behavioral therapy is to alleviate distress and foster adaptive emotions and behavior by accomplishing the change in maladaptive cognitions, i.e. how we feel and behave dependent on our thoughts and beliefs about stressful situation itself; the key is in overcaming cognitive distortion and dysfunctional thoughts by facilitating more effective and rational thinking.

Methods

The research was conducted during the 2016 and 2017 year and it was designed as a cross-sectional study, consisting of 97 breast cancer patients with average age of 57.43 years (range 29–78 years), who underwent breast cancer surgery, and who had one of the integrated psychological treatment with psychologist at the Institute of Oncology of Vojvodina. The study did not include those patients who were currently on chemotherapy and/or radiotherapy, as well as those in whom the disease was progressed (presence of distant metastases), in order to eliminate possible confounding effects on patients' health related quality of life. Most women (65%) were married and had secondary school (51%) and faculty (34%) as the level of education.

For the emotional distress, the total score of the Depression Anxiety Stress Scale (DASS-21) [22] was used, and included assessment of the level of depression (e.g. 'I couldn't seem to experience any positive feeling at all', 'I felt that I had nothing to look forward to', 'I felt that life was meaningless'), anxiety (e.g. 'I was aware of dryness of my mouth', 'I experienced trembling','I felt I was close to panic') and stress (e.g. 'I tended to over-react to situations', 'I found myself getting agitated', 'I found it difficult to relax') during the previous week. The internal consistency of the scale in our sample was high and ranged from $\alpha = 0.83$ to $\alpha = 0.89$ for subscales, and $\alpha = 0.93$ for the total score, which is a measure of emotional distress.

Specific cognitive emotion regulation strategies, that is, specific ways of thinking that are usually activated after the experience of a negative life event in order to regulate emotions, were measured by the Cognitive Emotion Regulation Questionnaire (CERQ) [23]. The questionnaire consists of 9 subscales: Self-blame (the tendency of a patients to blame themselves for a stressful life event), Acceptance (accepting thoughts and feelings about a stressful event), Rumination (intense thinking and preoccupation with thoughts and feelings related to a stressful event), Positive refocusing (redirecting thoughts from a stressful event to positive content), Planning (thinking about what to do to influence the possible consequences of a stressful event), Positive reappraisal (seeing the positive aspects of a stressful event), Putting into perspective (relativizing and decreasing the significance of the event itself), Catastrophizing (emphasizing negative aspects and consequences of a stressful event), and Other-blame (blaming others and the circumstances that led to the event itself). The internal consistency of the scale in our sample was high and ranges from $\alpha = 0.75$ to $\alpha = 0.81$.

Different aspects of patients' health related quality of life (physical well-being, social well-being, emotional well-being and functional well-being, i.e. state of being healthy and satisfied in those domains) were measured by the Functional Assessment of Cancer Therapy – Breast (FACT-B) [24], a questionnaire specified for the evaluation of breast cancer patients' health related quality of life. The internal consistency of this scale in our sample is also high and ranges from $\alpha = 0.72$ to $\alpha = 0.93$.

Data processing was performed using a macro INDIRECT for SPSS [25], which serves to determine multiple simultaneous mediations between variables. The method allows conducting an analysis of the total indirect effect, i.e. the join effect of all mediation variables included in the research and analysis of specific indirect effects, i.e. the effect of each mediator separately. It is possible to examine the total effect of the predictor variable on the criterion variable (c), and the direct effect of the predictor variable on the criterion variable when the mediators are controlled (c'), and the indirect effect, that is, the individual mediator effect of each mediator separately on the relationship between the predictor and mediator (ab). In addition to simultaneous introduction of a larger number of mediators in the analysis, this procedure also covers a bootstrapping method for calculating the confidence interval of an indirect effect. The logic behind this method is reflected in the inclusion of a larger number of

repetitions of the sampling itself, and the assessment of the indirect effect for each sample separately. Repeating this process 1,000 times, bootstrapping allows empirical approximation of sample distribution to the real population, and corrected bias builds confidence intervals for the indirect effect of the predictor on the criterion variable. The lower limit [lower 95% confidence interval (LCI)] represents the lowest value of the indirect effect (ab), and the upper limit represents the highest value (95% HCI). In order for the mediating effect to be significant, zero should not be included in the CI [25].

Results

Table 1 includes descriptive indicators of the variables used in the research and the Cronbach's alpha for each of the subscales. As the Other-blame subscale deviated from normal distribution, it was not included in further analysis. Patients showed a tendency to use adaptive cognitive emotion regulation strategies (planning, acceptance, positive reformulation, positive refocusing, putting into perspective), while maladaptive strategies were less pronounced. Among maladaptive strategies, ruminations and catastrophizing stood out. Table 2 shows results of the mediating analysis. Emotional distress was a predictor variable, various aspects of quality of life were criterion variables, while cognitive emotion regulation strategies were potential mediators of the relationship. In the relation between emotional distress and physical well-being, the total effect [ab = -0.46, standard error (SE) = 0.30; 95% LCI = 0.14, 95% HCI = 1.06] and the indirect effect of positive refocusing (a = -0.83, b = 0.50, ab = -0.42, SE = 0.14; 95% LCI = 0.17, 95% HCl = 0.83) were significant. Since the direct effect was not significant, positive refocusing was a complete mediator and has a positive effect on physical well-being (Figure 1).

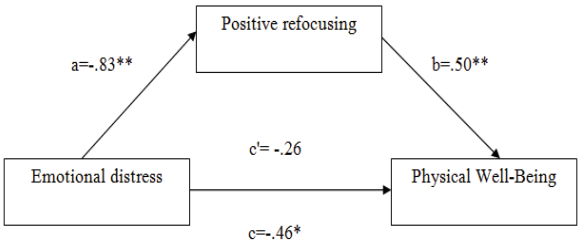

Fig. 1 – Relationship between emotional distress and physical well-being mediated by positive refocusing.

a – effect of predictor variable on mediator; b – effect of mediator variable on criterion; c' – direct effect of predictor variable on criterion variable when the effect of mediator is controlled; c – total effect.
***p* < 0.05; ****p* < 0.001.**

Table 1

Descriptive statistics and Cronbach's alpha coefficients (α) for study variables

Questionnaries	Subscale	Min	Max	M	SD	Sk	Ku	α
CERQ-36	Self- blame	4	18	9.14	3.73	0.57	-0.55	0.75
	Acceptance	5	20	14.40	3.72	-0.45	-0.44	0.76
	Rumination	4	20	10.70	3.95	0.11	-0.80	0.75
	Positive refocusing	5	20	13.76	4.14	-0.41	-0.83	0.80
	Planning	7	20	15.07	3.50	-0.42	-0.55	0.75
	Positive reappraisal	4	20	14.39	3.88	-0.26	-0.64	0.80
	Putting into perspective	4	20	13.70	3.67	-0.47	-0.07	0.69
	Catastrophizing	4	20	8.86	4.08	1.05	0.43	0.81
	Other-blame	4	18	6.90	3.03	1.69	3.36	0.76
	Physical well-being	0	28	12.35	9.01	0.34	-1.29	0.93
FACT-B	Social well-being	10	24	19.56	3.37	-0.35	-0.72	0.72
	Emotional well-being	0	24	12.04	6.76	0.21	-1.26	0.85
	Functional well-being	10	28	19.94	4.53	-0.16	-0.86	0.78
	Emotional distress	0	50	15.58	12.6	0.75	-0.06	0.94
DASS-21	Depression	0	16	4.75	4.10	0.65	-0.31	0.86
	Anxiety	0	18	4.26	4.02	1.00	0.70	0.83
	Stress	0	19	6.88	4.92	0.63	-0.27	0.89

CERQ – Cognitive Emotion Regulation Questionnaire; FACT-B – Functional Assessment of Cancer Therapy-Breast; DASS – Depression Anxiety Stress Scale; Min – minimum; Max – maximum; M – mean value; SD – standard deviation; Sk – skewness; Ku – kurtosis.

Table 2
Cognitive emotion regulation strategies as mediators between emotional distress and different aspects of health related quality of life in breast cancer patients

Cognitive emotion regulation strategies	Basic parameters			95% CI	
	Coefficient ab (SE)	Coefficient a	Coefficient b	Lower	Upper
Distress and Physical well-being					
Mediators and effects					
Direct effect (*c'*)	-0.26 (0.23)			-0.19	0.72
Total effect (*c*)	-0.46 (0.30)*			0.14	1.06
Indirect – Self-blame	0.01 (0.05)	0.18	0.08	-0.22	0.38
Indirect – Acceptance	0.02 (0.07)	0.08	0.30	-0.11	0.71
Indirect – Rumination	-0.20 (0.17)	0.75**	-0.27	-0.69	0.15
Indirect – Positive refocusing	-0.42 (0.14)**	-0.83**	0.50**	0.17	0.83
Indirect – Planning	0.03 (0.07)	-0.08	-0.31	-0.83	0.21
Indirect – Positive reappraisal	-0.04 (0.18)	-0.58**	0.07	-0.44	0.58
Indirect – Putting into perspective	0.10 (0.11)	-0.38*	-0.26	-0.69	0.17
Indirect – Catastrophizing	0.30 (0.19)	0.96**	0.32	-0.08	0.71
Distress and Social well-being					
Mediators and effects					
Direct effect (c')	-0.04 (0.16)			-0.35	0.28
Total effect (c)	-0.28 (0.12)*			-0.52	-0.030.
Indirect – Self-blame	-0.02 (0.04)	0.12*	-0.16*	-0.33	-0.01
Indirect – Acceptance	0.01 (0.03)	0.23	0.04	-0.20	0.27
Indirect – Rumination	-0.03 (0.12)	0.77**	-0.04	-0.19	0.20
Indirect – Positive refocusing	-0.05 (0.07)	-0.76**	0.07	-0.11	0.25
Indirect – Planning	-0.01 (0.04)	-0.05	0.13	-0.16	0.42
Indirect – Positive reappraisal	-0.05 (0.08)	-0.52**	0.09	-0.18	0.35
Indirect – Putting into perspective	0.00 (0.05)	-0.28	-0.01	-0.27	0.25
Indirect – Catastrophizing	-0.12 (0.13)*	0.96**	-0.12*	-0.33	-0.13
Distress and Emotional well-being					
Mediators and effects					
Direct effect (c')	-0.53 (0.27)*			0.01	1.06
Total effect (c)	-0.32 (0.20)*			0.07	0.72
Indirect – Self- blame	0.01 (0.04)	0.18	0.05	-0.21	0.32
Indirect – Acceptance	0.02 (0.05)	0.08	0.24	-0.12	0.61
Indirect – Rumination	-0.25 (0.16)*	0.75**	-0.33**	-0.71	-0.01
Indirect – Positive refocusing	0.24 (0.13)*	-0.83**	0.29*	0.01	0.58
Indirect – Planning	0.01 (0.05)	-0.08	-0.15	-0.62	0.32
Indirect – Positive reappraisal	-0.05 (0.16)	-0.58**	0.09	-0.37	0.55
Indirect – Putting into perspective	0.06 (0.09)	-0.38*	-0.16	-0.54	0.23
Indirect – Catastrophizing	0.23 (0.17)*	0.96**	0.24	-0.11	0.60
Distress and Functional well-being					
Mediators and effects					
Direct effect (c')	-0.19 (0.08)			-0.44	0.01
Total effect (c)	-0.53 (0.27)**			-0.73	-0.33
Indirect – Self- blame	0.01 (0.02)	0.18	0.04	-0.09	0.16
Indirect – Acceptance	-0.01 (0.02)	0.08	-0.09	-0.26	0.08
Indirect – Rumination	-0.07 (0.06)	0.75**	-0.09	-0.27	0.08
Indirect – Positive refocusing	-0.04 (0.08)	-0.83**	0.05	-0.09	0.19
Indirect – Planning	-0.02 (0.04)	-0.08	0.21*	0.01	0.42
Indirect – Positive reappraisal	-0.06 (0.07)	-0.58**	0.10	-0.11	0.31
Indirect – Putting into perspective	-0.01 (0.03)	-0.38**	0.01	-0.17	0.19
Indirect – Catastrophizing	-0.15 (0.09)*	0.96**	-0.16*	-0.32	-0.01

Coefficient ab – indirect effect of mediator in relation between predictor and criterion variable; a – effect of predictor variable on mediator; b – effect of mediator variable on criterion; c' – direct effect of predictor variable on criterion variable when the effect of mediator is controlled; c – total effect (all effects are non-standardized regression coefficients); CI – confidence interval.
***p* < 0.05; ****p* < 0.001.**

In the relation between emotional distress and social well-being, the total effect (ab = -0.28, SE = 0.12; 95% LCI = -0.52, 95% HCI = -0.03) and the indirect effect of catastrophizing (a = 0.96, b = 0.12, ab = -0.12, SE = 0.13; 95% LCI = -0.33, 95% HCI = -0.13) were significant. As the direct effect was not significant, catastrophizing was a complete mediator of the relation and negatively affected social well-being (Figure 2).

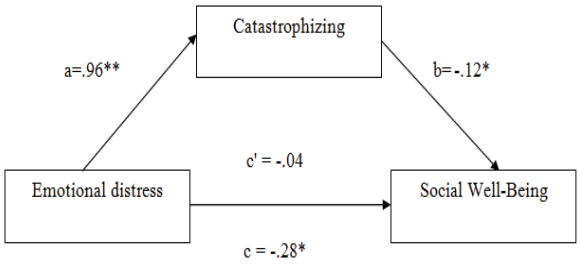

Fig. 2 – Relationship between emotional distress and social well-being mediated by catastrophizing.

a – effect of predictor variable on mediator; b – effect of mediator variable on criterion; c' – direct effect of predictor variable on criterion variable when the effect of mediator is controlled; c – total effect.
***p* < 0.05; ***p* < 0.001.**

When it comes to the relation between emotional distress and emotional well-being, significant were both the direct effect (ab = -0.53, SE = 0.27; 95% LCI = 0.01, 95% HCI = 1.06) and the total effect (ab = -0.32, SE = 0.20, 95% LCI = 0.07, 95% HCI = 0.72), as well as indirect effects of rumination (a = -0.75, b = - .33, ab = -0.25, SE = 0.16; 95% LCI = -0.71 , 95% HCI = -0.01) and positive refocusing (a = -0.83, b = 0.29, ab = 0.24, SE = 0.13; 95% LCI = -01, 95% HCI = 0.58). Given that both direct and total effects were significant, mediation was partial, i.e. distress remained a significant predictor of emotional well-being along with rumination that negatively affected emotional well-being and positive refocusing that positively affected the emotional well-being of patients (Figure 3).

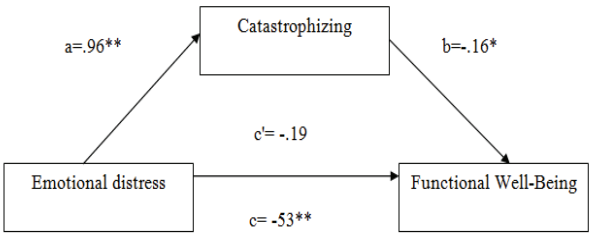

Fig. 3 – Relationship between emotional distress and functional well-being mediated by catastrophizing.

a – effect of predictor variable on mediator; b – effect of mediator variable on criterion; c' – direct effect of predictor variable on criterion variable when the effect of mediator is controlled; c – total effect.
***p* < 0.05; ***p* < 0.001.**

Finally, in the relation between emotional distress and functional well-being, the total effect (ab = -0.53, SE = 0.27; 95% LCI = -0.73, 95% HCI = -0.33) and the indirect effect of catastrophizing (a = 0.96, b = -0.16, ab = -0.15, SE = 0.09; 95% LCI = -0.32, 95% HCI = -0.01) were significant. As the direct effect was not significant, catastrophizing was a complete mediator of the relationship and negatively affected the functional well-being of patients (Figure 4).

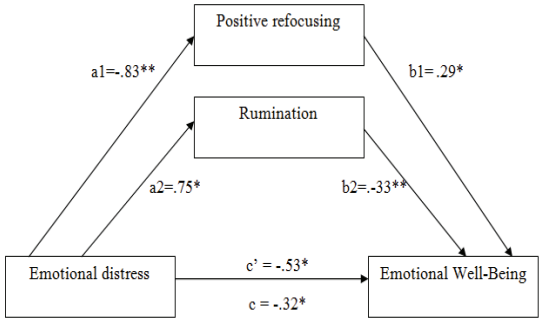

Fig. 4 – Relationship between emotional distress and emotional well-being mediated by rumination and positive refocusing.

a – effect of predictor variable on mediator; b – effect of mediator variable on criterion; c' – direct effect of predictor variable on criterion variable when the effect of mediator is controlled; c – total effect.
***p* < 0.05; ***p* < 0.001.**

Discussion

According to the aim of our study we started from the assumption that breast cancer, as one of the leading stressful events, provokes various unpleasant emotional states of patients, that, depending on the cognitive strategies used for regulation of the affect, can be intensified or mitigated, which then differently reflects on the physical, social, emotional and functional well-being of patients. Of particular interest to us was the cognitive aspect of emotion regulation, because it is an aspect that relates to conscious processes, including the perception of the situation itself, i.e. disease and treatment, which can be influenced by the training of patients in constructive techniques, or in other words, those that would allow them to make a successful adaptation to malignant disease and its treatment [26]. The obtained findings revealed that out of nine cognitive emotion regulation strategies, three strategies emerged as significant cognitive mediators of the relationship, and partially or completely changed the relationship between the negative emotional experience and the quality of life of patients.

Positive refocusing, a strategy that represents the redirection of thoughts, or the distraction of attention, from a stressful event to neutral or more enjoyable contents, proved to be beneficial for the emotional and physical well-being of patients. This means that when patients use positive refocusing, they reduce emotional distress by not focusing on it, but turning to positive stimuli, which positively reflect on the emotional well-being of patients, and it is also a strategy which reduces the influence of negative emotions on the perception of physical state caused by malignant disease. The obtained relation is also confirmed by the findings of earlier

studies which have showed that this technique of distraction, which is very often applied in health settings, is useful in the reduction of chronic pain and anxiety provoked by painful conditions [27], as well as in the induction of a positive affect [28], and that the application of this strategy over time can contribute to the reduction of depressive and anxious symptomatology [29], which all suggests that insisting on its use can be beneficial both to the emotional and physical well-being of patients.

On the other hand, rumination, or an alternative strategy to the previous one, which represents an intense focus on and preoccupation with one's own thoughts and feelings in relation to a stressful event, negatively affects the emotional well-being of patients. Therefore, those patients who tend to intensively deal with what they are currently experiencing, and even if their aim is to help themselves through better understanding what actually happened to them, leads to the escalation of emotional distress and contributes to even more negative evaluation of one's own emotional well-being. These are the most commonly intrusive thoughts in the form of brooding that lead to the intensification of the symptoms of anxiety, depression and stress [30], they inhibit effective problem solving and aim-oriented (instrumental) behavior [31], and rumination is significant cognitive factor that represents vulnerability for the development, maintenance, intensity and recurrence of depression [32, 33].

The tendency of patients to catastrophize or to overestimate the horror they have experienced, the negative aspects and possible consequences of a stressful event, negatively affects their functional and social well-being. This finding is not surprising, since catastrophizing is irrational and maladaptive thinking typical especially for the cognitive style of patients with anxious and depressive disorders, it is linked to intensification and maintenance of emotional distress [34, 35] and it is also related with experience of increased pain which may cause functional disability and impaired daily activities [35, 36]. Furthermore, the patients who tend to catastrophize, due to their social information-processing biases, will not usually notice supportive social relationships, nor the way in which the social environment accepts the disease positively, but will be sensitive to the negative interactions and they will overestimate non-acceptance and rejection [36], therefore, their health related quality of life in the social domain will be poorly perceived.

These findings are more than significant because they imply potential benefits of the implementation of cognitive behavioral therapy programs and interventions directed at rumination and catastrophizing as key mediators through which the negative impact of emotional distress is maintained and intensified, where insisting on positive refocusing can act therapeutically on the quality of life of breast cancer patients. For example, rumination-focused cognitive behavioral therapy, which starts from the assumption that rumination is a normal and understandable process that can be useful if used properly, through which patients are trained how to recognize ruminative thoughts related to the stressful event, which aspects of thoughts represent helpful, and which represent unhelpful thinking, how that reflects on their emotions and behavior, and how they can develop healthy alternatives in thinking (through relaxation, assertiveness, imagination, behavioral experiments), shows more and more importance and confirmation [37, 38]. Similarly, mindfulness-based cognitive therapy, which combines mindfulness-based stress reduction and cognitive behavioral therapy and implies awareness and nonjudgmental attitude as well as the acceptance of catastrophic and ruminative thoughts and negative mood, and then fostering to overcame them through the process of decentring and education, shows particular success in physically and chronically ill patients such as cancer patients, since focus is placed not only on emotions but also on painful bodily sensations [39, 40]. When it comes to positive distraction as a cognitive behavioral intervention, i.e., the effectiveness of the positive refocusing strategy, we proceed from the point that its application may be beneficial when it is followed by the acceptance of the disease and when it is used for the treatment of unhealthy rumination, and not when it represents an avoiding strategy that in long term can have a negative impact on emotional well-being, because a precondition for emotional well-being is contact with emotions, their acceptance, and understanding the meaning of unhealthy emotions [41]. Previous studies done with cancer patients have confirmed the effectiveness of cognitive behavioral interventions aimed at reducing the emotional distress and improving mental health [26, 42, 43], likewise, in controlling pain and painful conditions [44], as well as in optimizing functional status and reducing post-cancer fatigue [45], i.e. cognitive behavioral therapy shows beneficial effects for the overall health-related quality of life of breast cancer patients years after oncological treatment [46].

Given the fact that this study offers really significant implications for oncology settings, there are a few unanswered questions that at the same time represent a recommendation for the future research. Firstly, it was a cross-sectional study, so we can not suggest with certainty on the direction of the obtained relations, therefore the longitudinal monitoring of patients is indicated. Furthermore, it would be useful to examine the patients at different stages of malignant disease and its treatment, but also to compare the sample with those in different health situations in which the stressor at the time of the assessment is more controllable, since it is possible that the cognitive strategies, such as positive refocusing, in the regulation of the current distress, came to the fore because of the context itself, when the situation can be perceived as still uncertain and uncontrollable, and maybe it is still early for the more complex strategies such as positive reappraisal and putting into perspective. Also, it is very possible that female gender had an impact on the activation of these strategies, as earlier studies have shown that women are more likely to use rumination, catastrophizing and positive refocusing when dealing with stressful events [47]. Finally, we do not know how much the contribution is and whether this relation is moderated by neuroticism and a negative affect as personality traits in our sample, because these personality traits, along with the previous life experience are predispositions for the use of maladaptive cognitive strategies such as ruminations and catastrophizing [48], and therefore more intense emotional distress, so these variables should be also included and controlled in some of the future research.

Conclusion

Positive refocusing, rumination and catastrophizing are significant cognitive mediators of the relation between negative emotional experience and various aspects of the quality of life of breast cancer patients. Implementation of cognitive-behavioral interventions directed towards acquiring adaptive cognitive emotion regulation strategies would have positive effects on the improvement of the health related quality of life of breast cancer patients. For example, for patients who tend to use rumination, i.e. have intrusive thoughts such as 'Why the cancer has happened to me', 'What are the reasons that I deserve it', 'How will I cope', the interventions should be directed at refocusing attention on the present moment, accepting that disease has happened as also as the thoughts and feelings related to it, fostering healthy cognitions, finding solutions and planning the actions to manage possible consequences of cancer and its treatment. For patients who tend to catastrophize, e.g. 'My life is over, I won't succeed', 'When I lose my hair everyone will pity me', 'After mastectomy, I feel like I am not a whole person anymore', interventions should be directed at recognising the overestimation of negative predictions, relativizing and decreasing them through the prediction of possible positive outcomes, finding the meaning of stressful experience, increasing self- efficacy, self- esteem and self-confidence, fostering positive social support and relationships. These interventions are necessary part of oncological treatment and they are certainly more than beneficial for coping with malignant disease and the breast cancer patients' health related quality of life improvement.

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Association of factor II G20210A, factor V G1691A and methylenetetrahydrofolate reductase C677T gene polymorphism with different forms of myocardial infarction: ST segment elevation and non-ST segment elevation

Povezanost polimorfizama gena za faktor II G20210A, faktor V G1691A metilentetrahidrofolatreduktazu C677T sa različitim formama infarkta miokarda: sa elevacijom ST segmenta i bez elevacije ST segmenta

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Abstract

Background/Aim. Coagulation Factor II G20210A and Factor V G1691A variants are moderately associated with coronary artery disease. Polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene C677T is associated with myocardial infarction (MI) in some ethnical groups. At the present time there are rare studies which try to differentiate two forms of MI, ST-elevation MI (STEMI) and non ST-elevation MI (NSTEMI) according to the genetic background. The aim of the study was investigate the association of polymorphisms of Factor II G20210A, Factor V G1691A and MTHFR C677T with different forms of MI: STEMI and NSTEMI. **Methods.** The study included 82 patients, divided into two cohorts: patients with STEMI (49 patients) and NSTEMI (33 patients). Genetic factors that would be different in those two entities, included in response to plaque rupture and occlusion of coronary artery, were examined. The peripheral blood lymphocytes were used as DNA source. Genotypes were determined on the polymerase chain reaction (PCR) based methodology. **Results.** The frequency of MTHFR C677T CT genotype was higher in the patients with NSTEMI in comparison with the patients with STEMI [odds ratio (OR) 3.33; 95% confidence interval (CI) 1.22–9.15; $p = 0.02$]. Logistic regression analysis shows MTHFR CT genotype as an independent prognostic factor for development of NSTEMI (OR 3.15; 95% CI 1.20–8.29; $p = 0.02$). There were no differences between two patients groups in frequency of Factor II G20210A and Factor V G1691A gene polymorphism. **Conclusion.** MTHFR C677T CT genotype was significantly associated with the NSTEMI development examined patients.

Key words:
genes; factor v; prothrombin; st elevation myocardial infarction; non-st elevated myocardial infarction; polymorphism, genetic; risk factor.

Apstrakt

Uvod/Cilj. Varijante faktora koagulacije II G20210A i faktora V G1691A umereno su udružene sa koronarnom arterijskom bolešću. Polimorfizam gena za metilentetrahidrofolat reduktazu [*methylenetetrahydrofolate reductase* (MTHFR)] C677T udružen je sa infarktom miokarda u nekim etničkim grupama. Retke su studije koje pokušavaju da diferentuju dve forme infarkta miokarda, sa elevacijom ST segmenta [ST-*elevation myocardial infarction* (STEMI)] i infarkta miokarda bez elevacije ST segmenta [*non ST-elevation myocardial infarction* (NSTEMI)], u odnosu na genetičku osnovu. Cilj rad bio je da se utvrdi povezanost polimorfizama faktora II G20210A, faktora V G1691A i MTHFR C677T sa različitim formama infarkta miokarda: STEMI i NSTEMI. **Metode.** Ispitivanjem su obuhvaćena 82 bolesnika podeljena u dve kohorte: bolesnici sa STEMI (49) i bolesnici sa NSTEMI (33). Ispitani su genetički faktori kod ova dva entiteta, uključeni u rupturu plaka i okluziju koronarne arterije. Korišćeni su limfociti periferne krvi kao izvor DNK. Genotipovi su određivani po metodologiji zasnovanoj na lančanoj rekciji polimeraze. **Rezultati.** Učestalost MTHFR C677T CT ge-

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notipa je bila veća kod bolesnika sa NSTEMI u poređenju sa bolesnicima sa STEMI [*odds ratio* OR) 3.33; 95% *confidence interval* (CI) 1,22–9,15; $p = 0,02$]. Logistička regresiona analiza pokazala je da je MTHFR CT genotip bio nezavisan prognostički factor za razvoj NSTEMI (OR 3,15; 95% CI 1,20–8,29; $p = 0,02$]. Nije bilo razlike između dve grupe bolesnika u učestalosti genskih polimorfizama faktora II G20210A i faktora V G1691A. **Zaključak.** MTHFR C677T CT genotip je značajno povezan sa razvojem NSTEMI forme infarkta miokarda kod ispitivanih bolesnika.

Ključne reči:
geni; faktor v; protrombin; infarkt miokarda sa st elevacijom; infarkt miokarda bez st elevacije; polimorfizam, genetički; faktori rizika.

Introduction

Myocardial infarction (MI), and an ischemic heart disease are the leading mortality factor world-wide [1]. According to the third universal definition of MI, this disease is diagnosed upon specific electrocardiogram (ECG) patterns, as ST-elevation MI (STEMI) and non ST-elevation MI (NSTEMI) [2]. The clinical characteristics are different in these two forms of MI, as well as the histology of coronary plaques. Patients with STEMI show more severe plaque rupture of coronary culprit lesions than patients with NSTEMI [3]. Also, fibroatheromas in NSTEMI were more calcified than in STEMI [4]. If patients have recurrent infarctions, the type of infarction is often the same implying that an individual patient is prone for developing of either STEMI or NSTEMI [5]. There are established 50 genetic risk variants in the genome-wide association studies (GWAS) for coronary artery disease (CAD) and MI [6]. Among these genetic risk variants for CAD, there are some genes that are more related to the plaque rupture and thrombosis than atherosclerosis. Unfortunately, in many genetic studies there are no clear distinction between different forms of CAD and MI, leading to complicated conclusions of the impact of genetics in development of the particular disease. The literature is poor of genetic risk factors which differentiate STEMI and NSTEMI [7]. Most of reported single nucleotide polymorphisms (SNPs) were demonstrated to be associated with CAD, and not specifically with MI, so we presumed that genetic risk factors involved in atherosclerosis development, common condition for CAD and MI, are the same in STEMI and NSTEMI. We tried to examine genetic factors that would be different in these two entities, included in a response to plaque rupture, and an occlusion of coronary arteries. We supposed that SNPs of coagulation factors II and V, and polymorphism in the gene involved in metabolism, methylenetetrahydrofolate reductase (MTHFR), also known as risk factor for CAD [8] and vein thrombosis [9], would have different distribution in STEMI and NSTEMI. Factor V single base polymorphism, G1691A (factor V Leiden) leads to change in a functional protein which reduce protein C cleavage sites from three to only one site, and leads to an increased thrombin production. Prothrombin G20210A polymorphism affects a single base, but in a promoter region of the gene. This polymorphism increases the prothrombin production to levels of 30% and 70% higher in the heterozygous and homozygous individuals, respectively, than in those who does not have it [10]. It is shown in meta-analysis that polymorphisms in factor V Leiden and factor II are associated with CAD, and that per-allele relative risk is 1.17 for factor V and 1.31 for factor II mutation [11]. MTHFR C677T gene polymorphism (alanine to valin substitution) which results in a thermo labile form of the enzyme, is established as a risk factor for CAD developing with clear evidence for the TT genotype, and a trend towards an increased risk, for the CT genotype [12]. There are some studies which demonstrated association of MTHFR polymorphism with early onset of CAD [13], and others do not [14]. The homozygous form of the MTHFR C677T gene polymorphism is associated with elevated homocysteine in plasma. Experimental data have demonstrated that homocysteine is involved in an endothelial dysfunction and injury, followed by activation of platelets and thrombus formation [15, 16]. Some studies have shown mean homocysteine concentrations modestly increased in CT heterozygotes in comparison with CC homozygotes [17, 18]. In this study we analyzed factor II G20210A, factor V G1691A and MTHFR C677T variants in patients with STEMI and NSTEMI who underwent percutaneous transluminal coronary angioplasty (PCTA) with a bare metal stent implantation. The aim of the study was to determine impact of genetic polymorphisms of factor V, factor II and MTHFR on different forms of MI among patients with STEMI and NSTEMI.

Methods

Study population

In this observational, retrospective study, 82 patients with MI were included, 62 (76%) men and 20 (24%) women, subjected to PTCA, and the bare metal stent implantation. The patients were admitted to the Clinic of Emergency Internal Medicine, Military Medical Academy in Belgrade, in the period from 2008 to 2010. Patients with acute and chronic autoimmune conditions, and the malignant diseases were excluded from the study.

Among all patients, 49 (60%) were presented with STEMI and 33 (40%) were presented with NSTEMI.

Patients with STEMI and NSTEMI were diagnosed and treated according to the criteria of the European Society of Cardiology/American College of Cardiology Foundation/World Heart Federation Task Force for the Universal Definition of Myocardial Infarction [19, 20].

The main risk factors (elevated cholesterol, hypertension, obesity, smoking, diabetes mellitus and family history of CAD) were documented for each patient. Diabetes melli-

tus was diagnosed as elevated fasting plasma glucose level of more than 11 mmol/L, or self-reported by patients who used insulin or oral hypoglycemic agents. Hypertension was documented when a blood pressure was more than 140/90 mmHg, or when patients use antihypertensive therapy. Total cholesterol values used in the study were obtained from the fasting plasma samples and included values of more than 4.64 mmol/L for men and 4.76 mmol/L for women. Positive family history was defined if there was a history of CAD in at least one first or second relative degree. Obesity was categorized as body mass index (BMI) of more than 25 kg/m^2. Levels of C-reactive protein (CRP) were determined (normal range up to 4 mg/L) at the hospital admission, and every day up to the end of hospital stay. Creatine kinase-MB fraction (CK-MB) (normal range from 0.00 to 25 U/L) was measured at the hospital admission, and after that every 6 h in the follow-up to 48 h (CK-MB-maximal value). Levels of triglycerides and total cholesterol were measured at the time of hospitalization. Information about current smoking status were documented, too. Thrombolysis in MI (TIMI) flow grade at baseline was determined by an angiography. All the patients gave written informed consents, and the study was approved by the Ethics Committee of the Military Medical Academy in Belgrade.

Polymorphism analysis

Blood samples with sodium citrate as an anticoagulant, were obtained from each patient by a peripheral venipuncture. DNA was extracted by the salting-out method [21]. Factor II G20210A (rs 1799963), factor V Leiden G1691A (rs 6025) and MTHFR C677T (rs 1801133) were genotyped using the AttomolQuicktype PCR kit (Germany), according to manufacturer's instructions. Both alleles of a gene of interest were specifically amplified. Examinations of the amplified products were performed by an agarose gel electrophoresis.

Statistical analysis

Results were presented as means ± standard deviation, for the numerical data with a normal distribution, or median, with 25% to 75% percentile for numerical, nonparametric data, and frequency distributions for categorical variables. For numerical variables, the statistical significance was determined by the Student *t*-test, or Mann-Whitney test for nonparametric numerical data. Statistical significance within the categorical variables, genotype frequencies, between patients with STEMI and NSTEMI was tested by the χ^2 test. Association of factor II G20210A, factor V Leiden G1691A and the MTHFR C677T genotypes with different forms of MI was determined by odds ratio (OR) and 95% confidence interval (CI); *p* values less than 0.05 were considered significant. Logistic regression analysis was used to determine independent predictors in patients with STEMI and NSTEMI.

We use the Statistical Package for Social Science (SPSS), version 13.0, for Windows.

Results

Clinical characteristics of patients

Characteristics of patients with STEMI and NSTEMI are presented in Table 1.

The analyzed groups of the patients had significantly different triglyceride levels (1.95 ± 0.98 mmol/L in STEMI *vs* 1.36 ± 0.53 mmol/L in NSTEMI, $p < 0.01$) and total cholesterol levels (5.59 ± 1.10 mmol/L in STEMI *vs* 4.91 ± 0.94 mmol/L in NSTEMI, $p < 0.01$). The median value of maximal CRP levels (CRP max) measured up to 72 h from the hospital admission was 39.20 (21.00–52.90) mg/L in the STEMI group and 20.10 (11.90–35.10) mg/L in the NSTEMI group ($p < 0.01$). Median CK-MB max, measured up to 48 h from the hospital admission was 262.00 (150.00–384.00) U/L in the patients with STEMI and 59.00 (46.00–123.00) U/L in the patients with NSTEMI ($p < 0.01$). TIMI flow grade 3 at baseline was present in 13 (26.53%) of the patients with STEMI and in 17 (51.51%) of the patients with NSTEMI ($p = 0.02$).

The usage of all medications [beta blockers, aspirin, clopidogrel, heparin, angiotensin converting enzyme (ACE) inhibitors, calcium blockers and statins] was not significantly different between the STEMI group and the NSTEMI group.

The frequency of risk factors for MI (smoking, hypertension, obesity, hypercholesterolemia, and positive family history) was not significantly different between patients with STEMI and NSTEMI.

Association between genotypes and disease characteristics

Distribution of factor II G20210A, factor V G1691A and MTHFR C677T genotypes in patients with STEMI and NSTEMI is presented in Table 2.

The frequency of MTHFR CT genotype ($p = 0.02$) and combined CT and TT genotypes of MTHFR C677T polymorphism was higher in patients with NSTEMI ($p = 0.05$).

In the analyzed group of 82 patients with MI, GG genotype of factor II G20210A was found in 79 (96%) of the patients, while three (4%) of the patients had GA genotype. Factor V G1691A GG genotype was present in 76 (93%) and GA genotype in six (7%) of the patients. MTHFR C677T CC genotype was found in 33 (40%) of the patients, while 36 (44%) and 13 (16%) of the patients had CT and TT genotypes, respectively.

Association between factor II G20210A, factor V G1691A and MTHFR C677T genotypes and clinical characteristics (male *vs* female, age ≤ 45 years *vs* > 45 years, univessel *vs* multivessel disease, STEMI *vs* NSTEMI) was not statistically significant.

We used traditional risk factors which were different among our patients with STEMI and NSTEMI, along with genetic factors, for prediction of NSTEMI development. Logistic regression analysis revealed that MTHFR C677CT genotype alone was predictor for NSTEMI development (OR 3.15; 95% CI 1.20–8.29; $p = 0.02$) (Table 3).

Table 1

Characteristic of patients with different forms of myocardial infarction

Characteristics	STEMI (n = 49)	NSTEMI (n = 33)	*p*-value
Age (years)*	57.84 ± 9.99	57.97 ± 9.74	0.48
Male/female†	40/9	22/11	0.12
CAD risk factors			
hypertension†	31 (63.26)	22 (66.67)	0.75
diabetes mellitus†	16 (32.65)	14 (42.42)	0.37
positive family history†	22 (44.89)	18 (54.55)	0.39
current smoker†	22 (44.89)	12 (36.37)	0.44
obesity (BMI > 25 kg/m^2)†	32 (65.31)	28 (84.85)	0.05
hypercholesterolemia*	34 (69.38)	16 (48.49)	0.06
Laboratory data			
trigyceride (mmol/L)*	1.95 ± 0.98	1.36 ± 0.53	< 0.01
CRP (mg/L)‡	39.20 (21.00–52.90)	20.10 (11.90–35.10)	< 0.01
fibrinogen (g/L)*	3.95 ± 1.37	3.61 ±0.90	0.100
total cholesterol (mmol/L)*	5.59 ± 1.10	4.91 ± 0.94	< 0.01
CK-MB at hospital admission (U/L)‡	23.00 (14.00–34.00)	27.00 (14.00–33.00)	0.71
CK-MB Max (U/L)‡	262.00 (150.00–384.00)	59.00 (46.00–123.00)	< 0.01
Coronary angiographic data			
multivessel coronary disease†	22 (44.90)	11 (33.33)	0.29
TIMI flow grade 3 at baseline†	13 (26.53)	17 (51.51)	0.02
Medications at the time of PTCA			
aspirin†	49 (100.00)	33 (100.00)	1.00
clopidogrel†	49 (100.00)	33 (100.00)	1.00
heparin/enoxaparin†	49 (100.00)	33 (100.00)	1.00
beta blockers†	24 (48.97)	15 (45.45)	0.75
ACE inhibitors†	10 (20.41)	9 (27.27)	0.44
calcium blokers†	12 (24.49)	13 (39.39)	0.15
statins†	39 (79.59)	25 (75.76)	0.68

Data are expressed as mean ± standard deviation, median (minimum–maximum) or number (percentage).
CAD – coronary artery disease; ACE – angiotensin-converting enzyme; BMI – body mass index; CKMB – creatine kinase-MB; CKMB Max – creatine kinase-MB form (maximal value up to 48 h from hospital admission); CRP – C-reactive protein maximal values measured up to 72 h from hospital admission; STEMI – ST elevation myocardial infarction; NSTEMI – non-ST elevation myocardial infarction; TIMI – thrombolysis in myocardial infarction; PTCA – percutaneous transluminal coronary angioplasty;* – statistical significance determined by the Student *t*-test, † – statistical significance determined by the χ^2 test; ‡ – statistical significance determined by the Mann-Whitney test.

Table 2

Frequencies of analyzed genotypes in patients with STelevation myocardial infarction (STEMI) and non-ST elevation myocardial infarction (NSTEMI)

Genotype	STEMI group n (%)	NSTEMI group n (%)	OR (95% CI)	*p*-value
Factor II G20210A				
GG	47 (95.92)	32 (96.97)	1.36	1.00
GA	2 (4.09)	1 (3.03)	(0.12–15.66)	
Factor V G1691A				
GG	44 (89.80)	32 (96.96)	3.64	0.39
GA	5 (10.20)	1 (3.03)	(0.41–32.65)	
MTHFR C677T				
CC	24 (48.98)	9 (27.27)		
CT	16 (37. 21)	20 (60.61)	3.33 (1.22–9.15)	**0.02**
TT	9 (13.81)	4 (12.12)	0.84 (0.21–3.44)	1.00
CT+TT	25 (51.02)	24 (72.73)	2.56 (0.99–6.63)	**0.05**

MTHFR – methylemetetrahydrofolate reductase; OR – odds ratio; CI – confidence interval.
For testing null hypothesis by using the χ^2-test, GG genotypes were used as reference for Factor II G20210A and Factor V G1691A gene polymorphism, and CC genotype for MTHFR C677T gene polymorphism. There were no patients with AA genotype, for both Factor II and Factor V gene polymorphisms.

Table 3
Association of genetic and traditional risk factors with risk for NSTEMI development

Predictive factors for outcome	OR (95% CI)	*p*-value
Hypercholesterolemia	0.52 (0.11–1.42)	0.20
Factor II G20210A	1.01 (0.05–20.80)	1.00
Factor V G1691A	0.61 (0.06–6.14)	0.672
MTHFR C677T CT genotype	3.15 (1.20–8.29)	0.02
Trigyceride	0.47 (0.17–1.32)	0.15

NSTEMI – non-ST elevation myocardial infarction; OR – odds ratio; CI – confidence interval; MTHTR – methylenetetrahydrofolate reductase; MTHFR C677T CC+TT genotype and Factor II G20210A GG, factor V G1691A GG genotype were used as a reference for OR calculation in logistic regression model.

We also tried to use combined MTHFR C677T CT+TT genotypes, as a predictor for NSTEMI development. MTHFR C677T CC genotype was used as a reference for OR calculation in the logistic regression model. According to results of logistic regression analysis, combined genotypes CT and TT of MTHFR C677T polymorphism showed trend of increased risk for NSTEMI (OR 2.47; 95% CI 0.92– 6.62; $p = 0.07$), but without statistical significance.

Discussion

Patients from our study showed no difference according to the traditional risk factors (hypertension, diabetes mellitus, hypercholesterolemia, BMI, history of smoking and positive family history) for developing CAD and MI, in patients with STEMI and NSTEMI. Similar results were published in the study of Žaliaduonytė-Pekšienė et al. [21]. No difference according to the coronary risk factors among patients with STEMI and NSTEMI were obtained in the study of Miyachi et al. [22] too.

In our study, the patients with STEMI had mean total cholesterol and triglyceride levels higher than the patients with NSTEMI. In the previously mentioned study of Žaliaduonytė-Pekšienė et al. [21], there were no difference in mean total cholesterol and triglyceride levels in the two patients groups. In the study of Belle et al. [23], in France, patients with NSTEMI showed higher triglyceride levels than patients with NSTEMI. These differences can be explained by larger number of patients in the last study, which can increase a precision in statistics.

STEMI patients in our study had a higher absolute level of CRP, and maximal CK-MB levels, than the patients with NSTEMI. It may be due to a different inflammatory response to myocardial injury in these two MI groups, or a difference in some inflammation mediators which are included in pathogenesis of MI. Similar results were obtained in the study of Di Stefano et al. [24], where patients with STEMI had higher values of inflammatory markers at a hospital admission. Significant difference among STEMI and NSTEMI patients, according to the higher peak CRP levels, were demonstrated in the study of Habib et al. [25].

There are no many documented genetic data associated with MI that differentiate STEMI and NSTEMI. There are no genetic data which differentiate polymorphisms of coagulation factors II and V in STEMI and NSTEMI.

In our study, there were no differences in the frequency of polymorphism of factor II G20210A, and factor V G1691A between patients with STEMI and NSTEMI. In the study of Sode et al. [10], there was no association of factor V Leiden and prothrombin G20210A polymorphisms with the MI. In their study there were no differentiation of two categories of MI, STEMI and NSTEMI [27]. On the contrary, in the study of Ezzat et al. [26], in the Egyptian population, the prevalence of heterozygous factor V Leiden, and also recessive homozygous AA were higher in patients with MI than in the control group. In that study there was no differentiation between STEMI and NSTEMI patients. In one large GWAS study, factor V and factor II were documented as the risk factors for developing MI, but with no clear difference of an impact on STEMI and NSTEMI [27]. Among patients with STEMI, in our study, there were 5 of them (10.20%) who were heterozygous for factor V G1691A polymorphism, and only one (3.03%) patient was heterozygous among the NSTEMI cohort. According to the limited number of patients in our study, it is mandatory to include more patients to conclude about differences in factor V polymorphisms between STEMI and NSTEMI groups.

In our study, we found that there was a difference between patients with two forms of MI, STEMI and NSTEMI, in relation to genotypes of MTHFR C677T.

The frequency of CT genotype of MTHFR C677T was higher among the patients with NSTEMI in surveyed population, and lower in the STEMI patients.

When we used dominant model, namely CT+TT, in comparison with CC genotype, we found that these genotypes had increased risk for NSTEMI. There are no data about different genotypes of MTHFR in these two categories of MI, STEMI and NSTEMI. In the meta-analysis of Xuan et al. [28] by using the model TT versus CT for MTHFR gene, it was shown that there was significant risk for MI in Caucasian population. The results of our study are in accordance with the previously published study by Isordia-Salas et al. [13] in young Mexican patients (younger than 45 years). Polymorphism of MTHFR C677T was not associated with development of STEMI in young Mexican patients. It is known that MTHFR C677T genotypes have different ethnical and geographical distribution [29]. The TT genotype was common in Mexico (32%), southern Italy (26%), and northern China (20%). There was sort of a geographic increase in Europe (north to south) and China (north to south decrease). In the big meta-analysis of Alizadeh et al. [30], in the population of patients with MI, there were differences in MTHFR polymorphism according to the ethnical groups. Their results showed that T allele of C677T polymorphism is not associated with an increased risk for MI in the European, Asian and North American population, but is associated in the Af-

rican population. In that analysis, the CT genotype was associated with decreased risk of MI in the North American population and in elderly people. Again, this meta-analysis did not separate clinical forms of MI. Hyperhomocysteinemia is one of possible underlying mechanism for development of MI and CAD. Results of the study of Ho et al. [31] suggest that plasma homocysteine is important risk factor for CAD, and some other diseases, but it is also important to include factors, as MTHFR polymorphism, vitamin B12, triglycerides, total cholesterol, that can affect homocysteine metabolism.

The meta analysis of Kluijtmans et al. [32] has shown that all three MTHFR C677T genotypes confer different levels of atherothrombotic risks. CT heterozygotes have elevated risk in relation to CC homozygotes. The first explanation is that the CT genotype actively confers atherothrombotic risk. The second explanation proposed by these authors is that CC is a protective genotype for development of the atherothrombotic disease. We did not find studies which differentiated STEMI and NSTEMI according to polymorphism distribution of MTHFR C677T. Possible explanation for finding of no statistically significant difference between TT and CC MTHFR C677T genotypes is a small group of our patients, and, concequently, a small number of TT homozygous individuals. At the moment, it is not clear what underlines our finding of higher frequency of CT genotypes of MTHFR C677T in cohort of NSTEMI patients.

Discrepancies observed in all aforementioned studies demand better grouping of MI patients according to the age, gender, ethnic background, food intake, folic acid supplementation and, most important, different forms of the disease.

The limitation of our study was a small number of patients.

Conclusion

The MTHFR C677T CT genotype was significantly associated with development of NSTEMI among MI patients. As MI is a multifactorial disease in which combination of environmental factors and the genetic background both play role in its development, more studies are needed to determine clear association of MTHFR C677T gene polymorphism for development of NSTEMI.

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Three obstetric factors should be considered in umbilical cord blood donor selection

Tri akušerska faktora koja bi trebalo uzeti u obzir prilikom procesa selekcije donora umbilikalne krvi

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Abstract

Background/Aim. The umbilical cord blood (UCB) volume and hematopoietic stem cells count are used as indicators for hematopoietic potential of UBC units. These indicators are affected by a collection method and obstetric factors. It was established that birth weight and placental weight affect the volume and hematopoietic stem cells count in UCB units. The influence of other obstetric factors is less clear. The aim of this study was to investigate the impact of obstetric factors on hematopoietic potential of UCB units. **Methods.** The study involved 103 consecutive UCB units collected during 2013. Relationship of UCB volume, total nucleated cells, $CD34^+$ cells and Colony Forming Unit-Granulocyte Monocyte count with maternal and neonatal characteristics was retrospectively analyzed. **Results.** It was shown that birth weight, placental weight and umbilical cord length ≥ 31 cm significantly increased the volume of collected samples, total nucleated cells, $CD34^+$ cells and Colony Forming Unit-Granulocyte Monocyte count. Gestational age between 38−40 weeks increased significantly all umbilical factors (volume, total nucleated cells, $CD34^+$ cells, and Colony Forming Unit-Granulocyte Monocyte count). Gender did not have an influence on quality of UCB units except on total nucleated cells and $CD34^+$ cells count. Other obstetric factors did not affect significantly the quality of UCB units. **Conclusion.** Our study confirmed that birth weight, placenta weight, length of the umbilical cord and gestational age independently influenced the UCB unit volume, and absolute count of nuclear cells and hematopoietic stem cells. Due to a positive correlation between birth weight and placental weight, only birth weight, umbilical cord length and gestational age should be standard parameters in procedure of donor selection.

Key words:
fetal blood; hematopoiesis; stem cells; obstetrics; granulocyte-macrophage progenitor cells.

Apstrakt

Uvod/ Cilj. Zapremina umbilikalne krvi i broj matičih ćelija hematopoeze koriste se kao pokazatelji hematopoetskog potencijala jedinice umbilikalne krvi. Na ove pokazatelje utiču metode prikupljanja i akušerski faktori. Ustanovljeno je da porođajna masa i masa placente utiču na volumen i broj matičih ćelija hematopoeze u jedinici umbilikalne krvi. Uticaj drugih akušerskih faktora je manje jasan. Cilj ovog rada bio je da se istraži uticaj akušerskih faktora na hematopoetski potencijal jedinice umbilikalne krvi. **Metode.** Istraživanje je uključilo 103 uzastopnih jedinica umbilikalne krvi koje su sakupljene tokom 2013. godine. Retrospektivno su analizirani odnos volumena umbilikalne krvi, broja nuklearnih ćelija, $CD34^+$ ćelija i broja opredeljenih progenitorskih ćelija za granulocite i monocite sa karakteristikama neonatusa i majke. **Rezultati.** Pokazano je da veća porođajna masa, masa placente i dužina pupčane vrpce ≥ 31 cm značajno povećavaju volumen sakupljenih uzoraka, broj nuklearnih ćelija, $CD34^+$ ćelija i opredeljenih progenitorskih ćelija za granulocite i monocite. Gestaciona starost između 38–40 nedelje značajno povećava volumen, broj nuklearnih ćelija, $CD34^+$ ćelija i opredeljenih progenitorskih ćelija za granulocite i monocite. Pol ne utiče na kvalitet jedinice umbilikalne krvi, osim na broj nuklearnih ćelija i $CD34^+$ ćelija. Drugi akušerski faktori ne utiču značajno na kvalitet jedinica umbilikalne krvi. **Zaključak.** Naše istraživanje potvrđuje da porođajna masa, masa placente, dužina pupčane vrpce i gestaciona starost nezavisno utiču na volumen umbilikalne krvi, apsolutni broj nuklearnih ćelija i broj matičih ćelija hematopoeze. Zbog pozitivne korelacije između porođajne mase i mase placente, samo porođajna masa, dužina pupčane vrpce i gestaciona starost trebalo bi da budu standardni parametri u proceduri selekcije donora.

Ključne reči:
krv fetusa; hematopoeza; matične ćelije; porodiljstvo; granulociti-makrofagi progenitorske ćelije.

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Introduction

Traditional sources of hematopoietic stem cells (HSCs) are bone marrow (BM) and peripheral blood (PB). Umbilical cord blood (UCB) has been a known source of hematopoietic progenitor cells since 1988 [1]. The major disadvantages of UCB units are that they contain a limited number of HSCs and that additional cells cannot be obtained after the time of original collection. In addition, compared to BM and PB transplantations, the delayed hematopoietic reconstitution, higher risk of graft failure and increased transplantation-related mortality have been reported [2]. Clinical studies have shown that a cell dose of more than 2.0×10^7 total nucleated cells (TNCs)/kg recipient body weight and at least 1.7×10^5 $CD34^+$ cells/kg are the most significant predictors of the outcome [3]. It is also confirmed that regardless UCB collection, the average number of TNC per mL of UCB is equivalent [4]. This leads to the conclusion that an increased UCB unit volume provides increased TNC number and thus has a greater hematopoietic potential [5].

The volume of UCB unit is correlated with both a method used for UCB collection and obstetric factors [6]. Several authors have investigated the influence of obstetric factors on unit volume and HSCs count [7–10]. Based on contemporary study results, it was unambiguously established that birth weight (BW) and placental weight (PW) affect the volume as well as HSCs count in UCB samples, while the influence of other obstetric factors is less clear.

The aim of this study was to investigate the impact of obstetric factors on the hematopoietic potential of UCB units.

Methods

Umbilical cord blood collection

UCB units were collected from January 2013 to May 2013 with the institutional Ethics Committee approval. The selection criteria for UCB collection were: a signed informed consent for UCB collection and further usage in the experimental study, uncomplicated pregnancy and full-term vaginal delivery [gestational age (GA) 40 ± 2 weeks], the absence of neonatal asphyxia (Apgar score ≥ 8) and BW more than 2,500 grams. Pregnancies with more than one fetus were excluded. A total of 103 deliveries fulfilled the inclusion criteria. The infants were delivered according to normal obstetrical practices and UCB was collected while the placenta was still *in utero*. The original transfusion set (Syringe/Flush/Syringe) and original active method was used for the UCB sampling [6]. The UCB volume was determined as the actual net volume of UCB collected (i.e., not including the anticoagulant volume). UCB units were kept at 4°C and a further analysis was performed during the next 24 hours. The evaluation of the UCB unit quality was performed by measuring TNC, $CD34^+$ cells and Colony Forming Unit-Granulocyte Monocyte (CFU-GM) cells counts.

Cell quantifications

Nuclear cells and mononuclear cells (MNCs) count was determined by the flow cytometry Technicon H–3 (Technicon Corp, Tarrytown, NY, USA). TNC count was calculated by the multiplication with the UCB volume. A total MNC number was determined in the samples of collected UCB and cell suspension after the separation using Ficoll–Isopaque (density: 1.077 g/mL) as a density gradient (Pharmacia, Uppsala, Sweden) by centrifugation at 400 *g* for 35–40 min. The interface layer was collected and washed two times in phosphate-buffered saline (PBS) for 10 min. The MNCs concentration in 1 mL of the cell suspension was determined using the Spencer's chamber.

Total $CD34^+$ cell count was determined using the flow cytometer EPICS XL–MCL (Coulter, Krefeld, Germany). MNCs were incubated with mouse antihuman anti–CD34 monoclonal antibodies, and results were shown as a percentage of positive cells. The total $CD34^+$ cells number was calculated using the following formula: [(total MNCs number after Ficoll–separation/100) × $CD34^+$ cells count (in %)] [11].

Clonogenic assays were performed using the commercially available methylcellulose medium, Methocult GF H4434 (Stemcell Technologies, Vancouver, Canada). MNCs were added to the mentioned medium in the final concentration of 20,000 cells *per* mL. One mL of cells in methylcellulose medium was plated in duplicate into 35 mm diameter Petri dishes and incubated at 37°C and 5% CO_2 for 14 days. The colonies were counted on 14th day of incubation using an inverted microscope at 50× magnification. CFU–GM colonies were defined as the groups of 50 and more cells, while the clusters are defined as the groups of less than 50 cells.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (version 16.0) package (SPSS Inc, Chicago, IL, USA). The groups were compared using the Student *t*-test or Mann-Whitney *U* test, when appropriate. The relation between variables was analyzed using the Pearson's correlation and the multiple regression analysis. The level of significance was set at $p < 0.05$.

Results

Characteristics of UCB units

A total of 103 deliveries were analyzed in our study. The maternal, infant, placental, obstetric and cord characteristics are shown in Table 1. The mean UCB unit volume was 91.63 mL (range, 52–147 mL). The mean TNCs count was 11.35×10^8 (range, 6.2–42.82×10^8). The mean total $CD34^+$ cells count was 3.02×10^6 (range, 1.16–9.52×10^6) and the mean total CFU-GM count was 87.28×10^4 (range, 40.6–283.31×10^4).

The mean maternal age was 29.61 years, with a range from 19 to 41 years and the average GA was 39.13 weeks (range, 38–42 weeks). The mean BW was 3,347.24 g (range from 2540 g to 4870 g) and the mean PW was 723.71 g (range from 247 g to 1,220 g).

Table 1

Obstetric and umbilical cord blood (UCB) characteristics (n = 103)

Parameters	n (%)	Mean ± SD	Median	Min.	Max.
UCB volume (mL)	103	91.63 ± 24.54	89.6	52	147
TNCs ($\times 10^8$)	103	11.35 ± 5.27	10.37	6.2	42.82
$CD34^+$ cells ($\times 10^6$)	103	3.02 ± 2.81	2.94	1.16	9.52
CFU-GM ($\times 10^4$)	103	87.28 ± 28.92	82.39	40.6	283.31
Maternal age (years)	103	29.61 ± 5.24	28	19	41
GA (weeks)	103	39.13 ± 1.42	39	38	42
BW (g)	103	3347.24 ± 429.37	3324	2540	4870
PW (g)	103	723.71 ± 112.06	705	247	1220
Cord length (> 30 cm)	62 (60.19)				
Cord length (≤ 30 cm)	41 (39.81)				
Birth order (1)	59 (57.28)				
Birth order (> 1)	44 (42.72)				
Infants' gender					
male	53 (51.45)				
female	50 (48.54)				

TNCs – total nucleated cells; ‡CFU-GM – Colony Forming Unit-Granulocyte Monocyte; GA – gestational age; BW – birth weight; PW – placental weight; SD – standard deviation.

Table 2

Influence of obstetric factors on the UCB unit quality

Parameters		Volume (mL)		TNCs ($\times 10^8$)		$CD\ 34^+$ cells ($\times 10^8$)		CFU-GM ($\times 10^4$)	
	n	mean ± SD	r	mean ± SD	r	mean ± SD	r	mean ± SD	r
Gender									
male	53	98.9 ± 20.1		14.1 ± 6.6		4.0 ± 2.0		14.1 ± 6.6	
female	50	92.8 ± 26.3		14.4 ± 9.8		4.0 ± 2.7		14.4 ± 9.8	
GA (weeks)									
≤ 40	79	99.6 ± 24.3		15.2 ± 8.7		4.3 ± 2.5		15.2 ± 8.7	
≥ 41	24	84.2 ± 17.7†		10.8 ± 5.2*		2.9 ± 1.6*		10.8 ± 5.2*	
Cord length (cm)									
≤ 30	42	75.3 ± 13.9		8.9 ± 1.6		2.2 ± 0.9		8.9 ± 1.6	
≥ 31	61	110.2 ± 18†		17.9 ± 9.0†		5.2 ± 2.2†		17.9 ± 9.0†	
Birth order									
1st	59	96.2 ± 22.3		13.7 ± 7.2		3.9 ± 2.2		13.7 ± 7.2	
more	44	95.6 ± 25.9		14.9 ± 9.6		4.1 ± 2.5		14.9 ± 9.6	
BW			0.959†		0.868†		0.919†		0.932†
PW			0.901†		0.851†		0.889†		0.894†

*** – $p < 0.05$; †– $p < 0.01$; TNCs – total nucleated cells; CFU-GM – Colony Forming Unit-Granulocyte Monocyte; r – correlation coefficient; GA – gestational age; BW – birth weight; PW – placental weight; SD – standard deviation.**

Totally, 60.19% of the cords were longer than 30 cm and 39.81% were shorter than 30 cm. The number of previous live births (birth order) was classified into two groups: the first group included maternal first live birth and the second group included one and more than one previous live births. In 57.28% of the cases, it was the maternal first live birth and 50.48 % of births were male.

The impact of obstetric factors on the UCB unit quality

By using the bivariate analysis, it was shown that the greater BW and PW the larger was the UCB volume, and the higher were TNCs, $CD34^+$ cells, and CFU-GM counts ($p < 0.01$ and $p < 0.01$, respectively) (Table 2). Additionally, the multiple regression analysis showed that there was a positive correlation between BW and blood volume, TNCs, $CD34^+$ cells and CFU-GM counts ($p < 0.01$) (Tables 3–6). PW was also positively correlated with the volume of UCB, TNCs, $CD34^+$ cells and CFU-GM counts ($p < 0.05$) (Tables 3–6).

The cord length also impacted the quality of units as shown by the bivariate analysis. Cords greater than or equal to 31 centimeters had a larger volume of UCB units and a greater number of TNCs, $CD34^+$ positive cells and CFU GM cells ($p < 0.01$) (Table 2). Additionally, the multiple regres-

sion analysis showed that there was a positive correlation between the cord length and blood volume, but no correlation between the cord length and other factors (TNCs, $CD34^+$cells, CFU-GM counts) ($p > 0.05$) (Tables 3–6).

The bivariate analysis showed that GA had significant influence on the UCB volume and number of TNCs, $CD34^+$ positive cells and CFU-GM (Table 2). In children younger than 40 weeks, significantly larger the UCB volume and increased TNCs, $CD34^+$ positive cells and CFU-GM counts were found ($p < 0.01$ and $p < 0.05$, respectively) (Table 2). Additionally, the multiple regression analysis showed that there was a significant negative correlation between GA and TNCs, $CD34^+$cells and CFU-GM counts ($p < 0.05$) (Tables 3–6).

The gender did not influence any umbilical parameters (volume, TNCs, $CD34^+$ cells and CFF-GM counts) on the bivariate regression analysis ($p > 0.05$) (Table 2). In addition, when we used the multiple regression analysis, we observed a significant correlation between the gender and TNCs ($p < 0.05$) (Table 4) and the gender and $CD34^+$ cells ($p < 0.05$) (Table 5). On the other hand, there was no correlation between the gender and other umbilical factors (UBC volume and CFU-GM count) ($p > 0.05$), (Tables 3 and 6).

When we analyzed the birth order, we did not observe any correlation between the birth order and umbilical factors (UCB volume, TNCs, $CD34^+$ cells and CFU-GM cells) ($p > 0.05$), (Tables 2–6).

Table 3

Multivariate analysis of the UCB volume influence on other obstetric factors

Parameters	Unstandardized coefficients		Standardized coefficients	*t*	*p*	95% Confidence interval for B	
	B	Std. error	Beta†			lower bound	upper bound
(Constant)	-34.850	6.811		-5.117	< 0.001	-48.370	-21.330
Gender	1.885	1.435	0.040	1.314	0.192	-0.962	4.733
Birth weight	0.034	0.004	.747	9.281	< 0.001	0.027	0.041
Placental weight	0.016	0.008	.140	1.998	0.049	< 0.001	0.032
Gestational age	-4.330	1.598	-.077	-2.710	0.008	-7.502	-1.159
Cord length	4.987	2.050	0.104	2.432	0.017	0.917	9.057
Birth order	-2.099	1.269	-0.044	-1.653	0.102	-4.619	0.421

UCB – umbilical cord blood; B – regression coefficient; Beta – standardized regression coefficient.

Table 4

Multivariate analysis of total nucleated cell influence on other obstetric factors

Parameters	Unstandardized coefficients		Standardized coefficients	*t*	*p*	95% Confidence interval for B	
	B	Std. error	Beta			lower bound	upper bound
(Constant)	-29.332	4.104		-7.147	<0.001	-37.477	-21.186
Gender	2.128	0.864	0.129	2.462	0.016	.412	3.844
Birth weight	0.01	0.002	0.636	4.575	<0.001	0.006	0.015
Placental weight	0.012	0.005	0.311	2.579	0.011	0.003	0.022
Gestational age	-2.170	0.963	-0.111	-2.254	0.026	-4.081	-.259
Cord length	-1.509	1.235	-0.090	-1.222	0.225	-3.961	0.943
Birth order	1.105	0.765	0.066	1.445	0.152	-.413	2.623

B – regression coefficient; Beta – standardized regression coefficient.

Table 5

Multivariate analysis of CD^+ 34 cells influence on other obstertic factors

Parameters	Unstandardized coefficients		Standardized coefficients	*t*	*p*	95% Confidence interval for B	
	B	Std. error	Beta			lower bound	upper bound
(Constant)	-8.758	0.914		-9.581	<0.001	-10.573	-6.944
Gender	0.715	0.193	0.153	3.714	<0.001	0.333	1.097
Birth weight	0.028	0	0.625	5.706	<0.001	0.002	.004
Placental weight	0.032	0.001	0.282	2.967	0.004	0.001	0.005
Gestational age	-0.549	0.214	-0.100	-2.562	0.012	-0.975	-0.124
Cord length	0.212	0.275	0.045	0.770	0.443	-0.334	0.758
Birth order	0.025	0.170	0.005	0.147	0.884	-0.313	0.363

B – regression coefficient; Beta – standardized regression coefficient.

Table 6
Multivariate analysis of Colony Forming Unit-Granulocyte Monocyte influence on other obstetric factors

Parameters	Unstandardized coefficients		Standardized coefficients	*t*	*p*	95% Confidence interval for B	
	B	Std. error	Beta			lower Bound	upper bound
(Constant)	-243.956	23.356		-10.445	<0.001	-290.317	-197.595
Gender	9.089	4.919	0.073	1.848	0.068	-0.676	18.854
Birth weight	0.09	0.013	0.755	7.216	<0.001	0.066	0.116
Placental weight	0.067	0.028	0.219	2.411	0.018	0.012	0.123
Gestational age	-12.042	5.478	-0.082	-2.198	0.030	-22.917	-1.168
Cord length	-5.999	7.031	-0.047	-0.853	0.396	-19.955	7.958
Birth order	4.902	4.353	0.039	1.126	0.263	-3.739	13.542

B – regression coefficient; Beta – standardized regression coefficient.

Discussion

The number of HSCs is the most significant factor for transplantation success and overall prognosis. The possibility of finding of an increased number of these cells in samples taken from UCB led to an intensive research in this area. Several strategies have been developed for increasing cell number and overcoming the main obstacle in using UCB as the source of HSCs in the allogeneic transplantation. The most practical strategy is to improve a collection method and evaluate an influence of obstetric factors on the UCB sample quality.

With regard to obstetric factors, several studies have demonstrated that BW and PW correlate with both the HSCs number and UCB sample volume [12–16], likely due to the relationship between BW and circulatory volume in the fetal and neonatal period. Neonates with BW > 3,500 g had UCB units with greater CD34+ cells and CFU count [16] and also greater UCB volume and TNCs count [17]. Some authors showed a positive correlation between PW, BW and UCB volume, CD34+ cells and CFU count, and also a positive correlation was found between TNCs count and BW, but the statistically significant correlation between TNCs and PW was not found [18]. BW and PW were found to correlate significantly with the UCB volume. One gram of BW increase increases the UCB volume by 0.015 mL. Similarly, each gram increase in PW would contribute to a 0.013 mL increase in the UCB volume [19]. Our research confirmed that larger BW and PW result in a larger volume of collected UCB units as well as an increase in the absolute number of TNCs, $CD34^+$ cells and CFU-GM. In fact, all neonates with BW more than 3,300 g had PW more than 700 g (mean values of male and female neonates in our population – data not shown). Using these results, we suggest that in the process of donor selection measuring, BW is sufficient without the need for assessing PW. We recommend to collect UCB after a fetal delivery and before a placental delivery occurs. It would accelerate the procedure of UCB collection.

Our study also showed that the umbilical cord length had a significant impact on the UCB unit quality. This finding is consistent with the previous research [20] that the umbilical cord length has a positive correlation with the UCB volume. Umbilical cord lengths of more than 30 cm are associated with a greater UCB unit volume and the number of relevant cells. Our results are in agreement with those of other authors and reflect that a significant amount of UCB (about ¼) resides in the umbilical cord [9]. Therefore, our recommendation is to clamp umbilical cord as close to a neonate as possible with respect to the standard obstetric procedure.

Some data suggest that GA is correlated with the UCB sample volume, and that pregnancy duration (more than 40 gestational weeks compared to 38–40 gestational weeks) significantly decreases the sample volume. This can be explained by the relative placental insufficiency [13]. Also, some authors showed that neonates with younger GA have better quality of UCB (greater CFU count and/or CD34+ cells) [21, 22]. These relationships are probably due to mobilizing signals produced by placental tissue during the fetal development. On the other hand, other authors have concluded that older GA positively correlates with the UCB volume and TNCs count [23]. Some authors showed that there is not a positive correlation between GA and UCB unit quality (volume, CD34+ cells, CFU, TNCs count) [18]. Our results confirmed a significantly larger UCB volume and an increased number of TNCs, $CD34^+$ positive cells and CFU-GM cells in babies born at less than 40 weeks of gestation.

Gender of neonates and its influence on the UCB unit quality is still being clarified. In our study, UCB of male neonates had greater CD34+ cell count, which could be explained by the fact that the mean BW of male neonates was statistically significantly larger than the mean BW of female neonates [21, 23]. On the other hand, some authors did not find a difference between the male and female UCB unit quality [24], while other showed that UCB unit taken from a female neonate had a greater CD34+cell count [21]. Our study did not show any influence of gender on the UCB unit quality.

Some studies have concluded that UCB samples taken from first-time deliveries have an increased volume and HSCs count, because first-time newborns have larger BW on average [15]. Our study did not show any influence of the birth order of pregnancy on the UCB unit quality, which is consistent with earlier findings [25].

Conclusion

Our study showed that BW, PW, length of the umbilical cord and GA independently influence the UCB unit volume,

absolute count of nuclear cells, as well as HSCs, but only BW, umbilical cord length and GA should be standard parameters in procedure of donor selection, due to a positive correlation between BW and PW. Therefore, UCB should be collected after a fetal delivery and before placental delivery occurs and the umbilical cord should be clamped as close to a neonate as possible. This would lead to a shorter time needed for foundation of a public UCB bank and improve the quality of UCB units.

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Evaluation of chronological age based on third-molar development in the Serbian population

Procena hronološke starosti zasnovana na razvoju trećeg molara u populaciji Srbije

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Abstract

Background/Aim. Persons identification and their age assessment is necessary in vast number of cases and there are different methods used for such purposes. Numerous studies indicate that the third molar development could play a crucial role in identifying an individual`s age. The aim of this study was to determine the possibility for estimating the chronological age based on the third molar development stages in children and young adults in the Serbian population. **Methods.** A total of 570 Serbian patients aged 6–27 years were included in this study. Out of the total number of subjects, there were 248 males with an average age of 12.21 ± 3.91 years, and 332 females with an average age of 12.88 ± 4.06 years. Stages of dental formation were determined on orthopantomograms by comparing with standard Demirjian radiographic appearances. **Results.** Third molars mineralization occured more rapidly in males than in females. Most of the persons with third molar (the stage H development according to the Demirjian method) were older than 18 years. **Conclusion.** Third molar mineralization stages determination on orthopantomograms is useful additional method for determination of chronological age in living individuals. This finding might be important for forensic studies, focusing on the determination of the legally important ages. Variability among different ethnic groups has to be taken into consideration when applying this method. It is necessary to carry out extensive surveys on a larger sample in order to determine the norms for assessing the dental and chronological age of Serbian population.

Key words:
adolescent; age determination by teeth; child; forensic dentistry; molar, third; radiography, dental; serbia.

Apstrakt

Uvod/Cilj. Identifikacija osoba i procena njihove starosti su neophodni u velikom broju slučajeva i postoje različite metode koje se koriste u takve svrhe. Mnogobrojne studije ukazuju na to da razvoj trećeg molara može igrati ključnu ulogu u određivanju starosti osobe. Cilj studije bio je da se ispita mogućnost procene hronološke starosti na osnovu stadijuma razvoja trećeg molara kod dece i mladih u populaciji Srbije. **Metode.** Studijom je bilo obuhvaćeno ukupno 570 pacijenata iz Srbije uzrasta 6–27 godina. Od ukupnog broja ispitanika, 248 osoba bilo je muškog pola, prosečne starosti 12,21 ± 3,91 godina, dok su 332 osobe bile ženskog pola, prosečne starosti 12,88 ± 4,06 godina. Stadijumi dentalne zrelosti određeni su na ortopantomogramima poređenjem sa standardima po Demirjianu. **Rezultati.** Mineralizacija trećeg molara odvijala se brže kod muškaraca nego kod žena. Više osoba sa formiranim trećim molarima (stadijum razvoja H prema Demirjianu) bilo je starije od 18 godina. **Zaključak.** Određivanje stadijuma mineralizacije trećeg molara na ortopantomogramima je korisna metoda za određivanje hronološke starosti živih osoba. To može biti značajno za forenzička istraživanja, posebno zbog pravnog aspekta utvrđivanja odgovora na pitanje da li je osoba punoletna. Varijacije između različitih etničkih grupa moraju se uzeti u obzir kod primene ove metode. Neophodno je sprovesti obimnija istraživanja kako bi se odredili standardi za procenu dentalne i hronološke starosti stanovništva Srbije.

Ključne reči:
adolescenti; životno doba, određivanje po zubima; deca; stomatologija, sudska; molar, treći; radiografija, stomatološka; srbija.

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Introduction

Identification of persons and their age assessment are necessary in the number of cases, both in deceased individuals (airplane accidents, explosions, earthquakes, floods, fires), as well as in living persons (employment, retirement, wedding, voting right, health insurance, passport issuance and visa) [1]. To differentiate juvenile and adult status in criminal law cases, it is important to consider the age calculation[2]. The children and young adults chronological age estimation can be done by different methods that include: radiographic finding of the hand, radius and ulnar epidermis maturity, the combination of the cranial sutures as well as the assessment of secondary sexual characteristics [2]. Teeth could be considered as an indicator of person chronological age as well.

Such determinations are required in various clinical and scientific disciplines, such as orthodontics, pediatric dentistry, archeology and forensic dentistry [3]. The development of teeth is not only applicable for the age estimation as an addition to other parameters, but can be used as standalone parameter. Since teeth are the most resistant organs in the body, they survive significantly longer than other structures, even in the cases when bones and other tissues are distroyed [4]. By means of dental emergence or tooth formation stages observed in radiographs, the children's dental age can be estimated [5]. Numerous methods for the determination of the dental development from radiographs have been described [6, 7].

The most widespread method for the dental age estimation was initially described by Demirjian et al. [8] in 1973. It was based on a sample of French-Canadian children. They used eight stages of the crown and root development, denoting them with the letters of English alphabet from A to H. Until today this procedure has been tested in many populations all around the World and it is proven to be very applicable when it comes to Caucasian children [9, 10].

After the age of 14, age estimation becomes hindered given that all the permanent teeth, except the third molar, would have completed their development, rendering them to be the only clue used for age estimation [11].

The most common age involved in civil and criminal cases is 18 years. The hand and wrist development ends around the age of 18, while the development of third molars tends to continue over a longer period of time, even when the development of all other teeth is completed [2]. Third molars vary in size, formation time, outburst time, as well as in their position. Regardless of the previously mentioned facts, the third molar is the most stable biological indicator that can be used for determining the chronological age in adolescents aging from 15 to 25 [12, 13].

Several studies have been conducted in different populations to analyze whether the third molar was a reliable age indicator [14–18]. The studies concluded that dental development varies between different populations, indicating that population specific studies are necessary. The initial hypothesis was that Serbian children's rhythm of third molar maturation differentiates from that of the children in other countries where the standards were derived.

The aim of the study was to correlate chronological age with dental age based on the development of third molars in Serbian children and young adults and to compare third molar development by sex and age.

Methods

In this cross-sectional study, panoramic dental radiographs (orthopantomograms-OPGs) of 800 Serbian subjects with known chronologic age and sex were selected. Thirty-four films and 195 films were excluded because of poor radiographic quality and agenesis of the third molars, respectively. The final sample, consisted of 570 orthopantomograms from Serbian individuals aged 6–27 years were chosen for this study. There were 248 males with an average age of 12.21 ± 3.91 years, and 332 females with an average age of 12.88 ± 4.06 years. Additional data used for further statistical analysis were collected from patients' anamneses, clinical examinations and OPGs.

Subjects involved in this study did not have any medical history, as they had normal growth and dentition development. All OPGs were without image deformation. The subjects with anodontia, hiperdontia, hipodontia and/or narrowness were excluded from the study.

Examination and classification covered the development phase of the third right mandibular molar. Stages of dental formation in mandible were determined on OPGs by comparing the third molar appearance with radiographic appearance given by Demirjian et al. [8]. The third molar was scored "A" to "H" depending on the stage of calcification: A – Observed calcified areas of occlusal surface without their fusion; B – Fusion of the calcified areas occlusally, occlusal surface contoures recognizable; C – Calcification of the crown completed, dentine accumulation can be observed; D – Crown formation is completed to the enamel cemental junction; E – Radicular length is shorter than height of the crown; F – Radicular length is longer than height of the crown; G – Root formation is completed, apical opening is wide; H – Apical opening is closed, the periodontal membrane has a uniform width around the root and the apex.

Statistical analysis

The third molar formation process in mandible was examined using the Demirjian method and the obtained data were presented as mean values, standard deviation (SD), and range of chronologic ages for the eight stages of dental development. The comparison of ages between sexes was done by the Students *t*-test and Man-Whitney test. Statistical analysis was performed using SPSS V 15.0 program.

To test the reproducibility of the assessments of dental development stage, two investigators reevaluated randomly selected OPGs from 10% of the same subjects two months after the first evaluation. Inter- and intraobserver agreements were determined using the Wilcoxon matched-pairs signed-rank test.

Results

Repeated scorings of a subsample of 57 radiographs indicated no significant intra- or interobserver differences ($p > 0.05$). The intraobserver agreement was 96%, while the interobserver agreement was 95%.

The third-molar formation process was examined in both sexes, and the average ages ± SD for the Demirjian stages are given in Table 1, while mean values of dental age for both sexes are presented in Figure 1.

In males, mandibular third molar development commenced around 8.99 years, the root calcification started at 14.20 years and was completed by 20.87 years. In females third molar development started at 9.16 years, the root calcification started at 14.49 years and was completed at 21.11 years (Table 1).

In the present study development of the third molar in all stages was found slightly earlier in males than in females but the difference was not statistically significant ($p > 0.05$). The linear regression coefficient was provided to assess the correlation of the third molar development and chronologic age. Statistical analysis showed a strong correlation between age and the third molar development for both males (r = 0.62) and females (r = 0.63).

Regression formulas for the entire sample, as well as males and females separately, based on the number of third molar present were estimated (Whole sample: Age = 9.21 + 1.65 stage; Males: Age = 10.15 + 1.67 stage; Females: Age = 9.65 + 1.50 stage).

A comparative view of results of this study on the third molar formation in the Serbian population with those in other populations published earlier in the literature, is shown in Table 2.

Table 1

Descriptive values and statistical comparisons of Demirjian stages in the third molar formation in both sexes in the Serbian population

Demirijan stages*	Male			Female			*p*
	n	mean ± SD	min–max	n	mean ± SD	min–max	
A	34	8.99 ± 0.94	7.37–12.08	29	9.16 ± 1.26	7.49–13.49	ns
B	43	9.63 ± 1.11	7.81–12.56	48	9.90 ± 1.58	7.03–15.78	ns
C	39	10.27 ± 1.19	7.91–12.50	33	9.46 ± 0.83	7.81–10.99	ns
D	59	11.35 ± 2.03	6.11–15.35	77	11.70 ± 1.80	8.63–18.27	ns
E	25	14.02 ± 3.09	9.01–26.79	65	14.49 ± 2.93	7.22–23.29	0.01*
F	12	15.69 ± 1.23	13.92–17.46	24	14.93 ± 2.26	9.11–18.53	ns
G	16	17.77 ± 2.22	14.23–21.35	18	16.83 ± 3.47	10.63–21.74	ns
H	20	20.87 1.52	18.64–22.36	28	21.11 ± 3.21	18.26–26.58	ns

SD – standard deviation; *$p < 0.01$; ns – not significant.

***A – Observed calcified areas of occlusal surface without their fusion; B – Fusion of the calcified areas occlusally, occlusal surface contoures recognizable; C – Calcification of the crown completed, dentine accumulation can be observed; D – Crown formation is completed to the enamel cemental junction; E – Radicular length is shorter than height of the crown; F – Radicular length is longer than height of the crown; G – Root formation is completed, apical opening is wide; H – Apical opening is closed, the periodontal membrane has a uniform width around the root and the apex.**

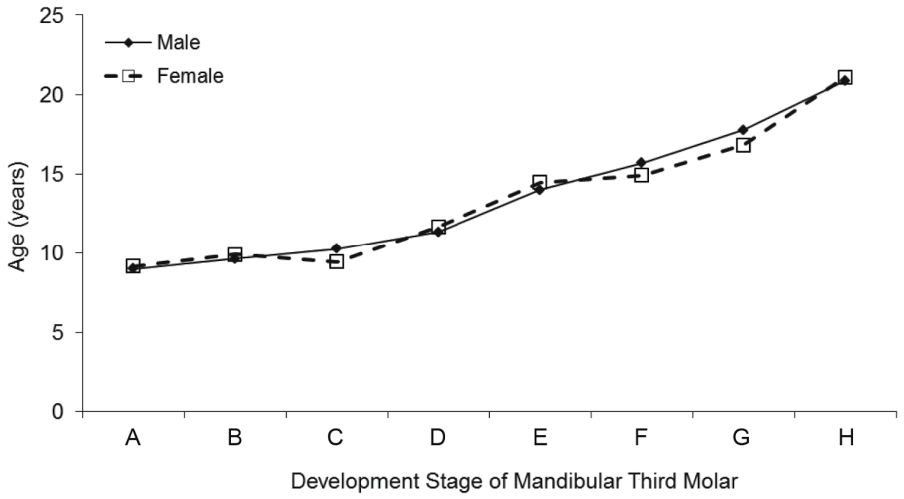

Fig. 1 – Mean ages of males and females in the Serbian population with different mandibular third molar development according to the Demirjian stages*. (*for explanation see under Table 1).

Table 2
Mean values and standard deviations (SD) in different populations estimated by the Demirijan method

Demirijan		German (Olze et al. [32])		Japanese (Olze et al. [32])		Spanish (Prieto et al. [14])		South African (Olze et al. [32])		Turkish (Sisman et al. [2])		Serbian (Present Study)	
stage*	Gender	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
D	M	16.3	3.1	18.2	3.3	15.08	1.04	15.08	1.04	12.9	1.5	11.35	2.03
	F	15.5	2.6	18	2.8	15.11	1	15.11	1	13.6	2.24	11.70	1.80
E	M	16.7	2.3	18.5	2.7	15.22	1.03	15.2	2.4	14.42	1.69	14.02	3.09
	F	16.8	2.3	18.6	2.3	16	1.43	15.9	2.3	15.42	2.4	14.49	2.93
F	M	18.3	2.2	20.4	2.4	16.42	1.34	18.7	2.3	16.9	1.5	15.69	1.23
	F	19.1	2.5	20.5	2.2	16.3	1.56	21.3	2.5	16.84	2.1	14.93	2.26
G	M	20.6	2.4	21.8	2.5	17.92	1.5	20.8	2.2	18.08	2.38	17.77	2.22
	F	21.7	2.1	21.8	2	18.41	1.44	19	2.3	19.29	2.32	16.83	3.47
H	M	22.7	1.9	22.7	2	19.74	1.09	22.6	1.9	22.1	2.87	20.87	2.22
	F	23	1.8	22.4	2.1	19.66	0.98	22.4	1.9	22.66	2.18	21.11	3.21

***For explanation see under Table 1.**

Discussion

Chronological age estimation based on teeth development has been used over a long period of time. Dental age estimation is particularly valuable given that teeth are highly resistant to mechanical, chemical or physical impacts and time. Dental aging was particularly used and received considerable attention within the field of dental anthropology, as well as in forensic medicine [20] and criminal law cases [2]. Since the increased number of adolescents and young adults with unknown date of birth is a current issue in justice and legal medicine, it is important to determine whether an individual was 18 years of age or older at the time the crime was committed [21, 22].

There have been a great number of different classifications (Gleiser and Hunt [23], Kullman [24]). However, the most frequently used one was given by Demirjian et al.[8] The Demirjian method is one of the simplest, the most effective and widespread methods. The advantage of this method is reflected in eight clearly defined stages and a precisely described changes occurring in crown and root shape within each stage. Liversidge et al. [25] reported that using Demirjian method one yields overestimated results, probably due to a positive trend in growth and development during the last 20–25 years. In children of the same chronological maturity, one can, very often, notice differences in various body parts growth and development rates. This is why biological age is defined, demarked by different stages in child development and maturity, whereas chronological age only roughly estimates child maturity [26]. Third molar development is important for dental age estimation in childhood, adolescence and in early adulthood. Several studies showed that chronological stages of wisdom molar mineralization vary slightly between different populations and races [10, 12, 27].

This study was strictly conducted on mandibular molars because in the evaluation of the maxillary molars a problem can arise due to the superposition of maxillary sinuses or maxillary tubes over the root of the molar. In the current study, no major differences could be analyzed between the different stages of root development, except for that boys were ahead of girls. The boys' teeth were reported to be calcified earlier than those of girls. Similar observations were noticed by numerous investigators [1, 2, 20, 28–31].

These observations are distinguishable from those of Kullman [24], who observed significant sex differences in 4 stages of root development. Rai et al. [32] found that third molar was calcified earlier in females. Levesque et al. [30] reported that besides being ahead of girls in the root development, the course of development was also faster in boys. This finding matches the results of the present study. It is surprising and unique for the third molar. A faster development for girls is usually seen for other permanent teeth.

When comparing these results with those in the Turkish [2], Japanese [19], German [19] and Spanish [14] population, the greatest similarities are seen with those from the Turkish one.

The study conducted on the Spanish population [14], with subjects 14–21 years old, showed that the wisdom teeth reached the same stages earlier than in the Scandinavian, American, German, Japanese and South African population. Comparing results of that study with the ones obtained here, it is clear that third molars development occurs earlier in the Serbian population.

Uzamiş et al. [33] found that the calcification of third molars begins between the age of 7 and 19 years in the Turkish population. It was also shown that the process of molar mineralization starts at the age of 8, and 12 months earlier in male than in female children. These results are similar with results of Sisman et al. [2] and Naik et al. [20]. On the other hand, the third molar development among the North India population was found to occur earlier relative to other populations and that there is a strong correlation between age and the third molar development for both sexes [32].

Authors of a study that included only males aged 13–23 years from the Saudi Arabian population, reported only mandible third molars development because there were no maxillary third molars in majority of subjects [34]. They also

reported that the difference between chronological and dental maturity ranged from 0.76 to 2.0 years, and concluded that the stage A of the third molars development was at 13.29 ± 0.76 years, while the stage H was at 22 ± 1.77 years [34].

Our results indicate that the Serbian population reaches the stage H at mean age of 20.87 years in males and 21.11 years in females. Orhan et al. [22] found that the Turkish population reaches the stage H at mean age of 20.1 years. Sisman et al. [2], in a study conducted also in the Turkish population, demonstrated that the stage H was reached at mean age of 22.1 years in males and 22.6 years in females. Results reporting the probability of an individual being older than 18 (at the stage H) are in accordance with previous studies [2, 14, 16, 35].

Rani Hamsa et al. [36] in a study that included males and females, children and adolescents at age of 8–23 years found that there was no difference between males and females in stages A, B, E, F, G and H. However, in stages C ($p < 0.05$) and D ($p < 0.01$), they reported that mineralization was occurring earlier in females than in males. On the other hand Golovencu et al. [27] estimated that in subjects from the Romanian population aged 11–25 years, no significant differences existed between the development of wisdom teeth in both sides of the jaws. The root calcification started at 15.1 years and was completed by 19.3–20 years [37].

Conclusion

Demirjian method could play an important role in determining the age of persons who need to be identified for different reasons.

In the Serbian population, third molars mineralization occurs earlier than in other population for almost all stages. Third molars mineralization occurs more rapidly in males than in females. Large percentage of persons with the third molar (the stage H) is older than 18 years, which might be important fact for forensic studies.

It is necessary to carry out extensive surveys on a larger sample in order to determine the norms for assessing the dental and chronological age within Serbian population.

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Risk factors for healthcare associated infections and in-hospital mortality in a neurological intensive care unit in a tertiary hospital in Belgrade, Serbia: A prospective cohort study

Faktori rizika od nastanka bolničkih infekcija i smrtnog ishoda u neurološkoj jedinici intenzivnog lečenja u tercijarnoj bolnici u Beogradu, Srbija: prospektivna kohortna studija

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Abstract

Background/Aim. Patients in a neurologic intensive care unit (ICU) are especially susceptible to healthcare-associated infections (HAIs). HAIs are cause of significant morbidity and mortality. Aim of this study was to assess the incidence of HAIs, to identify significant risk factors (RFs) and causative microorganisms for HAIs and to identify RFs for in-hospital mortality in a neurological ICU. **Methods.** A prospective cohort study was conducted in the six-bed ICU of the Clinic for Neurology, Military Medical Academy in Belgrade from January 1, 2014 to December 31, 2016. Active surveillance on HAIs was performed by the hospital infection control team, using methodologies of the European Centre for Disease Prevention and Control and the National Healthcare Safety Network/Centres for Disease Prevention and Control. **Results.** One hundred forty eight patients with a total of 2,708 patient-days were enrolled. There were 49 HAIs in 39 patients during the study period. The incidence and incidence density of HAIs were 26.3% and 18.1 per 1000 patient-days, respectively. The most frequent HAIs were urinary tract infections (15.5%), pneumonia (10.1%) and bloodstream infections (4%). RFs independently associated with HAIs in the neurological ICU were: urinary catheter [risk ratio (RR): 5.6; 95% confidence interval (CI): 1.153–27.632], urinary catheter-days (RR: 1.1; 95% CI: 1.057–1.188), central-line days (RR: 1.1; 95% CI: 1.010–1.150), and mechanical ventilation (RR: 0.3; 95% CI: 0.079–0.859). The most common microorganism was *Klebsiella* spp. RFs independently associated with in-hospital mortality in the neurological ICU were: mechanical ventilation (RR: 6.5; 95% CI: 2.868–14.116), Glasgow Coma Score (RR: 2.7; 95% CI: 1.135–6,396), and age (RR: 1.03; 95% CI: 1.005–1.055). **Conclusion.** Usage of invasive procedures during ICU hospitalization carries significant risk for development of HAIs. HAIs in ICU setting are most often caused by Gram-negative bacteria with substantial antimicrobial resistance. These results stress the importance of infection prevention.

Key words:
cross infection; neurology; critical care; risk factors; monitoring, physiologic; drug, resistance microbial.

Apstrakt

Uvod/Cilj. Bolesnici u neurološkim jedinicama intenzivnog lečenja (JIL) su u posebnom riziku od nastanka bolničkih infekcija (BI). BI uzrokuju značajan morbiditet i mortalitet. Cilj ovog istraživanja bio je da se utvrdi incidencija BI, identifikuju faktori rizika (FR) i uzročnici BI, kao i da se ustanove FR za smrtni ishod u neurološkoj JIL. **Metode.** U šestokrevetnoj JIL Klinike za neurologiju Vojnomedicinske akademije u Beogradu sprovedena je prospektivna kohortna studija od januara 2014. godine do decembra 2016. godine. **Rezultati.** U studiju je bilo uključeno 148 bolesnika praćenih tokom 2 708 bolesnik-dana. Registrovano je ukupno 49 BI kod 39 bolesnika. Incidencija BI bila je 26,3%, a gustina incidencije 18.1 na 1000 bolesnik-dana. Najčešće BI bile su: infekcije mokraćnog sistema (15,5%), pneumonija (10,1%) i sepsa (4%). FR povezani sa nastankom BI u neurološkoj JIL bili su primena urinarnog katetera [*risk ratio* (RR): 5,6; 95% *confidence interval* (CI): 1,153–27,632), dani primene urinarnog katetera (RR: 1,1; 95% CI: 1,057–1,188), dani primene centralnog vaskularnog katetera (RR: 1,1; 95% CI: 1,010–1,150) i primena mehaničke ventilacije (RR: 0,3;

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95% CI: 0,079–0,859). Najčešće registrovani uzročnik BI bila je *Klebsiella* spp. FR povezani sa smrtnim ishodom u neurološkoj JIL su bili: mehanička ventilacija (RR: 6,5; 95% CI: 2,868–14,116), Glasgov koma skor (RR: 2,7; 95% CI: 1,135 – 6,396) i starost bolesnika (RR: 1,03; 95% CI: 1,005–1,055). **Zaključak.** Upotreba invazivnih pomagala tokom boravka u neurološkoj JIL nosi značajan rizik od nastanka BI. U neurološkoj JIL BI su najčešće uzrokovane Gram negativnim bakterijama, koje ispoljavaju učestalu rezistenciju na antibiotike. Ovi rezultati naglašavaju značaj prevencije BI.

Ključne reči:
infekcija, intrahospitalna; neurologija; intenzivna nega; faktori rizika; fiziološke funkcije, praćenje; lekovi, rezistencija mikroorganizama.

Introduction

Healthcare associated infections (HAIs) are the cause of significant morbidity and mortality. This is especially important in intensive care units (ICUs) because of severe pathology and multitude of invasive diagnostic and therapeutic procedures [1]. Additionally, an infection risk increases with the length of stay in ICUs [2].

Patients in neurological ICUs are prone to HAIs because of underlying disease nature which is characterized by various disorders of consciousness, diminished protective reflexes, muscle weakness, concomitant immunosuppression, etc. These patients are often bedridden and immobilized, with indwelling central vascular catheter (CVC) and urinary catheter (UC), frequently requiring mechanical ventilation (MV) for extended period of time [3, 4]. They often receive intensive prolonged empirical antimicrobial therapy that could be potent driver of colonisation and infection by multidrug-resistant bacteria and *Clostridium difficile*. The surveillance on HAIs in this type of ICUs is widely accepted as control and prevention method and it can be valuable tool in risk factors (RF) identification and mortality and morbidity reduction [5].

Vincent et al. [2] found that there is a substantial international difference in infection prevalence, type of microorganisms and mortality. The most common microorganisms that cause HAIs in a neurological ICU are: coagulase-negative *streptococci*, *Escherichia coli, Staphylococcus aureus and Klebsiella* spp. [6].

The aim of this study was to assess the incidence of HAIs, to identify their significant RFs and causative microorganisms. We also wanted to identify RFs for in-hospital mortality in a neurological ICU.

Methods

This prospective cohort study was conducted in the six-bed ICU of the Clinic for Neurology at the Military Medical Academy (MMA) in Belgrade, 1,146-bed tertiary healthcare center, teaching hospital of the Faculty of Medicine of the University of Defence, Belgrade, Serbia. This neurological ICU was founded in 2013. A total of 148 patients hospitalized in the ICU for more than 24h from January 2014 to December 2016 were enrolled in this study. The patients whose length of stay was less than 24h or who died within the first 24h of admission into the ICU were excluded from the study. Active surveillance on HAIs was performed by the hospital infection control team, using methodologies of the European Centre for Disease Prevention and Control (ECDC) [7] and the National Healthcare Safety Network/Centres for Disease Prevention and Control (NHSN/CDC) [8]. Standardized form for data collection was used.

The data related to patients [age, gender, Glasgow coma score (GCS) on admission, primary diagnosis, the presence of underlying diabetes mellitus, neoplasms, presence of infections on admission] and those related to healthcare (CVC and central line days, MV and ventilator-days, UC and urinary catheter days, clinical outcome) and length of ICU stay were registered. The results of microbiological analysis and antimicrobial resistance were recorded on daily basis.

Only infections registered after 48h of admission were considered as HAIs. All HAIs were classified into following groups: urinary tract infection (UTI), pneumonia, bloodstream infection (BSI) and infection caused by *Clostridium difficile* (CDI). Microbiological analyses of the samples gathered from the patients with HAIs were performed in the Institute of Microbiology at the Military Medical Academy.

Incidence and incidence density were calculated as the number of HAIs *per* 100 patients and 1,000 patient/days or on 1,000 device/days (for specific devices associated with infection). The device utilization rate (DUR) was also calculated. DUR was determined using following formula:

$$\text{DUR} = \text{Number of device days} / \text{Number of patient days} * 100$$

Incidence rate of CDI was defined as the number of HAI CDI caused by *per* 10,000 patient-days. The in-hospital mortality rate was defined as the number of deaths per 100 patients.

Statistical analysis of data was performed using the SPSS software package (SPSS, Chicago, IL, USA, version 18.00). The results are expressed as mean ± standard deviation (SD) or as the proportion of the total number of patients. Testing for significant differences was conducted by the χ^2 test for categorical variables and the Student's *t*-test for continuous variables. The factors were considered to be significant at a *p*-value of ≤ 0.05. All *p*-values were two-tailed. RFs independently associated with HAIs and in-hospital mortality (poor clinical outcome) were identified by multivariate logistic regression analysis (MLRA) of variables selected by univariate logistic regression analysis (ULRA) with a limit for entering and removing variables of 0.05.

Results

During the 48-month study period, 148 patients with a total of 2,708 patient-days and mean length of ICU stay of

18.3 days (range, 3 to 97 days) were enrolled. The mean age of the patient population was 70.2 years (range, 15 to 94 years). Twenty-two or 14.9% of the patients had cerebral hemorrhage, 94 or 63.5% had ischemia, while other diagnoses (epilepsy, Parkinson's disease, multiple sclerosis, CNS tumors, polyneuropathies, traumas) accounted for 32 or 21.6% of all treated patients.

There were 49 HAIs in 39 patients during the study period. Thirty one patients (20.9%) had one, 6 patients (4%) had two and 2 patients (1.3%) had 3 infections during their stay in the neurological ICU. The incidence and incidence density of HAIs were 26.3 % and 18.1 *per* 1,000 patient-days, respectively.

UTIs (23 or 15.5%) were the most frequent HAIs. Incidence density was 0.8 UTIs per 1,000 patient-days. There were 10.4 UTIs *per* 1,000 catheter-days. Pneumonia (15 or 10.1%) was the second most common type of HAIs with incidence density of 5.5 *per* 1,000 patient-days. There were 12.3 ventilation associated pneumonias (VAPs) *per* 1,000 ventilator-days.

BSI (6 or 4%) had incidence density of 2.2 per 1,000 patient-days.

The DUR values were 81.3, 35.7 and 17.8 for UV, MV and CVC, respectively.

Risk factors for healthcare-associated infections acquisition

Demographic and clinical characteristics in the group with and the group without HAIs according to ULRA are shown in Table 1. According to ULRA, several characteristics were more frequent in the group with than in the group without HAIs: lenght of ICU hospitalization, CVC, MV, central line-days, ventilator-days and urinary catheter-days.

MLRA identified 4 RFs independently associated with HAIs in the neurological ICU: UC [risk ratio (RR): 5.6; 95% confidence interval (CI): 1.153–27.632), urinary catheter-days (RR: 1.1; 95% CI: 1.057–1.188), central line-days (RR: 1.1; 95% CI: 1.010–1.150), MV (RR: 0.3; 95% CI: 0.079–0.859).

The total of 55 microorganisms was detected in 39 HAIs (Tables 2 and 3).

CDI incidence rate was 14.8 *per* 10,000 patient-days.

Table 1

Distribution of patients with and without HAIs according to demographic and clinical characteristics: results of univariate logistic regression analysis

Variable	Patients with HAIs (n = 39)	Patients without HAIs (n = 109)	*p* value
Male, n (%)	26 (66.7)	60 (55.0)	0.207
Age (years), mean ± SD	67.1 ± 18.9	71.4 ± 16.5	0.216
Primary diagnosis, n (%)			0.640
hemorrhage	4 (10.2)	18 (16.5)	
ischemia	26 (66.7)	68 (62.4)	
other	9 (23.1)	23 (21.1)	
Glasgow coma score, n (%)			0.659
3–9	10 (25.6)	32 (29.4)	
10–15	29 (74.4)	77 (70.6)	
Diabetes mellitus, n (%)	2 (5.1)	18 (16.5)	0.131
Neoplasm, n (%)	0 (0)	1 (0.9)	/
Infection at admission, n (%)	7 (17.9)	9 (8.3)	0.130
Survived, (%)	23 (59.0)	66 (60.6)	1.000
ICU hospitalization days, mean ± SD	32.5 ± 19.6	13.2 ± 11.9	< 0.001
CVC, n (%)	15 (38.5)	14 (12.8)	0.001
Central line days, mean ± SD	8.5 ± 14.7	1.4 ± 4.1	< 0.001
MV, n (%)	26 (66.7)	45 (41.3)	0.011
Ventilator days, mean ± SD	14.7 ± 21.3	3.6 ± 9.3	< 0.001
UC, n (%)	35 (89.7)	94 (86.2)	0.777
Urinary catheter days, mean ± SD	26.4 ± 17.6	10.8 ± 10.4	< 0.001

HAIs – healthcare associated infections ; ICU – intensive care unit; CVC – central vascular catheter; MV – mechanical ventilation; UC – urinary catheter.

Table 2

Distribution of microorganisms isolated from patients with HAIs

Microorganisms	Total n (%)	BSI n	Pneumonia n or n (%)	UTI n or n (%)	Diarrhea n or n (%)
Klebsiella spp.	11 (19.0)	1	3	7 (25.9)	0
Acinetobacter spp.	10 (17.2)	1	5 (25.0)	4	0
Enterococcus spp.	8 (13.8)	1	0	7 (25.9)	0
Proteus spp.	8 (13.8)	0	3	5	0
Pseudomonas aeruginosa	6 (10.3)	1	3	2	0
Clostridium difficile	4 (6.9)	0	0	0	4 (100)
Escherichia coli	3 (5.1)	0	1	2	0
Staphylococcus aureus	2 (3.4)	0	2	0	0
Coagulase negative *staphylococci*	2 (3.4)	2	0	0	0
Streptococcus spp.	1 (1.7)	0	1	0	0
Serratia spp.	1 (1.7)	1	0	0	0
Coryneform bacteria	1 (1.7)	0	1	0	0
Hemophilus spp.	1 (1.7)	0	1	0	0
Total	58 (100)	7	20	27	4

HAIs – healthcare associated infections; BSI – bloodstream infection; UTI – urinary tract infection.

Table 3

Antimicrobial resistance of isolated strains

Microorganism	n	AMR
Klebsiella spp.	11	11 to 3G (100%), 5 to CBP (45.4%)
Acinetobacter spp.	10	All to CBP (100%)
Enterococcus spp.	10	2 to vancomycin (20%)
Pseudomonas aeruginosa	6	4 to CBP (66.7%)
Proteus spp.	5	4 to 3G (80%), 3 to CBP (60%)
Clostridium difficile	4	–
Staphylococcus aureus	2	All were MRSA
Escherichia coli	2	–
Serratia marcescens	1	1 to 3G and CBP

AMR – antimicrobial resistance; 3G – third-generation cephalosporins; CBP – carbapenems; MRSA – methicillin resistant *Staphylococcus aureus.*

Risk factors for poor clinical outcome of treated patient

While overall mortality rate was 39.9%, mortality rate in patients with HAIs was 59%. Demographic and clinical characteristics in patients with poor *versus* patients with favourable clinical outcome according to ULRA are shown in Table 4.

RFs independently associated with in-hospital mortality in the neurological ICU were: MV (RR: 6.5; 95% CI: 2.868–14.116), GCS (RR: 2.7; 95% CI: 1.135–6,396), and age (RR: 1.03; 95% CI: 1.005–1.055).

Increasing morbidity and mortality associated with HAIs in the neurologic ICU is a topic of serious concern today. A few studies addressed these issues during first two decade of 21st century. A comparative review of our results and those of these studies is given in Table 5.

Table 4

Distribution of survived patients and died patients according to their demographic and clinical characteristics: results of univariate logistic regression analysis

Characteristics	Survived patients (n = 89) n (%)	Died patients (n = 59) n (%)	*p*
Male	59 (68.6)	27 (31.4)	0.021
Age (years)			
< 65	33 (37.1)	11 (18.6)	0.018
≥ 65	56 (62.9)	48 (81.4)	
Primary diagnosis			
hemorrhage	11 (12.4)	11 (18.6)	
ischemia	54 (60.7)	40 (67.8)	0.124
other	24 (27.0)	8 (13.6)	
Glasgow coma score			
3–8	16 (18.0)	22 (37.3)	0.01
9–15	73 (82.0)	37 (62.7)	
Diabetes mellitus	9 (10.1)	11 (18.6)	0.215
Neoplasm	1(1.1)	0 (0)	/
Infection at admission	13 (14.6)	3 (5.1)	0.120
HAIs	23 (25.8)	16 (27.1)	1.000
Duration of hospitalization in ICU, mean ± SD	18.3 ± 16.6	19.3 ± 15.6	0.004
CVC	18 (20.2)	11 (18.6)	0.979
MV	29 (32.6)	42 (71.2)	0.000
UC	79 (88.8)	50 (84.7)	0.642

CVC – central vascular catheter; MV – mechanical ventilation; UC – urinary catheter; HAIs – healthcare-associated infections; ICU – intensive care unit.

Table 5

Results from current and other relevant studies (mean values)

Parameters	Current study	Zolldan et al. [9] study	Tekin et al. [6] study	Dettenkofer et al. [4] study	Djordjevic et al. [13] study
Patients (n)	148	338	11772	505	537
Patient days (n)	2,708	2,867	133,992	4,873	6,549
Length of stay (days)	18.3	8.5	34.3	9.6	–
Incidence (%)					
UTI	15.3	36.6	32.0	8.7	13.78
pneumonia	10.1	29.6	25.1	11.7	0.74
bloodstream infection	4	15.5	17.2	1.4	2.05
VAP (*per* 1000 days of MV)	12.3	12.8	–	20.4	–
Overall incidence (incidence density) (%)	26.3 (18.1)	21.0 (24.8)	3.7 (3.2)	24.2 (25.0)	18.81 (15.42)
Device use rate (%)					
UC	81.3	92	–	86	–
CVC	17.8	69	–	75	–
MV	35.7	57	–	22	–
Dominant microorganism	*Klebsiella* spp.	*Escherichia coli*	*Coagulase negative staphylococci*	*Acinetobacter* spp.	*Enterobacter cloacae*
In-hospital mortality rate (%)	39.9	–	–	–	–

UTI – urinary tract infection; VAP – ventilation associated pneumonia; UC – urinary catheter; CVC – central vascular catheter; **MV – mechanical ventilation.**

Discussion

Tekin et al. [6] found that during fourteen year surveillance of patients who had cerebrovascular diseases and epilepsy, treated in the Clinic for Neurology in southeast of Turkey, overall incidence of HAIs was 3.7% (range 1.0–7.7) and overall incidence density was 3.2 (range 0.8–7.2). The rates reported in studies conducted in patients in neurological ICUs were higher. Dettenkofer et al. [4] reported that the incidence was 24.2% and incidence density was 25.0 *per* 1,000 patient days. Zolldann et al. [9] found overall incidence and incidence density as 18.5% and 25.0 respectively, while Abdulhasan et al. [10] presented results acquired during 6-year surveillance study with 227 HAIs that were identified for a rate of 10.9/1000 ICU days. Highest incidence rate were registered for subdural hematoma and intracerebral/intraventricular hemorrhage, 21.3 and 21.1 *per* 1,000 patient days, respectively. In the present study, overall incidence was 26.3% and incidence density was 18.1 *per* 1,000 patient-days.

DUR was calculated as ratio of devices-days to patients-days for each location type. These data may serve as marker of severity of illness of patients or measure of use invasive devices which constitute extrinsic RF for HAIs [11].

The most relevant database documenting HAIs in ICUs is provided by the NHSN in the United States (US). Pooled data of the surveillance activities in participating US hospitals are published annually. In 2011, US neurological ICUs reported pooled mean urinary catheter-associated UTI rates of 3.4 (21 ICUs, 116 catheter-associated UTI, 34,422 urinary catheter-days) and pooled mean urinary catheter DUR of 0.66 (21 ICUs, 34,422 urinary catheter-days, 48,549 patients-days) [12]. In our neurological ICU, we recorded 10.4 UTI *per* 1,000 catheter-days and DUR for urinary catheters was 0.81. MLRA confirm that use of UC (RR: 5.6; 95% CI: 1.153–27.632) and duration of urinary catheterization (RR: 1.1; 95% CI: 1.057–1.188) were independent RFs for HAIs. These data bear high correlation with those of similar studies conducted in Serbia [13].

VAP refers to hospital-associated pneumonias (HAP) that develops among patients on MV and presents more than 48 hours after endotracheal intubation [14]. Our results also show significant incidence of VAP (incidence density was 12.3) which corresponds with results published by Zolldan et al. [9]. Also, we found that patients with HAIs more often had presence of MV and higher number of ventilator-days ($p = 0.011$ and $p < 0.001$, respectively). In 2011, the US neurological ICUs reported pooled mean VAP of 3.6 (19 ICUs, 64 VAPs, 17,656 ventilator-days) and pooled mean ventilator DUR of 0.36 (19 ICUs, 17,656 ventilator-days, 48,822 patient days) [12]. Our results showed the same ventilator DUR as in theUS neurological ICUs, but rate of VAP was far higher.

We detected low incidence of BSI (4%) in our study, but patients with HAIs more often had presence of CVC and higher number of central line-days than patients without HAIs ($p = 0.011$ and $p < 0.001$, respectively). Some underreporting cannot be ruled out, because blood cultures were missed in few cases of febrile episodes in patients with CVC in place.

According to data for 2014, the ECDC reported the relative contribution of Gram-negative bacteria as a cause of HAIs in ICUs in European hospitals, with higher proportions of HAIs caused by *Klebsiella* spp. and *Acinetobacter* spp. in some countries [15]. The results of our study conducted in the neurological ICU confirmed that the most commonly cause of HAIs was *Klebsiella* spp., followed by *Acinetobacter* spp.,

Proteus spp. and *Enterococcus* spp. Dettenkofer et al. [4] also reported isolation of *Acinetobacter* spp. as most common cause of HAIs in neurological ICU patients, especially as the cause of pneumonia (22.4%). *Acinetobacter* spp. was the most frequent cause of pneumonia (25%) in our patients, too.

In their study, Zolldan et al. [9] found that UTIs were predominately caused by *Escherichia coli* (33.3%) and *Enterococcus* spp. (33.3%), while UTIs in our patients were caused by *Klebsiella* spp. (25.7%) and *Entercoccus* spp. (25.7%).

During 2014 in European ICUs resistance to third generation cephalosporins was reported in 44% of *Klebsiella* spp. isolates; carbapenem resistance was reported in 8% of *Klebsiella* spp. isolates, in 28% of *Pseudomonas aeruginosa* isolates and 64% of *Acinetobacter* spp. isolates [15]. Significantly high resistance to third generation cephalosporins for *Enterobacteriaceae* isolates in our study gives us limited treatment options for Gram-negative bacterial infections and making carbapenems as the treatment of choice. High percentages of resistance to carbapenems of Gram-negative bacteria reflect challenges for treatment of our neurological ICU patients.

CDI is a major cause of nosocomial illness worldwide. The disease occurrence in the US has doubled during 2001–2010 [16]. Increased ward-level prescriptions for antimicrobial drugs have have been shown to increase CDI in hospitalized patients [17]. We identified CDI incidence rate of 14.8 *per* 10,000 patients-day during study period. According to data from North America and Europe, registered incidence rate in ICUs varied from 8.7 [18] to 53.9 [19] cases *per* 10,000 patient-days.

A recently published study, in which results of two large studies (the SOAP – Sepsis Occurrence in Acutely ill Patients and the ICON – Intensive Care Over Nations) were compared, showed that overall mortality rate in all ICUs involved decreased over time from 18.5% to 16.8%, although diseases severity increased [20]. Colpan et al. [21] conducted prospective study in three surgical and one medical ICUs and found overall mortality rate of 46.7%, significantly higher in patients with HAIs than in patients without HAIs ($p < 0.001$). Another study from Turkey, that specifically followed neurological ICU patients, found that overall mortality rate was 60% with higher mortality rate in patients with than in patients without HAIs [3]. In our ICU, overall in-hospital mortality rate was 39.9% and was similar in patients with and those without HAIs (59.0% vs 60.6%, respectively). Compared to the Turkey study we registered lower ICU mortality rate, but compared with those in ICUs involved in the SOAP and ICON studies, it is significantly higher. This may be explained by the fact that patients in neurological ICUs are especially severe and prone to complications and thus should be evaluated independently.

Cevik et al. [3] have shown that HAIs, MV, two or more underlying diseases and low GCS, as independent factors increase mortality in the neurologic ICU. Colpan et al. [21], on the other hand, have found that HAIs, mean age, mean Acute Physiology and Chronic Health Evaluation (APACHE II) score, MV, and stay in the medical/surgical ICU, enteric nutrition, tracheostomy and use of steroid or chemotherapy are independent RFs for in-hospital mortality. Our study confirmed MV, age and low GCS (3–8) as independent RFs for in-hospital mortality.

There are several limitations of our study. The main limitation is that it was performed at the single ICU of tertiary healthcare centre. Second limitation is the possibility of confounding variables that were not examined in our study. Some parameters were not included, e.g. existence of different underlying diseases (we analyzed only diabetes mellitus) enteric nutrition, steroid therapy, localization of ischemic/haemorrhagic lesions and analyzing these factors could enhance the relevance of our results. Lastly, we did not include appropriateness of antimicrobial therapy in analysis of in-hospital mortality.

The strength of our study is that it was prospective and could be generalized to all neurologic ICU patients.

Conclusion

The results of this study can be used to guide local prevention efforts in patient care areas that are shown to have highest incidence of invasive devices-associated HAIs and high DUR. Further studies involving burden of HAIs caused by Gram-negative carbapenem resistant bacteria in neurological ICUs and their antibiotic treatment are needed.

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Significance of the pulsatility index in the evaluation of hemodynamic changes in peripheral arterial circulation in obese persons treated with orlistat

Značaj pulsatilnog indeksa za procenu hemodinamskih promena u perifernoj arterijskoj cirkulaciji gojaznih osoba lečenih orlistatom

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Abstract

Background/Aim. Prolonged hyperinsulinemia accelerates the process of endothelial dysfunction and arteriosclerotic changes affecting the development of cardiovascular and cerebrovascular diseases. There are various measuring techniques for the evaluation of early functional changes in the arterial wall such as: flow-mediated endothelium-dependent vasodilation, impulse wave analysis, intima-media thickness assessment, venous plethysmography and so on; however, each has certain limitations in results interpretation. The aim of this study was to indicate the association of the pulsatility index (PI) that shows peripheral arterial contractility, with the changes in metabolic parameters under insulin resistance conditions. **Methods.** The study included a total of 30 healthy obese subjects with the values of body mass index more than 30 kg/m^2, randomized with double blind design into two groups: placebo and orlistat. The extent of insulin sensitivity was calculated on the basis of glycemia and insulinemia values using an appropriate formula. **Results.** The obtained results suggest a statistically significant improvement in the PI index in the orlistat group ($p < 0.002$), while there was no such improvement in the placebo group. **Conclusion.** The results obtained in this study indicate the improvement in insulin sensitivity within early arteriosclerosis is a significantly favorable effect of orlistat on peripheral arterial circulation additionally supported by the reduction in levels of lipid fractions, especially triglycerides. Early hemodynamic changes under conditions of the reduced insulin resistance are characterized by the increase in arterial wall contractility evaluated by the PI determination.

Key words:
arteries; atherosclerosis; blood flow velocity; insulin resistance; obesity; orlistat; prognosis; triglycerides.

Apstrakt

Uvod/Cilj. Dugotrajna hiperinsulinemija ubrzava proces endotelne disfunkcije i nastajanja arteriosklerotskih promena, što utiče na nastanak kardiovaskularnih i cerebrovaskularnih oboljenja. Za procenu ranih funkcionalnih promena u arterijskom zidu koriste se različite metode merenja kao što su: protokom posredovana endotel-zavisna vazodilatacija, analiza pulsnog talasa, procena debljine intime i medije, venska pletizmografija i drugo; međutim, svaka od njih ima određena ograničenja u tumačenju rezultata. Cilj ove studije bio je da se ukaže na povezanost pulsatilnog indeksa (PI), koji pokazuje kontraktilnost perifernih arerija, sa izmenjenim metaboličkim parametrima u uslovima insulinske rezistencije. **Metode**. Ispitivanjem je bilo obuhvaćeno 30 zdravih gojaznih osoba, sa vrednostima indeksa telesne mase iznad 30 kg/m^2, koje su po metodi duplo slepog dizajna bile randomizovane u dve grupe – placebo i orlistat. Na osnovu vrednosti glikemije i insulinemije, primenom odgovarajuće formule bio je izračunat nivo insulinske senzitivnosti. **Rezultati**. U orlistat grupi, došlo je do statistički značajnog poboljšanja PI ($p < 0,002$), dok u placebo grupi nije bilo značajnog uvećanja PI. **Zaključak**. Dobijeni rezultati ukazuju na to da je poboljšanje insulinske senzitivnosti u fazi rane ateroskleroze značajan faktor povoljnih uticaja orlistata na perifernu arterijsku cirkulaciju, čemu dodatno doprinosi redukcija nivoa lipidnih frakcija, posebno triglicerida. Prve hemodinamske promene u uslovima smanjene insulinske rezistencije karakteriše porast kontraktilnosti arterijskog zida, procenjeno određivanjem PI.

Ključne reči:
arterije; ateroskleroza; krv, brzina protoka; insulin, rezistencija; gojaznost; orlistat; prognoza; trigliceridi.

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Introduction

The majority of obese people are commonly characterized by the increase in endogenous insulin secretion, reduced response of peripheral tissue to its effects and the occurrence of insulin resistance (IR) – metabolic syndrome. Recent studies point out a relationship between IR and morphofunctional endothelial changes mostly responsible for the occurrence of early and accelerated atherosclerosis [1–5].

It has been proven that endothelium is not only semipermeable barrier between blood and the layer of smooth muscle of blood vessels. It is a multifunctional highly active endocrine organ, one of its major functions being keeping balance between vasodilatory and vasculoprotective agents on one side, and vasoconstrictive and proliferative ones, on the other side [6–17].

Especially interesting is the fact that early hemodynamic and morphologic disorders in arteries occur much earlier than the picture of metabolic syndrome manifests itself. In some individuals, particularly in the so-called healthy obese ones, prolonged unrecognized hyperinsulinemia and endothelial dysfunction could cause sudden vascular disorders, such as myocardial infarction (MI) and stroke [18–24].

Methods for evaluation of peripheral artery disease pathophysiology

Contrast angiography could not be used for reliable measuring of preclinical atherosclerotic lesions. For the last two decades, various methods have been in use such as: flow-mediated endothelium-dependent vasodilation, and intima-media thickness (IMT) assessment, while venous plethysmography, and impulse wave analysis are less dependable. Magnetic resonance is mainly used for arterial compliance and large blood vessels analysis [25–33].

The aim of this study was to indicate the presence of an association of some other hemodynamic parameters such as the pulsatility index (PI) with some metabolic syndrome components and changes in morphofunctional characteristics of peripheral arteries [34, 35]. The PI is mainly used for the evaluation of arterial subocclusal and occlusal diseases [36]. Modern ultrasound diagnostics has not enough data on the association of this parameter with functional changes in peripheral arteries under the conditions of IR. The determination of PI implies also the study of arterial wall properties. Considering the known facts on comorbidity of endothelial changes and arterial compliance damage, it could be supposed that the changed PI values reflect not only advanced morphologic changes, but also early functional changes in the wall of peripheral arteries [37–39].

Methods

The study included a total of 30 healthy obese subjects with the values of body mass index (BMI) more than 30 kg/m^2, randomized with double blind design into two groups: placebo and orlistat. The subjects of the placebo group were given placebo capsules three times daily, per one with main meals, while those from the orlistat group were given per one orlistat capsule of 120 mg also with main meals. Each subject was on individually evaluated hypocaloric diet. Inclusion criteria were age of 35 to 60 years, BMI of 30 to 35 kg/m^2, low density lipoprotein (LDL) cholesterol of 4 mmol/L, triglyceride less than 4.5 mmol/L, normotensive nonsmokers, no surgery nor myocardial infarction six months prior to the study, and no other diseases.

Using standard techniques, body height, body mass, BMI of the subjects were measured, as well as the Oral Glucose Tolerance Test (OGTT) with 75 g of glucose and determination of the value of insulin and C-peptide. The level of HbA1c was also determined. Insulin sensitivity index (ISI) was calculated on the basis of the values of glycemia and insulinemia using the formula as follows:

$$ISI = \frac{10{,}000}{\sqrt{(G_0 \times I_0)\,(G_x \times I_x)}}$$

where: G_0 is glycemia on an empty stomach, I_0 is insulinemia on an empty stomach, G_x is average glycemia within the test, I_x is average insulinemia within the test [40].

Each subject was submitted to the determination of triglyceride concentration, total and LDL cholesterol.

Echoangiographic measurements were performed with the ultrasound apparatus type Hewlett Packard equipped with a linear probe of 7.5 MHz in an air conditioned room at 20°C. Prior to that, the subjects were at the state of rest to adapt to microclimatic conditions. IMT measurement was done on the femoral artery surface (AFS) of the right leg, in the middle of the line that connects the inguinal ligament to the inner extension of the proximal edge of tibia (Hunter's canal). The PI was determined at the same site by activating impulse Doppler and applicable commands on the ultrasound apparatus. Each echoangiographic measurement was repeated three times, and the average value was used as a final result at the beginning and at the end of three months, while in 10 subjects also after six months.

Results

There were 30 patients involved in the study, which were divided into two groups (orlistat and placebo). The orlistat patients were 48.25 ± 7.32 years old on the average, overweight with BMI 32.4 ± 2.71 kg/m^2, with changes in lipid fractions (cholesterol 6.78 ± 1.56 mmol/L; LDL cholesterol 4.11 ± 0.98 mmol/L; triglycerides 3.65 ± 1.9). The examined subjects were not treated for diabetes or arterial hypertension. The average level of systolic arterial blood pressure (Tas) was 135 ± 10 mmHg and that od diastolic arterial blood pressure (Tad) 80.0 ± 8 mmHg. In the orlistat group the average glycemia was 6.6 ± 1.4 mmol/L. The fasting insulin level was 23 ± 2.2 mU/L, and the index of the insulin sensitivity was 42.2 ± 21.6. The mentioned parametres did not have any statistical differrence in relation to the placebo group (Table 1).

Table 1

Data on the subjects at the beginning of the study

Parameters	Groups, mean ± SD		*p*
	Orlistat (n = 20)	Placebo (n = 10)	
Age (year)	48.25 ± 7.32	52.73 ± 8,87	0.094
BMI (kg/m^2)	32.40 ± 2.71	32.31 ± 2.40	0.930
WHR	0.97 ± 0.1	0.98 ± 0.1	0.078
Tas (mmHg)	135 ± 10	130 ± 10	0.207
Tad (mmHg)	80 ± 8	80 ± 5	1.000
TA/impulse (mmHg)	55 ± 6	50 ± 9	0.080
Triglycerides (mmol/L)	3.65 ± 1.9	3.75 ± 1.92	0.893
Cholesterol (mmol/L)	6.78 ± 1.56	6.90 ± 1.70	0.848
LDL-cholesterol (mmol/L)	4.11 ± 0.98	4.10 ± 0.96	0.979
Glucose (mmol/L)	6.60 ± 1.40	6.02 ± 1.12	0.265
Insilin on an empty stomach (mU/L)	23 ± 2.20	21.40 ± 1.7	0.054
IMT (mm)	1.9 ± 0.25	1.7 ± 0.23	0.758
MN (cm/s)	18.2 ± 4.8	18.1 ± 3.0	0.205
ISI	42.2 ± 21.6	66.9 ± 26.2	0.221
PI	5.43 ± 1.96	4.74 ± 0.5	0.36

BMI – body mass index; WHR – waist-hip ratio; TAs – systolic arterial blood pressure; TAd – diastolic arterial blood pressure; IMT – intima-media thickness; MN – mean velocity of blood flow; ISI – insulin sensitivity index; PI – pulsatility index; SD – standard deviation.

Table 2

Effects of three and six-month treatment with orlistat *versus* placebo on the studied risk factors

Parameters	After three months		*p*	After six months		*p*
	Orlistat	Placebo		Orlistat	Placebo	
BMI (kg/m^2)	-3.24	-1.51	0.0001	-5.06	-3.41	0.021
WHR	-1.90	-1.60	0.281	-5.50	-3.20	0.0001
Tas (mmHg)	-5	-1,5	0.0001	-15	-5	0.0001
Tad (mmHg)	-2	-0.5	0.0001	-5	-2	0.0001
TA/impulse (mmHg)	-3	-1	0.0001	-10	-3	0.0001
Triglycerides (mmol/L)	-1.55	-1.14	0.301	-2.43	-1.60	0.121
Cholesterol (mmol/L)	-0.68	-0.20	0.0001	-1.58	-0.80	0.006
LDL cholesterol (mmol/L)	-0.71	-0.20	0.0001	-1.21	-0.70	0.002
Glucose (mmol/L)	-0.90	-0.07	0.0001	-1.00	-0.22	0.738
Insulin on an empty stomach (μU/L)	-6.50	-0.50	0.0001	-11.90	-3.30	0.0001
ISI	16.2	-5.9	0.005	26.9	-7.14	0.0001

***Note*: Results are presented as differences of values after three and six months in relation to the values at the beginning of the study.**

BMI – body mass index; WHR – waist-hip ratio; TA – arterial blood poressure; Tas – systolic arterial blood pressure; Tad – diastolic arterial blood pressure; LDL – low density lipoprotein; ISI – insulin sensitivity index.

The morphofunctional parametres on the right femoral artery were determined intially, as well: IMT 1.9 ± 0.25 mm; mean velocity of blood flow (MN) 18.2 ± 4.8 cm/sec, and the PI 5.43 ± 1.96.

After three month period, BMI in both groups was significantly reduced. In the orlistat group, levels of blood pressure and lipid fractions were stastically significantly reduced, especially values of triglycerides (Tas -5 mmHg; Tad -2 mmHg; cholesterol -0.68 mmol/L; LDL cholesterol 0.71 mol/L; triglycerides -1.55 mmol/L). The values of the observed parametres in the orlistat group after three months of the treatment were in most cases statistically significantly different compared to the values of the same parametres in the placebo group (Table 2). As the result of IR reduction, the rise of ISI values especially stood out in the orlistat group (+16.20 ± 22.8), which was not the case in the placebo group (-5.9 ± 8.5) (Table 3).

Table 3

Insulin sensitivity index (ISI), mean ± SD

Group	ISI-1	ISI-3	Δ	*p*
Orlistat	42.2 ± 21.6	58.4 ± 27.7	16.2	0.005
Placebo	66.9 ± 26.2	60.9 ± 22.1	-6.0	0.018

ISI-1– values at the beginning of the study; ISI-3 – values after three months of the treatment; Δ **– mean difference between ISI-3 and ISI-1 values; SD – standard deviation.**

Table 4
Morphofunctional parametres determined on the right femoral artery in the orlistat and the placebo group (mean ± SD)

Parameters	At the beginning	After 3 months	*p*	After 6 months	*p*
Orlistat group (n = 20)					
IMT (mm)	1.9 ± 0.25	1.8 ± 0.91	0.132	1.8 ± 0.24	0.001
MN (cm/s)	18.2 ± 4.8	15.4 ± 5.1	0.039	14.2 ± 3.8	0.021
PI	5.40 ± 1.9	6.30 ± 1.5	0.002	6.80 ± 1.6	0.002
Placebo group (n = 10)					
IMT (mm)	1.7 ± 0.23	1.7 ± 0.11	0.071	1.6 ± 0.93	0.068
MN (cm/s)	18.1 ± 3.0	17.7 ± 3.9	0.435	17.0 ± 2.34	0.361
PI	4.70 ± 0.5	5.00 ± 1.0	0.213	5.10 ± 1.0	0.292

IMT – intima-media thickness; MN – mean velocity of blood flow; PI – pulsatility index; SD – standard deviation.

As the reflection of peripheral artery vasodilation, in the orlistat group, MN is reduced in relation to the beginning of the study (-2.7 ± 5.6 cm/sec) (Table 4). Finally, all together it contributed to the improvement AFS contractility in the orlistat group (PI +1.3 ± 1.6) (Table 4).

All registrated statistical significances in the orlistat group after three month observing were maintained or improved even after 6 months, but not in the placebo group (Table 4).

Statistical analysis showed that the rise of IR and the reduction of triglyceride levels had the biggest significance (importance) for the PI improvement as the reflection of contractility of peripheral arteries.

Discussion

It is known today that IR condition precedes type 2 diabetes. As early as in that period vascular disorders with the damage of endothelial function appear, often followed with hypertension, dyslipidemia, disordered fibrinolysis, and most often associated with obesity. In prediabetes stage we could find more or less changes in the relation between vasoconstrictive and vasodilatory, proatherogenic and antiatherogenic, procoagulant and anticoagulant agents, as well as stimulators and inhibitors of growth factor of endothelial cells [40–45].

Endothelial damage causes the production of numerous vasoactive substances such as: soluble vascular adhesion molecules, intercellular adhesion molecules, E-selectin, P-selectin, endothelin, thrombomodulin, and von Willebrand factor. As a response to inflammation and adhesion of circulating leukocytes, cellular adhesion molecules appear on the surface of endothelial cells. These endothelial factors, at the phase of prediabetes, could be the markers of endothelial activation [46, 47].

Arterial stiffness is partially regulated with basal release of nitric oxide as a regulator of vascular tonus in arteriolar resistance. Numerous studies emphasize the role of endothelium in arterial stiffness regulation, and the majority of them refer to the correlation of nitric oxide and endothelin [48–59].

The PI is calculated out of e values of waves amplitudes and the mean blood velocity. Each arterial level has its normal values of this parameter. The normal value of PI for the AFS ranges from 5 to 10. There is the association of changes in the PI with the changes of individual components of the IR syndrome estimated on the AFS [47].

The obtained results indicate a statistically significant improvement of the PI ($p = 0.002$) in the orlistat group within three months, while there was no significant PI increase in the placebo group. One of the main facts that affect the improvement of pulsatility is a reduced blood flow velocity, especially pronounced in the orlistat group ($p = 0.03$), while it was not so pronounced in the placebo group ($p = 0.435$). Univariate regression analysis in the orlistat group showed the presence of a significant correlation of changes in the level of insulin sensitivity ($p = 0.025$) and triglyceride ($p < 0.05$) with changes in the PI. Multivariate regression analysis showed the changes in insulin sensitivity ($p= 0.02$) and triglyceride levels ($p = 0.04$) also as independent predictors of PI changes. All the subjects with a reduction in IR had increased PI values. In a number of subjects of the placebo group, there was no improvement of insulin sensitivity, but there was a reduction in triglyceride levels, with no improvement in the PI. These finding that changes in IR mainly affect the contractility of arterial wall regardless of levels of lipid fractions.

Reaven et al. [60, 61] reported the results on the influence of orlistat and a reduced body mass on decreasing the risk of coronary artery disease in those with syndrome x. In the group with syndrome x there was a significant reduction of plasma insulin concentrations, decrease of triglyceride levels and an increase of HDL cholesterol levels as compared with the group with no characteristics of metabolic syndrome. Our results are in compliance with these results since there was a significant positive correlation between improvement of insulin sensitivity, reduction of triglyceride levels, lowering of blood pressure, and improvement of hemodynamic parameters, particularly in the group of those treated with orlistat ($p < 0.001$).

There were no studies published till now on effects of orlistat and hypocaloric diet on the reduction of hyperinsulinemia, as well as effects on hemodynamic parameters and atherosclerotic changes in peripheral arteries evaluated with ultrasound measurements. The results of our study confirmed that already in three months orlistat leads to the increase in insulin sensitivity (42%) together with the correction of majority of metabolic parameters changed. The reduction of IR significantly correlates ($p < 0.001$) with decreasing of the level of glycemia on an empty

stomach, triglyceride levels, impulse pressure, and with an improvement of the pulsatility of arterial wall.

Conclusion

The obtained results indicate that an improvement of insulin sensitivity in the stage of early arteriosclerosis is a significant factor of favorable effects of orlistat on peripheral arterial circulation, additionally supported by the reduction of lipid fractions, especially triglycerides. The first hemodynamic changes in the conditions of reduced IR are characterized with the increase of contractility of arterial wall, evaluated by the PI determination.

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Antimicrobial drug-nutrition interactions: Consistency of information for generic drugs

Interakcije antimikrobnih lekova i hrane: konzistentnost informacija za generičke lekove

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Abstract

Background/Aim. Antimicrobial drug-nutrition interactions can compromise the efficacy and safety of therapeutic regimen, as well as the nutritional status of a patient. In order to prevent them, health professionals consult the reference information sources. Summary of Product Characteristics (SmPC) is the basis for reliable and objective informing, and in the case of generic products, the content of documents should be consistent. The aim of the study was to compare information on antimicrobial drug-nutrition interactions for generic products, and to consider the influence of relevant factors (the time of the first authorization and the number of generic products) on the outcome of evaluation. **Methods.** SmPCs for all generic antimicrobial products for systemic use were retrieved from the Medicines and Medical Devices Agency of Serbia website, and statements of interest were extracted from different sections and were compared. The comparison was based on classification of statements on interaction into one of five classes: "effect of nutrition status on drug action", "effect of food in general on drug action", "effect of specific nutrient on drug action", "effect of drug on nutrient and metabolic status", or "effect of drug on nutrition status". **Results.** A total of 160 SmPCs were evaluated for 30 antimicrobial drugs corresponding to 46 dosage forms [mean number 3.48, standard deviation (SD) = 1.68; median 3.00, interquartile range (IQR) = 2; range: 2-9]. Nine (30%) antimicrobials (azithromycin, clarithromycin, cefazolin, cefepime, pipemidic acid, ciprofloxacin, levofloxacin, moxifloxacin and gentamicin) had inconsistent information. The inconsistency was related to different classes of interactions, and in some cases it could have clinically important implications (gentamicin, fluoroquinolones). The existence of a larger number of generic products was related to identified differences ($p = 0.003$). **Conclusion.** One third of generic antimicrobial products had inconsistent drug-nutrition interaction statements. Given the potential clinical implications, strategies for further harmonization of this information should be considered.

Key words:
anti-infective agents; drugs, generic; food-drug interactions; databases, factual; serbia.

Apstrakt

Uvod/Cilj. Interakcije antimikrobnih lekova i hrane mogu kompromitovati efikasnost i bezbednost terapijskog režima, kao i nutritivni status bolesnika. Kako bi prevenirali iste, zdravstveni profesionalci konsultuju referentne izvore informacija. Sažetak karakteristika leka (*Summary of Product Characteristics – SmPC*) je osnov pouzdanog i objektivnog informisanja i, u slučaju generičkih proizvoda, sadržaj dokumenata bi trebalo da je konzistentan. Cilj istraživanja je bio da se za generičke antimikrobne proizvode uporede informacije o interakcijama sa hranom, i razmotri uticaj relevantnih faktora (vreme prve autorizacije i broj generičkih proizvoda) na ishod analize. **Metode.** SmPCs za sve generičke antimikrobne proizvode za sistemsku upotrebu su preuzeti sa *web*-sajta Agencije za lekove i medicinska sredstva Srbije i iskazi od značaja su ekstrahovani iz različitih sekcija dokumenta i upoređeni. Komparacija je bila bazirana na klasifikaciji iskaza o interakcijama u jednu od pet klasa: „efekat nutritivnog statusa na dejstvo leka", „efekat hrane kao obroka na dejstvo leka", „efekat specifičnog nutrijenta na dejstvo leka", „efekat leka na status nurijenta" i „efekat leka na nutritivni status". **Rezultati.** Ukupno 160 SmPCs je analizirano za 30 antimikrobnih lekova što je korespondiralo sa 46 doznih oblika [prosečan broj 3,48, standardna devijacija (SD) = 1,68); medijana 3,00, interkvartilni raspon (IKR) = 2); opseg: 2-9). Devet (30%) antimikrobnih lekova (azitromicin, klaritromicin, cefazolin, cefepim, pipemidinska kiselina, ciprofloksacin, levofloksacin, moksifloksacin i gentamicin) je imalo nekonzistentne informacije. Nekonzistent-

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nost je bila u vezi sa različitim klasama interakcija i, u izvesnim slučajevima, mogla je imati klinički važne implikacije (gentamicin, fluorohinoloni). Postojanje većeg broja generičkih proizvoda je bilo povezano sa identifikovanim razlikama (p = 0,003). **Zaključak.** Jedna trećina generičkih antimikrobnih proizvoda je imala nekonzistentne iskaze o interakcijama lek-hrana. S obzirom na potencijalne kliničke implikacije, trebalo bi razmotriti strategije za dalju harmonizaciju ovih informacija.

Ključne reči:
antibiotici; lekovi, generički; hrana-lekovi interakcije; baze podataka, faktografske; srbija.

Introduction

Antimicrobial therapy is highly effective, nevertheless many factors can affect the efficacy and safety of therapeutic regimen as adherence, pharmacokinetic processes (absorption, metabolism, excretion) and drug interactions [1]. Interactions may occur between drugs used specifically for treating the infection as well as drugs used for treating unrelated conditions. Furthermore, interactions can occur between antimicrobial agents and nonprescription drugs, supplements or nutritional substances, such as interactions between saquinavir and herbal preparations containing *Hypericum perforatum* which may lead to loss of virologic response and possible resistance to saquinavir; therefore, simultaneous use is not recommended by the manufacturer of saquinavir with additional warning related to the effects of *Hypericum perforatum* that may persist for at least 2 weeks after discontinuation of the treatment [2]. Lastly, clinically significant interactions can be caused by food-induced changes in the bioavailability of antimicrobials. For example, the bioavailability of ciprofloxacin is reduced by 30% to 36% when it is taken with dairy products (like milk or yogurt); since concentration resulting from standard dosage of ciprofloxacin often only marginally exceeds the minimal inhibitory concentration, the interaction may result in the treatment failure [3]. Therefore, if milk cannot be avoided, milk ingestion and ciprfofloxacin ingestion should be separated by several hours. To prevent drug therapy failures and subsequent bacterial resistance, health professionals consult the reference sources of information. Summary of product characteristics (SmPC) is the basis of reliable and objective informing, and content and format of the document is defined by Standard Operating Procedure [4, 5]. In case of generic products, the SmPC content should be consistent with the reference medicinal product, and any differences should be justified [6]. Despite requirements, certain variations in the composition have been reported. For example, Theuretzbacher [7] compared product information for parenteral colistin in 21 European countries, and highlighted that the posology and pharmacokinetic sections for special patient populations varied substantially requiring careful review and updating. Analyzing SmPCs for the most commonly prescribed antibacterials in the United Kingdom (UK) with respect to dosing guidance for obese patients, Boyd et al. [8] pointed out a lack of advice provided by pharmaceutical companies. Furthermore, Sillo et al. [9] evaluated conformity of prescribing information to regulatory requirements among selected branded and generic antimicrobials (albendazole, ciprofloxacin, amoxicillin, artemether/lumefantrine, metronidazole) on the East African market and revealed the existence of a significant number of medical products without necessary compliance in some parameters (handling and disposal, container package description, excipients used, clinical pharmacology of medicines, and directions regarding overdosage).

Considering antimicrobials as high-risk agents in the context of drug-nutrition interactions (DNIs) [10, 11], so as the fact that all aspects of informing about medicinal products should be accurate, relevant and timely in order to support health professionals in making informed choices about therapy [12], the aim of the present study was to compare information on DNIs for generic antimicrobial products authorized in Serbia. Additionally, it was considered the influence of relevant factors, the time since the first authorization and the number of generic products, on the outcome of evaluation.

Methods

Data collection (sources and extraction)

Information about all nationally authorized antimicrobial drugs for systemic use (n=97) was obtained from Register of Drugs [13]. In the case of generic products, all corresponding SmPCs were retrieved from the Medicines and Medical Devices Agency of Serbia website (https://www.alims.gov.rs). The collecting was conducted between July 2017 and October 2017. A total of 199 SmPCs were identified for 37 antimicrobial drugs. SmPCs of different package size and different content of active substances were combined for appropriate products [5]. Seven drugs were excluded beacuse their SmPCs did not contain a statement in line with DNIs. Different dosage forms (DFs) of antimicrobials were considered separately because formulation can influence on the magnitude of DNI. For example, when two fast-dissolving azithromycin capsules were administered to fed subjects, a decrease of azithromycin bioavailability was great (and probably complete) [14]. However, research with tablets and suspension showed a little effect of a high-fat meal on azithromycin absorption [15]. Finally, 160 SmPCs associated with 30 antimicrobial drugs corresponding to 46 DFs were included in the analyisis.

SmPCs were consulted and statements referring to DNIs were extracted from different sections: “Interaction with other medicinal products and other forms of interactions” (4.5), “Pharmacokinetic properties” (5.2), “Posology and method of administration” (4.2), “Special warnings and precautions for use” (4.4), “Undesirable effects” – “Metabolism and nutrition disorders” sub-section (4.8) and “Contraindications” (4.3).

Data analysis

The extracted statements were assigned to one of five classes (Table 1) [16]. A distinction was made between the effects of nutrition status, foods or specific nutrients on drug action, and conversely, the effects of drug use on determinants of nutrition status. Specifically, class 1 was related to the efffect of overweight or malnutrition on drug action. Class 2 was about impact of food in general to absorption and bioavailability of certain drug. Class 3 was associated with benefit or risk of simultaneously use of certain nutrient or specific food and drug. Class 4 was referred to the effect of drug on the status of specific nutrient (e.g. hypokalaemia, hypocalcaemia, hypomagnesaemia) or metabolic status (e.g. hypertriglyceridaemia, hyperglycaemia). Finally, class 5 was related to the effect of drug on overall nutrition status (e.g. weight gain, weight loss).

Table 1
A classification of statements on antimicrobial drug-nutrition interactions

DNI Class	Classification aspect
Class 1	Effect of nutrition status on drug action
Class 2	Effect of food in general on drug action
Class 3	Effect of specific nutrient or food component on drug action
Class 4	Effect of drug on nutrient and metabolic status
Class 5	Effect of drug on nutrition status

DNI – drug-nutrition interaction.

Classification procedure was repeated two months later and the intrarater agreement was estimated by calculating linear weighted kappa (κ) coefficient. In order to minimize factors (prevalence, bias) that could influence on the magnitude of κ, prevalence-adjusted bias-adjusted kappa (PABAK) coefficient was also calculated [17]. The κ (0.97; 95% confidence interval, 0.94 to 1.00) and the PABAK (0.98; 95% confidence interval, 0.95 to 1.00) values indicated almost perfect degree of agreement. In interpreting of coefficients, guidelines proposed by Landis and Koch (≤ 0 = poor, 0.0–0.20 = slight, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial and 0.81–1.00 = almost perfect) was used [18]. Calculations were performed using software WinPepi version 11.65 (http://www.brixtonhealth.com).

In relation to a practical context, disagreement was resolved by additional considering of statements.

In the case of generic products, classified statements were compared in relation to inconsistency as the outcome of evaluation. A given SmPC was worded in clear and standardized language (e.g. the Medical Dictionary for Regulatory Activities Terminology was being applied in section 4.8) [5], inconsistency was defined as the totally or partially different fact listed under information across paired SmPCs for the same drug, e.g. drug can be given without regard to meals vs. give drug at least 1 hour before or 2 hours after a meal, or e.g. the risk of hypo- or hyperglycemia vs. the risk of hyperglycemia, respectively. In addition, inconsistency was defined as the presence or the absence of particular information in at least one of the paired SmPCs for the same drug, e.g. the risk of a disulfiram-like reaction when drug and alcohol are coingested vs. the risk of a disulfiram-like reaction was not listed.

Statistical analyses

For the purposes of statistical analysis, data were entered into an Excel spreadsheet (Microsoft Excel 2007; Microsoft Redmond, Washington, the United States (US)) and subsequently imported into the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM, US). Descriptive statistics was performed to calculate central tendency (mean and median) and dispersion (standard deviation, interquartile range) for quantitative variables, and frequency and proportion for categorial ones. The influence of the time since the first authorization of antimicrobial agents on the Serbian market and the number of generic products on the inconsistency in informing was analysed by the non-parametric (Mann-Whitney) test. Data normality was previously assessed using the Kolmogorov-Smirnov test. The tests were two tailed and p value < 0.05 was regarded as statistically significant.

Results

A total of 160 SmPCs were analyzed for 30 antimicrobials corresponding to 46 DFs; thirteen antimicrobials had more than one authorized DF. The mean number of SmPCs per DF was 3.48 [standard deviation (SD) = 1.68], the median was 3.00 [interquartile range (IQR) = 2), and the range was: 2–9; the mean time since the first authorization per DF was 222.78 months (SD = 133.63), the median was 176.00 (IQR = 201), and the range was: 38-553.

In different sections of SmPCs, 126 individual statements were evaluated and the mean number per DF was 2.74 (SD = 1.53), the median was 2.50 (IQR=2), and the range was: 1–7.

Inconsistency was indentified in 9 (30%) antimicrobials (azithromycin, clarithromycin, cefazolin, cefepime, pipemidic acid, ciprofloxacin, levofloxacin, moxifloxacin and gentamicin) corresponding to four classes of DNIs (Table 2). The examples of inconsistency are presented in Table 3.

The Kolmogorov-Smirnov test revealed that both the number of generic products ($p < 0.001$) and the time since the first authorization were not normally distributed ($p < 0.001$).

In line with inconsistent informing, the number of generic products exerted statistically significant influence (Mann-Whitney $p = 0.003$); oppositely, the time elapsed since the first authorization did not show statistical significance (Mann-Whitney, $p = 0.220$).

Table 2

Inconsistency among antimicrobial generic products in accordance to DNI classes

DNI Class*	Number of drugs		Example of drugs with inconsistent statements
	Consistent statement	Inconsistent statement	
Class 1	1	2	Cefepime powder for solution for injection; Gentamicin solution for injection
Class 2	20	2	Azithromycin tablets and powder for oral suspension; Pipemidic acid capsules
Class 3	17	1	Cefazolin powder for solution for injection
Class 4	12	5	Clarithromycin tablets and film coated tablets with extended releas; Ciprofloxacin tablets and solution for infusion; Levofloxacin tablets and solution for infusion; Moxifloxacin tablets and solution for infusion; Gentamicin solution for injection
Class 5	13	0	-

***See Table 1.**

Note: **Generic products were considered as products which have the same qualitative and quantitative composition in active substance(s), the same pharmaceutical form and the same or very similar bioavailability/bioequivalence, inactive ingredients may not be the same, in accordance to the definition contained in the National Medicines Registry – NRL 2017, The Medicines and Medical Devices Agency of Serbia (https://www.alims.gov.rs). The distinction between generic and reference products was not made. The reference products were available for ≈ 30% (8 of 30) of antimicrobial agents (azithromycin, co-amoxiclav, linezolid, fluconazole, voriconazole, tenofovir, emtricitabine/tenofovir, ribavirin).**

DNI – drug-nutrition interaction.

Table 3

The examples of inconsistent statements in SmPCs of generic antimicrobial products*

Drug and DF	Brand Name: Statement in SmPC
Azithromycin tablets	Azibot , Azitromicin Krka, Hemomycin, Sumamed: Can be given without regard to meals
	Azitromicin Sandoz : Can be taken with food
	Azitromicin Pharma S, Azitromicin Special Product's Line SPA: Give at least 1 hour before or 2 hours after a meal
Cefazolin powder for injection/infusion	Cefazolin, Cefazolin Pharmanova, Primaceph: The possibility of a disulfiram-like reaction in the presence of alcohol
	Cefazolin-MIP: Not reported interaction with alcohol
Ciprofloxacin tablets	Ciprinol, Ciprofloxacin Remedica, Marocen: Decreased appetite, hypo- or hyperglycemia
	Ciprocinal, Citeral: Decreased appetite, hyperglycemia
Gentamicin solution for injection/infusion	Gentamicin HF, Gentamicin Krka: In cases of significant obesity gentamicin serum concentration should be closely monitored and a reduction in dose should be considered
	Gentamicin B.Braun: In obese patients the initial dose should be based on ideal body weight plus 40% of weight excess
	Gentamicin: Not reported dosage regimen in obese patients

***The comment was listed under Table 2.**

SmPC – Summary of Product Characteristics; DF – dosage form.

Discussion

Analyzing different sections of SmPCs in relation to information about DNIs it was found that one-third of generic antimicrobial products had various statements. Similarly, San Miguel et al. [19] assessed quantity and quality of information about food-drug interactions contained in SmPCs of medicinal products authorized in Spain and concluded that SmPCs were a suboptimal source of informing. Namely, interactions were mentioned in only 72.7% of all SmPCs where it should be; and the description and agreement of information with the European recommendations for different sections was between 31.8% and 49.0%.

In the present analysis inconsistency was in line with effects of the nutrition status, food in general or specific nutrient on drug action, as well as effects of drug on the nutrient status. For instance, with respect to the effects of nutrition status on drug action, manufacturers' recommendations for dosage regimen of gentamicin in obese patients were "in obese patients, the initial dose should be based on ideal body weight plus 40% of weight excess" or "in cases of significant obesity gentamicin serum concentration should be closely monitored and a reduction in dose should be considered" or not reported. The absence of information is not only a feature of the Serbian market; namely, as it is above mentioned, analyzing the UK SmPCs, Boyd et al. [8] pointed out a lack of ad-

vice on dosing of commonly prescribed antibacterials in obese patients. From clinical point of view, the exact information is of importance because obesity can significantly alter the tissue distribution and clearence of gentamicin and implicates modification of loading and/or maintenance doses; and dose-adjustment is particularly important due to small difference between therapeutic and toxic dose of this drug [20]. Concordance in findings is not a surprise given that key information included in SmPCs in Serbia is harmonized with the European Union directives and regulations [4].

Statements on food-induced changes in the oral bioavailability of antimicrobials were commonly identified in analyzed SmPCs. Confusion existed regarding the absorption of azithromycin from azitromycin tablets and azithromycin suspension. In keeping with manufacturers' recommendations, "drug can be given without regard to meals" or "drug can be taken with food" or "drug should be administered at least 1 hour before or 2 hours after a meal", however, available data reveals an insignificant effect of food on the bioavailability of azithromycin from tablets and an oral suspension. Thus, in the study of Foulds et al. [15], the mean relative bioavailability of azithromycin after ingestion of standard high-fat breakfast was 96% [90% confidence interval (CI), 82–113%) and 113% (90% CI, 103–124%) for tablets and an oral suspension, respectively; 90% CI has met the limit of 80–125% indicating an insignificant effects of food on the bioavailability. Based on these results, Pfizer (the patent holder in the US) has created recommendations to administration of Zithromax® tablets and oral Zithromax® suspension. On the other hand, Pliva (the patent holder in Yugoslavia and Eastern Europe) has stated differently in the case of administration of oral Summamed® suspension. This unusual case with double patenting for the same active ingredient (azithromycin dihydrate) by two different pharmaceutical companies could contribute to inconsistency. Namely, information contained in generic versions of a drug could be based on it in one or the other reference product.

Disulfiram-like reaction is the most important interaction between antibiotics and alcohol. Thus, metronidazole, thrimethoprim/sulfamethoxazole, chloramphenicol and some cephalosporins decrease alcohol elimination and elevate acetaldehyde concentrations increasing risk of unpleasant but not life-threatening symptoms (nausea, flushing of face, headache, tachycardia, hypotension). In the last compounds, disulfiram like-activity is explained by chemical structure or more precisely by the presence methyltetrazolethiol side-chain at position 3 of the cephem ring (like in cefoperazone, cefamandole, cefotetan). In the case of cefazolin, cephalosporin with 1H-tetrazol group at position 7 of the cephem ring, the study published in 1986 showed that it also had disulfiram-like activity [21]. However, other experimental studies demonstarted that cefazolin did not significantly influence alcohol metabolism [22, 23]. These conflicting findings could contribute inconsistent labeling; namely, in opposite to others, this interaction in the Cefazolin-MIP® SmPC was not reported.

It is well-known that fluoroquinolones upset glucose homeostasis, precipiting both hypoglycemic and hyperglycemic episodes. This adverse effect is rare but may lead to emergency department visits or hospitalizations especially in elderly patients taking sulfonylureas [24–26]. In the present study, evaluating "Metabolism and nutrition disorders" subsection across matched fluoroquinolone SmPCs, certain inconsistencies were identified. Dysglycemia was mainly stated for ciprofloxacin with exception of oral Ciprocinal®, oral and parenteral Citeral® where hyperglycemia was reported as a rare adverse drug reaction, and risk of hypoglycemia was entirely considered during simultaneous administration of ciprofloxacin and glibenclamide in "Interaction with other medicinal products and other forms of interactions" section. Most SmPCs of levofloxacin also reported dysglycemia, whereas oral Leflogal® and parenteral Levoxa® Pharmathen SA only stated the risk of hypoglycemia. The moxifloxacin SmPCs contained statements on hyperglycemia or dysglycemia; furthermore, it is interesting to note that labeling of oral and parenteral Moloxin® was not consistent. In general, inconsistency related to statements in "Undesirable effects" section has also recognized in other studies with a focus on several other pharmacotherapeutic classes [27–29]. This inconsistent medical information is not useful for health professionals who need relevant safety data to make informed risk-benefit decisions across alternative antimicrobial therapies.

A lot of drugs are implicated in electrolyte imbalance. For instance, gentamicin precipitates transient hypomagnesaemia enhancing renal excretion of magnesium that is essential for the normal metabolism of potassium and calcium [30, 31]. In line with mentioned effect, statements for four gentamicin products authorised in Serbia were heterogeneous. So, the Gentamicin HF, Gentamicin Krka, Gentamicin B. Braun and Gentamicin SmPCs stated "hypomagnesaemia", "hypomagnesaemia", "hypokalaemia, hypocalcaemia and hypomagnesaemia" and "hypokalaemia and hypocalcaemia", respectively.

Inconsistency reported in the present study was associated with a number of generic products ($p < 0.003$). The present results confirmed results of Duke's et al. [32] study about a positive correlation between a number of bioequivalent medications and the proportion of different labels ($p < 0.001$), and they may imply lack in collecting, evaluating and/or arbitrating of evidence. Namely, SmPC, as part of the documentation required in procedure of applications for drug authorization, is prepared by a pharmaceutical company and it is ultimately approved by a regulatory agency. In case that an agency considers that information contained is suboptimal, explanation should be requested from a pharmaceutical company.

The lenght of the presence of antimicrobials on the Serbian market was not in line with inconsistent informing ($p = 0.220$). Findings in previous studies are conflicting. San Miguel et al. [19] reported that in SmPCs in Spain, the influence of time of authorization on quality of information on food-drug interactions was close to a statistical significance ($p < 0.0526$), and 1998 was considered as referent year. Namely, the principles of presenting information about food-drug interactions are provided in two European Documents-

Guideline on the investigation of drug interactions (European Medicines Agency, Committee for Human Medicinal Products) and Guideline on Summary of Product Characteristics (European Commission, Enterprise and Industry Directorate-General. Consumer Goods, Pharmaceuticals) that came into force in 1997 and 1999, respectively. Focusing on comparison of statements on adverse drug reactions at international level, Eriksson et al. [27] reported higher consistency of information for drugs approved after 2000 ($p < 0.003$). These results highlight that the quality of safety information included in SmPCs has been improved along the years; nevertheless, there is still room for a further harmonization (e.g. particular aspects of antimicrobial drug-nutrition interactions as effects of nutrition status, foods or specific nutrients on drug action).

The present study was limited to evaluation of information about interactions of antimicrobial drugs only; hence, generalization of findings to other drug groups is restricted. However, other studies also highlighted certain inconsistencies in safety information provided in SmPCs for drugs that were not antimicrobials [27, 28]. Although these studies were focused on adverse drug reactions and hence are not comparable directly to the present study, their findings indicate that differences identified in the present study could be extrapolated to drugs other than those studied. Furthermore, the evaluation was confined to SmPCs of drugs authorized in Serbia; nevertheless, it is expected that the present findings could be of international interest considering the harmonization of Serbian regulations with European ones. Another limitation is associated with the influence of excipients on nutritive and metabolic state, aspect that was not considered in the study; simply, comparison accross generic products was not possible because differences in qualitative and quantitative profile of products related to this subject. Lastly, statements about macro- and micronutrient disturbances contained in "Interference with laboratory tests" subsections were not analysed supposing that they were a consequence of chemical influence on the testing process that is analytical interference [33].

Drug-nutrition interactions are a permanently evolving area of study and interest particularly in high-risk patients as the elderly, obese, those with chronic diseases who use multiple medications as well as those taking high-risk medications (antimicrobials, antiepileptics, warfarin, drugs with narrow therapeutic index); individuals with well-known genetic polymorphism in drug transporters, receptors, or enzymes may also be at higher risk [10]. Data to improve current knowledge about prevention of undesirable effects will continue to emerge in clinical trials and patient care [34, 35], and attention to documentation and relevant statements are of primary importance to produce evidence-based information for those involved in patient care. Lastly, harmonization of safety data is crucial not just for clinical reasons but also for legal ones (e.g. court cases Pliva, Inc. v. Mensing, Mutual Pharmaceutical Co. v. Bartlett, Rafferty v. Merck & Co., Inc.) [36–38]. Therefore, it would be interesting in future work to evaluate if necessary revisions are done.

Conclusion

Analyzing SmPCs of antimicrobials for systemic use authorized at the Serbian market it was found that one third of generic products had inconsistent information about DNIs. Inconsistency was in line with a number of generic products. In some cases, it could have clinically significant implications. Hence, it should be considered strategies for a further harmonization of information on interactions between antimicrobial agents and nutrition.

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Influence of chorionicity on healthy twin pregnancy outcome

Uticaj horioniciteta na ishod zdrave blizanačke trudnoće

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Abstract

Background/Aim. It is still under debate in what sense and extent can chorionicity impact the pregnancy outcome of twins without gestational complications specific for monochorionicity. The study aimed to evaluate the effect of chorionicity on healthy twin pregnancy outcome. **Methods.** The study included patients with uncomplicated twin pregnancies after first trimester that were checked-up and delivered at the Clinic of Obstetrics and Gynecology, Clinical Center of Serbia, Belgrade during three years (2010–2013). Data regarding mother's age, comorbidities, parity, presence and type of gestational complications, chorionicity, mode and time of pregnancy ending, birth-weight and Apgar score of twins were determined. Obtained data were compared and statistically analyzed. **Results.** The study included 361 women with mean age of 33 years. Regardless of chorionicity, twins were mostly born during the 36th gestational week and received Apgar score ≥ 8. Only three monochorionic twins were stillborn, two preterm (29 and 32 gestational week) and one in term (35 gestational week) delivery. Contrary, no intrauterine fetal deaths were recorded. Monochorionicity negatively correlated with having live-born twins (OR = 0.023; CI = [0.001–0.609]; p = 0.024), but was not associated with twins condition at birth, i.e. Apgar score (p = 0.345), pregnancy ending time (p = 0.578) or any other twins characteristic. However, premature preterm membrane rupture and earlier gestational week of pregnancy ending were important confounding factors for relationship between chorionicity and pregnancy outcome. **Conclusion.** Monochorionicity increases risk for adverse pregnancy outcomes even for uncomplicated, healthy twin pregnancy, but has no influence on the condition of twins who survive until term. If appropriate surveillance and therapy are applied, both healthy twins can be delivered at term regardless of chorionicity.

Key words:
chorion; pregnancy, twin; pregnancy outcome.

Apstrakt

Uvod/Cilj. Još uvek se raspravlja u kom smislu i do kog stepena horionicitet može uticati na ishod blizanačke trudnoće bez gestacijskih komplikacija specifičnih za monohorionicitet. Studija je imala za cilj da se proceni efekat horioniciteta na ishod zdrave blizanačke trudnoće. **Metode.** Studijom su bile obuhvaćene sve trudnice sa nekomplikovanom blizanačkom trudnoćom nakon prvog trimestra koje su kontrolisane i porođene na Klinici za ginekologiju i akušerstvo Kliničkog centra Srbije u Beogradu tokom tri godine (2010–2013). Utvrđeni su podaci o starosti majke, komorbiditetima, paritetu, prisutnosti i tipu gestacijskih komplikacija, horionicitetu, načinu i vremenu završetka trudnoće, težini na rođenju i Apgar skoru blizanaca. Dobijeni podaci su upoređeni i statistički analizirani. **Rezultati.** Studijom je bila obuhvaćena 361 žena prosečne starosti od 33 godine. Bez obzira na horionicitet, blizanci su uglavnom bili rođeni tokom 36. gestacijske nedelje i dobili Apgar skor ≥ 8. Samo tri monohorionska blizanca su bila mrtvorođena, dva pre termina (29. i 32. nedelja gestacije) i jedan u terminu (35. nedelja gestacije). Nasuprot tome, nisu registrovane intrauterusne smrti plodova. Monohorionicitet je negativno korelisao sa živorođenošću blizanaca (OR = 0,023; CI = [0,001–0,609], p = 0,024) i nije bio povezan sa stanjem blizanaca na rođenju, tj. Apgar skorom (p = 0,345), vremenom završetka trudnoće (p = 0.578) ili bilo kojom drugom karakteristikom blizanaca Međutim, prevremena ruptura vodenjaka i ranije gestacijske nedelje završetka trudnoće su važni "konfaunding" faktori koji su uticali na odnos između horioniciteta i ishoda trudnoće. **Zaključak.** Monohorionicitet povećava rizik od loših ishoda trudnoće čak i kod nekomplikovane, zdrave blizanačke trudnoće, ali nema uticaj na stanje blizanaca koji prežive do termina. Uz primenu odgovarajućeg nadzora i terapije, oba zdrava blizanca mogu biti porođena u terminu bez obzira na horionicitet.

Ključne reči:
horion; trudnoća, blizanačka; trudnoća, ishod.

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Introduction

Careful monitoring and management of twin pregnancy is the basis of modern perinatology because multiple pregnancies carry an increased risk of perinatal morbidity and mortality compared with singleton pregnancies. This risk arises as a result of complications such as high incidence of preterm birth, fetal growth restriction, preeclampsia, etc. [1]. Twin pregnancies are classified according to either zygosity or chorionicity, and chorionicity rather than zygosity determines the outcome. Twin pregnancies can be divided into monochorionic (MC) or dichorionic (DC) according to placentation, and MC twins are classified as monoamniotic or diamniotic ones [2]. Apart from usual pregnancy complications, monochorionic twins develop unique type-specific perinatal complications, more often twin-twin transfusion syndrome. Consequently, it is well determined by numerous studies that monochorionicity poses the highest risk for both morbidity and mortality of twins [3]. However, it is still under debate in what sense and extent can chorionicity impact the pregnancy outcome of uncomplicated healthy twin pregnancy. Therefore, increased detailed antenatal fetal surveillance with precise first-trimester diagnostics of chorionicity is suggested for twins [4]. Determining chorionicity at early pregnancy can help obstetricians to plan the care of these patients in managing twin pregnancies and in counseling according to the local perinatal outcome [5].

The study aim was to evaluate the effect of chorionicity on outcome of uncomplicated healthy twin pregnancy.

Methods

The study prospectively included all patients with twin pregnancies who were checked-up and delivered at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade in three years period (2010–2013). The study was approved by the Clinic's Ethical Board and all women signed informed consent to participate in the study. After ultrasonographic confirmation of twin pregnancy and determination of chorionicity (single placental masses with T sign – monochroionicity vs. separate placentas and lambda sign – dichorionicity), women were closely monitored throughout pregnancy. Exclusion criteria for this study were first trimester miscarriage, development of twin-to-twin transfusion syndrome (TTTS), fetal growth restriction, placental pathologies and other complications specific for monochorionicity.

On the initial examination, detailed history regarding age, number of previous pregnancies and chronic illnesses (cardiovascular, endocrinologic, etc.) were taken from each patient. If patients had chronic illnesses that were not adequately treated, they were excluded from the study.

Obstetrical complications (presence and type) were registered throughout pregnancy such as presence of hypertension in pregnancy (HTA), gestational diabetes mellitus (GDM), placental problems (placental abruption, retro placental hematomas, placental insufficiency detected by small diameter and higher than expected grade of placenta on ultrasound and by pathological Doppler measures of placental vascularization), fetal growth restriction (IUGR) (fetal weight at delivery below the 10th percentile for gestational age), TTTS (presence of placental blood vessels anastomoses), perinatal asphyxia (assessed by cardiotocographic findings), other and combined comorbidities. In case of complications development, patients were excluded from the study.

Twins delivery was either vaginal or urgent or elective Caesarean Section (CS). The time of membranes rupture was noted and classified as during delivery or premature (PPROM) while the characteristics of amniotic fluid were expressed as clear or meconial. According to the gestational week in which pregnancy ended (miscarriage/ delivery), twins were considered as term or prematurely born (before 36th week of gestation). After birth, sex (male or female), weight and Apgar score of both twins were determined. We also noted if both twins were live-born or if one or both twins were stillborn. Moreover, all cases of intrauterine fetal death (IUFD) of one or both twins were registered.

Finally, as the primary outcome of this study we assessed the survival of twins throughout the whole pregnancy. Therefore, if both twins were live-born, pregnancy outcome was good while as adverse outcome we regarded IUFD or stillbirth of one or both twins. Moreover, the condition at birth of twins was assessed through their Apgar scores and if both twins had Apgar scores ≥ 8, their condition was regarded as good.

For statistical analysis, methods of descriptive (frequencies, mean value, standard deviation) and analytical (Spearman's correlation, Kruscal Wallis χ^2-test, logistic regression) statistics were applied. All assessed data were correlated with chorionicity and pregnancy outcomes. Binary logistic regression was used to construct models of relationship between pregnancy outcome and chorionicity.

Statistical significance was defined as $p < 0.05$. The SPSS ver. 15.0, Chicago IL, USA software was used for analysis.

Results

Initially study included 435 women with twin pregnancies who had 17 to 46 years of age at the time of birth [mean ± standard deviation (SD) = 33.18 ± 6.61 years]. Patients were mostly primiparous. Majority of women had no chronic illnesses, while women who reported comorbidities were regularly checked-up and on adequate therapy (Table 1). In our sample there were significantly more dichorionic twins (Table 2).

During examined pregnancy we registered hypertension in 51, diabetes in 24, placental pathologies in 8, TTTS in 8, IUGR in 15, malformations in 4, asphyxia in 13, chorioamnionits in 17 and other complications altogether in 43 cases. All of these gestational complications were more frequent in monochorionic than dichorionic twins ($p = 0.001$). According to the study criteria, due to complications we excluded 74 women from the study, while remaining 361 cases underwent further analysis.

Table 1

Frequency of assessed parameters of examined women

Parameters and their categories	Overall sample n (%)	Overall sample *p* between categories	Dichorionic (n)	Monochorionic (n)
Mothers parity				
1	278 (77)	< 0.001	262	16
2	61 (17)		56	5
3 and more	22 (6)		21	1
Mothers comorbidities				
no chronic illnesses	213 (59)	0.039	203	10
yes, but on therapy	148 (41)		136	12

Table 2

Frequency of assessed parameters of twins

Parameters and their categories	Overall sample n (%)		Overall sample *p* between categories	Dichorionic (n)	Monochorionic (n)
Chorionicity					
dichorionic	339	93.9	< 0.001	339	/
monochorionic	22	6.1		/	22
Delivery mode					
vaginal	145	40.2	< 0.001	139	6
CS planed	137	38.0		128	9
CS urgent	79	21.9		72	7
PPROM					
no	270	74.8	< 0.001	257	13
yes	91	25.2		82	9
Amniotic fluid					
clear	339	93.9	< 0.001	317	22
meconial	22	6.1		22	0
Sex twin I					
male	186	51.5	0.563	176	10
female	175	48.5		163	12
Sex twin II					
male	175	48.5	0.563	165	10
female	186	51.5		174	12
Apgar score (twin I)					
< 8	148	41.0	0.001	141	7
≥ 8	213	59.0		198	15
Apgar score (twin II)					
< 8	160	44.3	0.031	152	8
≥ 8	201	55.7		187	14
Both twins condition					
bad	183	50.7	0.792	174	9
good	178	49.3		165	13
Pregnancy outcome					
stillborn/IUFD	3	0.8	<0.001	0	3
both alive	358	99.2		339	19
Pregnancy ending time					
preterm	135	37.4	< 0.001	128	7
in term	226	62.6		211	15

PPROM – premature preterm rupture of membranes; CS – Caesarean Section; IUFD – intrauterine fetal death.

Examined healthy twins were mostly delivered by CS, usually elective. Regardless of chorionicity, twins were in average born during the 36th gestational week (range = 27 to 40; mean ± SD overall = 35.68 ± 2.56; MC = 35.81 ± 1.95; DC = 35.67 ± 2.59 gestational weeks). Only few twins (n = 27) were delivered before the 30th gestational week, all dichorionic. Moreover, significantly more twins were born in term (≥ 36th gestational week). Preterm membrane rupture

occurred in 25% of the cases, but the amniotic fluid usually had appropriate characteristics (Table 2).

In our sample of healthy twins without gestational complications only three twins were stillborn and all of them were from monochorionic pregnancies. These adverse outcomes occurred in two cases preterm (29th and 32nd GW) and in one case in term (35th GW) delivery. Contrary, no IUFDs were recorded. First twins had the mean birth weight 2,298.16 ± 621.48 grams and the mean Apgar score was 7.32 ± 1.85 (MC = 7.86 ± 0.99; DC = 7.28 ± 1.88). Second twins had the mean birth weight 2,237.53 ± 651.49 grams and the mean Apgar score was 7.28 ± 1.86 (M = 7.86 ± 0.99; DC = 7.24 ± 1.91). Nevertheless, majority of twins both monochorionic and dichorionic ones, were in good condition at birth and 59% of the first and 55.7% of the second twins received Apgar score ≥ 8 (Table 2). Therefore, the overall outcome of investigated twin pregnancies was good.

Chorionicity was correlated negatively with pregnancy outcome and positively with delivery mode (Table 3). Dichorionic twins had better survival rates. Monochorionic twins were at higher risk for adverse perinatal outcome [odds ratio (OR) = 0.023; 95% confidence interval (CI) = 0.001–0.609] and had a higher chance to be delivered by Cesarean Section (OR = 1.88; 95% CI = 1.05–3.38). Pregnancy outcome was associated with amniotic fluid characteristics, delivery time and twin birthweight, but not chorionicity. Moreover, there were no significant differences in mothers and twins characteristics or other assessed parameters regarding chorionicity.

Table 3

Correlations and differences of assessed parameters and chorionicity

Parameters		Chorionicity	Pregnancy outcome		Chorionicity
Mothers age	ρ	-0.036	0.049	χ^2	0.463
	p	0.497	0.351	*p*	0.496
Mothers parity	ρ	0.021	0.054	χ^2	0.164
	p	0.686	0.310	*p*	0.685
Chronic illnesses of the mother	ρ	0.088	-0.012	χ^2	2.785
	p	0.095	0.819	*p*	0.095
PPROM	ρ	0.092	-0.075	χ^2	3.055
	p	0.080	0.156	*p*	0.080
Amniotic fluid characteristics	ρ	-0.065	**-0.112**	χ^2	1.516
	p	0.219	**0.033**	*p*	0.218
Delivery mode	ρ	**0.776**	-0.002	χ^2	**6.266**
	p	**0.039**	0.964	*p*	**0.042**
Sex twin I	ρ	0.031	-0.025	χ^2	0.345
	p	0.558	0.631	*p*	0.557
Weight twin I	ρ	0.013	**0.401**	χ^2	0.061
	p	0.805	**0.001**	*p*	0.805
Apgar score twin I	ρ	0.042	**0.785**	χ^2	0.634
	p	0.427	**0.000**	*p*	0.426
Sex twin II	ρ	0.015	0.048	χ^2	0.085
	p	0.771	0.368	*p*	0.770
Weight twin II	ρ	0.025	**0.395**	χ^2	0.231
	p	0.631	**0.001**	*p*	0.631
Apgar score twin II	ρ	0.050	**0.837**	χ^2	0.902
	p	0.343	**0.001**	*p*	0.342
GW of delivery	ρ	-0.012	**0.316**	χ^2	0.052
	p	0.819	**0.001**	p	0.819
Pregnancy ending time (pre/in term)	ρ	0.029	**0.350**	χ^2	0.310
	p	0.578	**0.001**	p	0.577
Twins condition at birth	ρ	0.050	**0.624**	χ^2	0.895
	p	0.345	**0.001**	*p*	0.344
Pregnancy outcome	ρ	**-0.831**	/	χ^2	**7.369**
	p	**0.026**	/	*p*	**0.018**

***Note*: Significant differences are bolded.**
PPROM – premature preterm rupture of membranes; GW – gestational week; ρ – coefficient of correlation.

A significant binary logistic regression equation of relationship between pregnancy outcome and chorionicity was obtained ($\chi^2 = 5.343$; $p = 0.021$; B = 3.750; Wald = 137.356; Exp(B) = 42.5; R^2 Nagelkerke = 0.851; total classification % = 80). Model was adjusted for PPROM, delivery time and mode as well as mother's age, previous parity and illnesses: Twin pregnancy outcome = – 0.792 + 0.038 × PPROM – 0.012 × gestational week of delivery – 0.084 × chorionicity

From the obtained equation it could be seen twin pregnancy outcome was significantly associated with chorionicity. Still, occurrence of PPROM and earlier gestational week of delivery were important confounding factors that could influence the relationship between chorionicity and pregnancy outcome. Therefore, prevention of gestational complications and preterm birth are crucial to minimize the potential negative impact of monochorionicity on twins' survival and condition.

Discussion

According to the results of our study chorionicity can influence survival of otherwise healthy twins placing MC twins at greater risk. However, chorionicity does not significantly impact the condition at birth of live-born twins as well as growth and development of twins who endure up to term.

Most literature data show that monochorionic twins have higher rate of very preterm birth (before 33rd gestational week), very low birth weight (birth weight < 1,500 g), the first minute Apgar < 7 and hospitalization [1, 4]. Perinatal mortality is usually also significantly higher as well as intrauterine fetal death [6]. The prospective risk of IUFD is much higher for MC twins at all gestational ages but the highest risk is before 24–28 gestational weeks [7]. Monochorionic pregnancies carry an increased risk of both a single fetal demise and a double twin demise mostly occurring before the third trimester [8]. MC twins were also two times more likely to be stillborn than DC twins. However, the prospective risks of stillbirth is found to be low for both MC and DC twins after 32 weeks of gestation and decreases even more at higher gestational weeks especially for MC twins [9, 10]. If both fetuses are alive at 24–28 weeks, the chance of their survival until term is similar in MC and DC pregnancies [2]. Some studies have shown that mortality of MC and DC twins did not differ in deliveries after 30 weeks of gestation, probably due to the fact that modern obstetrics is more effective in reducing mortality in both MC and DC twins [1, 11]. Still, after 38 gestational weeks MC twins have a higher risk for perinatal mortality compared to DC twins. Placental vascular malperfusion is the usually the main complication of dichorionic while IUGR, placental vascular abnormalities and TTTS, abnormal cord insertion and adverse neonatal outcomes are more common in monochorionic twins [12]. In our study, no IUFDs were registered in uncomplicated healthy twin pregnancies regardless of their chorionicity. On the other hand, adverse perinatal outcomes occurred only in case of monochorionicity. All adverse outcomes happened during or close to delivery implying an additional risk for monochorionic twins that might be caused by issues of the cord (entanglement in case of single amniotic sac, etc.) or some still unknown processes that needs further investigation. However, it should be mentioned that the rate of adverse outcomes was very low and that majority of investigated twins were live-born and in good condition with high Apgar scores.

The optimal time for delivery of monochorionic twins to prevent cord entanglement, growth discrepancies and intrauterine fetal death is still controversial [13]. MC twins are mostly delivered preterm [4]. As the highest morbidity for monochorionic twins is registered in 35th and 36th GW some authors support delivery of uncomplicated monochorionic twins at completed 34 gestational weeks [14, 15]. Conversely, others think that, with a strategy of close fetal surveillance, both monochorionic and dichorionic pregnancies can be continued to ninth lunar month (36–37 gestational weeks) with minimal perinatal morbidity [16, 17]. Studies have shown that median gestational age is mostly one week longer in DC twins than in MC ones. In uncomplicated dichorionic twin pregnancies delivery should happen at 37 weeks of gestation and in monochorionic ones at 36 weeks [16, 18]. Although delivery closer to term was associated with better pregnancy outcome, chorionicity did not have any significant influence on time of delivery in our study. Investigated twins were successfully delivered mostly in the 36th gestational week regardless of their chorionicity. Consequently, it can be said that if there are no gestational complications that might indicate induction of preterm delivery, both MC and DC healthy twins should not be delivered before ninth month of gestation.

Some studies indicate that there is usually no difference in the delivery mode of twins as a function of chorionicity and consequently intrapartum management should not vary due to chorionicity [19]. Twins we followed were generally more often delivered by CS. However, MC twins had a higher chance to be delivered by CS than DC twins. On the other hand, delivery mode did not influence pregnancy outcome, so the optimal delivery mode of twins should be more thoroughly investigated in further studies.

Most literature data show that mean birth weight in DC twins is usually significantly higher than in MC ones and that MC twins are almost two times more likely to have fetal growth restriction or severe birth weight discordance (> 20%) [4, 16]. Still, other studies show that severe birth weight discordance occurs equally in twins regardless of their chorionicity [1]. There were no significant differences of birth weight between MC and DC twins in our sample.

In studies using multivariate analysis, lower gestational age at delivery, monochorionicity and growth restriction were independent predictors of adverse neonatal outcome [12]. The model we constructed showed that chorionicity did not impact pregnancy outcome even in healthy uncomplicated pregnancies. However, more attentions should be paid to prevention of PPROM and preterm birth as these two parameters were found to be significant confounding risk factors of adverse pregnancy outcome.

Conclusion

Monochorionic twins, even if healthy and uncomplicated, are at high risk for perinatal mortality. Nevertheless,

chorionicity does not significantly impact the condition at birth, growth and development of twins who manage to survive nine lunar months. Therefore, it can be said that if appropriate prenatal and peripartum surveillance and adequate therapy are applied, both healthy twins can be delivered even in term regardless of their chorionicity.

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Overdenture in terms of preparation and restoration of supporting teeth

Supradentalna proteza sa aspekta pripreme i restauracije potpornih zuba

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Introduction

Overdenture (OD) is applied in removable prosthodontics and also for the prevention of edentulous alveolar ridge reduction [1–7]. Magnets insertion into the OD results in significant slowing down of periodontal disease [2] and osteoporosis [4, 5] and produces an antimicrobial effect in the oral cavity [7]. Tooth extraction presents irreversibile mutilation that patients unwillingly agree especially in case of the last remained teeth. Of that reason, the suggestion about keeping those remained teeth is being gladly accepted in case where patients are very amenable and prepared to any cooperation. In this respect, patients psychologicaly very gladly agree with cutting the tooth crown and creation of appropriate abutment in regard to OD that is followed because now they keep their "hopeless" teeth [8]. Subtotal edentolousness might be solved in many ways where the main existing problem is almost always the residual alveolar ridge reduction. The extraction of remained natural teeth and rehabilitation of such status by conventional full denture (D) is nowadays very often, but the most unwillng solution, where every therapeutist is aware of everlasting problem on retention and stabilization. On the other hand, rising usage of implant-supported ODs offers many possibilities of prosthodontic rehabilitation of subtotal edentulousness. Regarding beforementioned, up-to-date literature offers various solutions for fabrication of implant-bearing OD both for lower and upper jaw. Here many tasks are to be planned such as implant load by suprastructure and precision attachments (connectors) considering partially edentulous alveolar ridge. A decade before, a very few dentists were brave to indicate OD fabrication in fear of prognosis on the remained, partially endangered teeth. The near future and epidemiology studies will answer the question wether partially edentulous jaw would be solved by conventional acrylic plate denture or ODs. Besides many functions, teeth in oral cavity present the symbol of youth, sound health and beauty from a psychological point of view. Their loss in number and shape might be considered as endangered health, power loss and sign of aging predominantly in rural areas where negligence is more present than in urban ones. Teeth loss sometimes disturbs emotional tranquility exposing often depression. In those cases full denture is unacceptable as a foreign body and loss of identity that is one more reason to prefer OD solution respecting remained teeth-abutments [7–13].

Bearing this in mind, the patients who have perodontologically weakened teeth in dental arch could count on their keeping and specific preshaping/restauration thus ready to accept OD that follows. By the way, tactile discrimination is preserved by teeth keeping as much as possible [14]. All this motivates a patient to the success of the whole OD treatment. In favour of indications for OD planning goes the fact about possibility of magnet incorporation in its base [15, 16]. Encouragements were found about magnet antiosteoporotic [6] and antimicrobial effects [7]. Periodontal tissues around remained preserved teeth, pulp and alveolar bone under OD can be further stimulated biologically by installing micro-magnets and permanent effect of the magnetic field.

The aim of this paper is to present the different approaches in planning, choice, preparation and way of restauration of the remained teeth for OD acceptance.

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General considerations

Based on the assessment of the clinical status and teeth radiographies, the decision about retaining the last remaining teeth is to be made. The criteria for hopeless teeth are: less than 5 mm of health alveolar bone and poor endodontic prognosis (narrow, curved, obstructed or caries-destructed tooth-root) [17]. The remained teeth before OD fabrication might be conservative or prosthodontically prepared/restored by filling in calotte shape [amalgam, composite, glass ionamer cement (GIC), casted cap] [17, 18].

Considering the justification for OD, its significant benefits could be characterized through financial factor and preservation of supporting tissue around crown-destructed tooth, also maintaining the satisfied bone density, very important in elderly population prone to osteoporotic process [19–21].

In the moment of the change of submaximal partial toothlessness and conventional acrylic denture to OD, the half-time life period of the remaining teeth is prolonged because patients are able to adapt to new OD [17, 22].

In the case of spacing set of teeth or anodontia of several teeth, it is important to keep each of them or apply quality treatment and restoration [22].

A layer of saliva, which still exists between the depressions formed in OD by impression of retentive restored tooth stump (abutment) and outer surfaces of the restoration, beneficially acts by amortizing the mastication forces that tend to move OD in the horizontal plane. It also has a favourable impact to the adhesion to OD in terms of minimizing the displacement in the vertical direction. This phenomenon is also obtained by fabrication of larger surface of the restoration and preforming of the occlusal tooth surface to be at the level with the surface of the definitive spherical occlusal plane.

Restoration of teeth defects, regardless of origin (caries, erosion, attrition, abrasion, fracture) always has to be planned the best way taking into account the retention, resistance to pressure, its solubility, distance to gingiva and endodontium condition as well [22, 23].

Considering less favourable aesthetic solutions of ODs, they are indicated with extreme wear of tooth substance (bruxists, some professions) [23]. ODs may be indicated in cases of extreme tooth wear because it is the easy way to increase the vertical dimension of occlusion [24].

Regardless of the choice of the appropriate type of restoration and material during the crown preparation it is important to estimate large undercuts and neutralize them, then preciously plan the type and design of coronal restoration for OD acceptance [18].

Retention of OD can be based on resting on the shape retention of milled fixed restoration such as cast cap (more usually in practice), creating duplicate – telescopes [3] or installation of specific milled precision attachments and bars.

Tooth selection for restoration

From the standpoint of stability, bilateral remaining tooth position is always a better choice than unilateral. However, even the survival of only one incisor can have a very positive impact on the OD stabilization and alveolar ridge preservation in the frontal region which is very prone to the resorption changes [25]. Hence, during the selection of the remaining teeth, priority should be given to the canines, then premolars and optionally incisors [26]. We should bear in mind the survival prognosis of teeth with periodontal disease or osteoporosis [5]. Molars are rarely candidate for the OD bearer due to endodontic reasons (high risk of failure, more complicated and long-term treatment, unsafe guarantee for tooth survival in the jaw or pulp-periodontal complex relation). The incisors are also extremely rare for selection because of unfavourable crown-root ratio and might be included only when there is no other option (markedly reduced alveolar ridge when incisors are only remaining teeth, preserved satisfactory). Restored teeth have a reduced height of the crown thus achieving a favorable ratio to the abutment root. This also reduces harmful effects of the lateral forces when stimulation positive effect of the masticatory forces might be archived [22].

The most favourable teeth for OD support, assuming topography, are canines (angle teeth) and molars [27] because their mass enable greater stabilization and retention owing to the root morphology, huge root surface, vast and wide junctional epithelium in comparison to the other teeth groups, as well as large root canal space very wishfull for endo treatment. Mascola [28] and Basker et al. [29] in their own clinical studies prefered canines for OD support. The same is for premolars, eventually dislocated mesially, more often in cases of prematurary loss of frontal teeth. Our longitudinal research pointed out canines as the most long-lasting teeth under OD after ten years of check-ups (49%). The exposed premolars have shorter survival upon six (48%) [28] and ten years of surveillance (18%) [30].

Periodontology

Periodontal tissue that surrounds preserved remaining teeth, intact pulp and alveolar bone below OD may be additional positive biologically stimulated by installation of small magnets and their permanent irradiation [4, 16]. The teeth with greater alveolar support and coronal mass should enter the list. They also have to fulfill one condition – to be inserted in alveolar bone 5 mm at least [22]. The confirmation for these long-lasting teeth in the jaw gave the six-year study that recorded the average loss of alveolar bone height of only 1.5 mm [30]. A ten-year study of American authors points to the just 5.9% of failure due to bad periodontal condition as the cause of tooth extraction [2]. The other groups of teeth are considered insufficiently strong to withstand the mastication forces and have to be extracted [31].

Materials and modes for direct tooth restoration

Extraalveolar parts of teeth are cared for conservative or prosthetic restorations as calotte-hemispherical shape of amalgam, composite, glass ionomer cement (GIC) of cast-caps depending on the size of the retention and restoration of gingival shoulder junction. Supplemental retention of some fillings might be presented by canal post or parapulpal pins.

A short and rounded tooth crown of a sphere shape without fillings is abandoned method to adapt to OD. It is potential carrier for new or already existing microbes in the dentinal tubules. Microbes have to be captured with the appropriate long lasting adhesive agent whether at vital or non vital teeth [32]. In the study of Ettinger and Krell [32], the authors obtained 17% of failure in teeth preservation and teeth vitality where after cutting crown of dentinal stump, surface was only polished without capping or any type of protection. For such cases the most appropriate methods of restoration are liquid or hybrid composites because of their micro penetration and good adhesion to the conditioned substrate (dentine surface of the tooth stump). If the coronal restoration of the abutment tooth is not properly rounded, due to unfavourable distribution of forces and the fact that the OD base is thinner in dental then in the edentulous area, denture fracture could occur.

Although the roots of teeth served as early as 1856 to stabilize the prosthesis [3], this doctrine began to increasingly apply only since by the merit of Miller (1958), the first time in the US, using the vital tooth with the preparation of annular cap without tooth devitalization. It was not until 1969 when Morrow and collaborates suggested a reduction of the tooth crown to a few millimeters above the free gingival margin which of course usually require root canal therapy [32]. In shallow cavity of vital teeth, when it is assumed that the micromechanical retention will be insufficient, parapulpal pins are desired (class III, V and erosion) respecting the pulp topography. Where the situation demands, in "slot cavities", it is possible to improve the retention by preparation of moderate convergence of walls outwardly (mild undercuts). The drying up of tooth substance before relining and filling should be avoided especially in the application of composite, GIC and compomer materials. It is particularly stressed in non-vital teeth due to weakening of chemical bonds of tooth-filling whose chemistry requires minimal presence of moisture in the dentinal tubules [33].

It is necessary to round off the remaining tooth stump after the reduction of the tooth crown in order to reduce the impact of adverse forces.

Novelty for amalgam application on the tooth stump assumes, besides additionl macromechanical retention (dovetail, notch within the the cavity), also the micromechanical retention that involves the application of conditioning of enamel margins and peripherial dentine tissue with amalgam bonding substances [34].

Even improved GICs, up-to-date formulations are recomended for restoration in low stress-bearing areas. Hence, in the case of non-vital tooth, making the deobturation of the first 2–4 mm and applying GIC barrier enable extended amalgam filling with good retention in the coronal portion of canal with the possible implementation of preferred amalgam-bonding agent. Nonvital tooth has lower percentage of water then vital one, thus attention should be focused on the mild drying where remainig water contributes the qualitative chemical reaction during bonding process.

Narrow indication for GIC restoring is abutment teeth prone to caries occurence and other defects under gingival margin with mandatory ecartation (cauterization, haemostiptic caustic) and restoration varnishing upon finishing and polishing. The low solubility of type II (around 0.4%) favours GIC restorations in the gingival third cavities at abutment tooth [35].

In those cavities, varnish layer is mandatory and rubber dam technique due to the water imbalance. The second reason to apply GIC is easyness of removing of restoration if necessary. Up to date GIC and composite filling owe adhesive systems as well as conditioning of cavity walls by polyacrylic and other organic acids for appropriate chemism they need [25]. If the remaining abutment teeth (premolars, molars) under OD are prone to exessive force in masseteric muscular type of chewing, the choice for restoration could be GIC type II–2, silver-reinforced or "cermet" GIC, either as a filling base or a dentine base (dentine substitute) that is further can combine by a cast cap. Silver-reinforced GIC restoration emerged in the early eighties of the last century while still is in very successful use especially in vital teeth as temporary filling [36].

With increased quality of contemporary adhesives and their bond strength to the tooth hard tissues, the interests among dentists were arising particulary for type of OD prosthetic rehabilitation concerning good composite restoration for abutment teeth. This way of restrictive preparation does not follow the Black rules of preventive extension and extension due to retention when modern types of composites are used. Their elasticity modulus similar to dentine and more superior resistance to pressure in constant contact with dynamic movements of OD should have entered them (ceramcor, ormocer, compomer) on the final checklist and shortlist. In addition, we should uphold strictly to a manufacturer's protocol regarding preparation (conditioning) of cavity walls and composite/adhesive systems application to the treated surfaces [37].

Where necessary, additional macroretention should be set by canal composite post and composite adhesive for fix it by light cure.

In favour of the use of new generation of adhesives for enamel and dentine substrate, combined with aesthetic composite resins is increased bond strength (15–25 MPa) [37]. They simplify the procedure (one bottle system) especially at gingival and subgingival tooth defect, very often cases in OD wearers. In elderly population, prompt action of acid etching, washing and conditioning should be done in relative or absolute dry operation where one bottle adhesive system is required.

The latest investigations have suggested the best bond enabled in the adhesive agent hybrid composite system regarding conditioned dentine creating very thick hybrid layer. This was confirmed by Micro Raman spectroscopy and scanning electron microscope (SEM) analysis where adhesive system of "etching and rinsing" creates thicker bonding hybrid layer in hard tooth tissues in comparison to the next adhesive combinations: self-etching one step and self-etching by two bottle system [38]. As the abutments present solitary remaining teeth in the jaw (without agonists neighbours), they are directly exposed to the stressful thermodynamical

changes from all sides where the best solution for their restoration present hybrid and microfilled composit resins [37].

At vast and shallow lesions (cavities), regardless of taking care on the composite applying in thin layers, as well as gradual amplifying on the curing light, material contraction still occurs at some degree. For those cases, the best choice is composite consisting of the great deal of filler and less monomer content as well as resin that lessen the viscosity and thus create superior contact to the outermost and distant surfaces of the cavity [37].

With the advancement of composite technology regarding their physical properties, nowadays is possible to do patching correction at aesthetic fillings. The advantage of patching old composite over its entire removing is unwishfull adhesive fractures that might occur within resin material. Also, regarding tooth resistance to fracture, the correction should be placed as much as distant to the gingiva and antagonist contacts, closely intended for low-caries risk patients who "promise" proper oral hygiene and regular checkups [37].

Some authors point out that combining of persisted amalgam and/or amalgam repair by composite filling increases the resistance to fracture around 51% in comparison to entire amalgam replacement. They conclude that rebonding and patching of old composite by new one is beneficial in improving superior resistance to pressure and fracture [39].

The vital teeth tissues should be avoided of long and excessive drying (manufacturers' instructions) because necessary remained wetness is wishfull to create good bondage to the composite filling.

At nonvital teeth, precaution of over dessication should be more pronounced due to the less wetness in hard tooth tissues, i.e. enough water content is prerequisite for bonding chemism. In those teeth of decreased flexure strength, final restauration surrounded by enough dentine wall thickness would be of rounded shape to resist the masticatory forces [40].

The newest studies concerning microleakage of composite resins have revealed increased leakage in the deep cavities of great diameter, what is more often at abutment teeth under OD.

Even solo use of dentine adhesive agent without comosite restoration for nuded root surfaces, often in OD bearers, could prevent further progress in depth [41].

Indirect restorations

Molded golden or Ag-Pd alloy caps restorations are indicated in abutment teeth with destroyed extraalveolar structure, especially where it is impossible to make preparation with healthy supragingival solid surface of tooth neck [3], i.e. when gingival wall of tooth is missing or partially destroyed. It is then usually made as a fixed post in root canal for better retention.

By that, the cap follows the shape of neighboring alveolar ridge regardless its design. The cap design can posses additional retention ornament or not above marginal edge and demarcation [18].

Short dimensioned caps require the preparation of retentive (canal) posts or dentine (parapulpal) pins. This is not necessary for the medium-sized caps, usually dome or long ones of thimble shape. This type of restoration can be applied directly to the prepared tooth stump or previously prepared and restored stump (amalgam, composite, GIC, polycarboxilate or oxiphosphate cement) [42, 43].

At vital and nonvital teeth, chamfer form of demarcation should be precisely defined in order to completely rest the cap margin fully on the prepared tooth part.

The advantage of amalgam and cast fillings over other materials in the gingival third of the supporting teeth, because of the constant presence of moisture during filling, is reflected in resistance to solubility over time. Contact of these fillings with oral fluids immediately after implantation is less susceptible to dimensional changes than other restorative materials (composites, GIC), which is difficult to provide anaerobic conditions without the presence of moisture in the first few hours or days during their setting-curing time (crystallization, conversion, maturation).

Restoration luting

Immediately before cementing, overdrying should be avoided when disinfection and insulation should be done by appropriate liners/varnishes of big wetting angle to enable theirs deep tubule penetration [44]. The best properties for luting exposed surfaces has GIC-type 2 due to the favourable features (viscosity, adhesion, solubility) [42, 45, 46].

Endodontic treatment of abutment teeth in the scope of overdenture manufacturing

From the standpoint of modern endodontics there is a need to save and keep each tooth that shows great chances for successful treatment, for example simple canal system. When molars need endodontic treatment, indications for their keeping become much wider. Sometimes, the current unsatisfactory status of filled root canal (canal with bad filling) does not necessarily show the failure (e.g. a female patient, age of 73, who has been followed for 12 years). Her teeth, with insufficiently filled canals (all three lower incisors) still persisted in the jaw without manifested focus followed by well-preserved alveolar structure [31].

At the end of the previous century, when endotreatment had reached high results, whether alone or by periapical surgery, this form of subtotal edentulousness began to atract more and more OD supporters. Regardless of the endodontic mode of solving the pulpal/periapical pathology (cases of inflammed or infected tissues), the standpoint of abutment tooth longevity should bear in mind as a safe support for OD. The 3–4 months period of follow-up and tooth radiology control are considered mandatory for the full security. In minor endo cases, the excpectation period of possible flareup is of several days when significant relief of symptoms is expected [1].

Periodontal lesions combined with endo pathology presents specific entities but also a high risk for inclusion of

such teeth as OD carriers. For those teeth, primary periodontal treatment involves periodontal and then endo treatment as much as possible compulsory. When endo arousses perio lesion, endo therapy is premier without parodontal therapy. Almost invisible radiological canal silhouette does not involve endo treatment in elders except in extreme rare cases when retentive canal post is unavoidable solution [47].

Endodontic retreatment should be done in case when canal entrance comunicates for 4–40 days at least with oral cavity even if good canal obturation exists on radiogram due to saliva diffusion [47, 48]. Some authors have informed risk period of 20–90 days even if proper compaction obturation technique is used [48, 49]. The best prognosis at periapical lesion cases gives endo treatment where $Ca(OH)_2$ canal dressing is applied [50, 51]. Mandatory check-ups are protocolar (6 and 12 months) where Ettinger and Quian [1] recorded around 13% postprocedural failures during 23 years at 626 endo tretmants, of which one third were due to secondary caries, loose of restorative adherence and microbial leakage. Authors found 31% cases of vertical fractures, predominantly at upper teeth due to lower amortization in maxila rather than mandibula which was connected by scull base with soft tissue of temporomandibular joint (TMJ). The fractures were noted in teeth without caps when occluded to antagonists. Periapical lesions failed in endo therapy numbered 3.8% extractions of all teeth under OD in the 10-years study [2]. That research cited prevalence of vertical tooth fractures and extractions mostly in men, probably because of stressed superior bite force.

Overdenture and tooth with attachement or implant combination

If more stability and retention of OD are required, attachments should be planned on the pair of distal teeth (pair of premolars) at Kennedy I case where the rest of teeth might bear indirect or direct fillings. This combination (for example with supraradicular attachments) and other simple tooth restorations are very thankfull for OD fabrication. Advantages of such combination are: good stabilization, chance of dental bear of mastication forces, preservation of alveolar ridge and periodontal receptors as well as quick patient adaptation to new oral condition [11]. Besides attachments on the strong teeth abutments, the implant units might be involved for better OD stability reason, especially in mental disordered patients (hyperkinetism) [52].

Check-ups

Periodical controls are mandatory for teeth-abutments at six and 12 months and every six months later on, due to possible restoration failures, missing and fractures [2], assuming suspective symptoms, secondary caries and perodontal tissue condition. Perialveolar pockets lead to the tooth loose by provoking the uncurable periapical process [32].

Up to date, digital radiology systems might be of great help during radiogram analysis where strong softer enables easy and comfortable follow of alveolar bone density in comparison to the conventional devices. This is enabled through the histogram of bone part along the drown line what is possible to follow in the course of time by memorize the data at check-up controls [5, 53, 54].

The partial or total loss of coronal restoration after a few (3–7) days can cause diffusion of microorganisms and their products which is particularly unfavorable for curved/narrow root canal what some *in vitro* studies revealed [48, 55]. Often controls could prevent such cases when endodontic retreatment must be avoided. If control check-up predicts resection of root, the crown/root ratio has to be not less than 1:1.3.

Tooth restorations and OD itself should be controlled often. There is a need to make an increased effort to maintain them with occasional fluorination of teeth resulting in lower caries incidence [1]. Undetected defect of non-vital tooth fillings when controls miss, allows diffusion of undesirable agents of the oral cavity through dentinal tubules (lateral periodontium) and the apical canal portion (apical periodontium). Hence, there is a need to fulfill at least the first three millimeters of coronal canal portion with GIC as a gold standard by well adaptation to the walls of the access cavity [35]. The modern view is that retreatment must be done if microleakage persisted for at least three months (canal medication by $Ca(OH)_2$ suspension for a period of 2–4 weeks) with a consequent obturation of compaction techniques of gutta-percha. Contemporary findings suggest that undetected coronal filling defect enables human salivary flora penetration even the entire length of the hermetic root canal obturation by proper compaction technique for a period of 4–40 days [48, 55].

Restored nonvital teeth are particularly susceptible to fractures (along root and crown) that can occur spontaneously or accidentally and should be monitor by six month intervals by the help of loupe and clinical microscope. Those teeth often expose pathognomonic signs of sensitivity to bite and percussion that persist even after the disarticulation of prosthesis even after retreatment or apicotomy. Those teeth should be extracted in case of inadequate therapy, i.e. patients after extraction were immediately alleviated of painful percussion sensation.

The advantage of OD is the fact that the supporting restored teeth allow patients greater mastication force [22] than other solutions of submaximal edentulousness (classic partial or full denture after extraction of the last remaining teeth). Some authors recorded an increase in the strength of occlusal forces up to 120% in the case of OD over the abutment teeth [56]. In addition, OD preserves facial contour mimic muscles and prevents the appearance of wrinkles which meet aesthetics too, slowing the process of aging.

If one considers quality/function of speech in conventional denture bearers, it is much better with OD over supporting teeth whereby better coordinated and an interjaw relation is achieved.

Further preservation of supporting teeth and neighbouring alveoli by OD can be provided by inserting of small magnetic cylinders in its base under the remaining teeth. The constant magnetic field is beneficial in reducing the dental plaque index values [7] and food retention around the support-

ing teeth due to the poor oral hygiene in elders, what was confirmed in the 12-month study [4].

Long-term monitoring of patients rehabilitated with OD, by implementation of the above-mentioned restoration procedures, recorded a low level of tooth loss (about 10%) at the end of the studies [3, 4, 24, 30, 31].

In addition to the financial aspect, the advantage of this kind of restoration and conservation of the supporting apparatus of crown-destroyed teeth is evident. These teeth also contribute to maintaining density of the jawbone bearing in mind the pronounced presence of osteoporosis in elders [5].

Conclusion

Whatever kind of tooth restoration is planned, the most appropriate mode must be chosen for the current situation, it should be processed properly to enable long abutment life and an adequate OD support. By keeping and restoring of the remaining teeth, their periodontium must be protected too, enabling proprioceptors to protect the abutment teeth of excessive force during the chewing function and help to control the position and movement of the lower jaw. Patients are therefore subjectively much more comfortable with several own teeth combined with OD if compare to the classic partial denture they have. All in all, if one can summarize the overall benefits for patients, wearer of ODs, owing to the our long lasting experience as well as extensive data from foreign literature, the importance of preserving the remaining teeth is really huge. This implies an inevitable and significant advantage of OD over other forms of prosthetic treatment of subtotal edentulousness. Positive developments in restorative, both laboratory and clinical prosthetic dentistry, are increasingly noticeable, obvious and inevitable, whereof the improvement of OD is limitless and depends on the imagination and practitioner's skill. It can be concluded that if you understand the essence of its existence and many restoration factors that determine its design, OD can be considered as a simple and easily manageable job. In fact, if we start from a simple item concerning its creation, the rest will come by itself. Hence, master the simple, the rest follows.

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Unilateral, frontal polymicrogyria and supratentorial white matter microcysts in fetus with Joubert syndrome and related disorders: Prenatal diagnosis with magnetic resonance imaging

Unilateralna frontalna polimikrogirija i mikrociste supratentorijalne bele mase fetusa sa znacima Joubert-ovog sindroma i povezanih bolesti: prenatalna dijagnoza magnetno rezonantnim snimanjem

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Abstract

Introduction. Joubert syndrome (JS) and related disorders (JSRD) are a group of rare multiple congenital anomalies syndromes, defined by complex midbrain-hindbrain malformation that creates the "molar tooth sign" (MTS) on brain imaging and may be associated with multisystem organ pathology, mainly of the retina, kidney, liver and skeleton. Prenatal diagnosis of JSRD has proved difficult because of the rarity of the condition and low sensitivity of ultrasound in evaluation of the fetal posterior fossa (PF) in most affected fetuses. **Case report.** We presented an unusual case of JSRD, pure Joubert syndrome with unilateral frontal polymicrogyria and supratentorial white matter microcysts, diagnosed by magnetic resonance imaging (MRI), in fetus aged 30 gestational weeks. The distinctive MRI features of this rare ciliopathy were confirmed by 6-months postnatal MRI study. The postnatal outcome was poor; clinical follow-up in the first 6 months of life confirmed hypotonia, developmental delay, oculomotor apraxia and seizures. **Conclusion.** To the best of our knowledge, fetal MRI features of the coexistence of pure JS and supratentorial abnormalities leading to postnatal cerebellar dysfunction and epilepsy, have never been reported before. Presented case may contribute to the broadening of the spectrum of sparse prenatal features of JRSD, and support the stand that presence of neuronal migration abnormalities can affect the clinical outcome and prognosis of fetuses with JSRD.

Key words:
cerebellum; congenital abnormalities; diagnosis, differential; epilepsy; neurologic manifestations; growth disorders; prognosis.

Apstrakt

Uvod. Joubert-ov sindrom (JS) i povezane bolesti (*Joubert syndrome and related disorders* – JRSD) su objedinjena grupa retkih urođenih, razvojnih sindroma srednjeg i zadnjeg mozga, sa tipičnim neuroradiološkim „znakom kutnjaka", često udruženih sa multiplim anomalijama mrežnjače, bubrega, jetre i skeleta. Prenatalna dijagnoza JSRD je teška zbog veoma retke pojavnosti oboljenja i niske senzitivnosti i specifičnosti fetalnog ultrazvuka u proceni struktura zadnje lobanjske jame. **Prikaz bolesnika.** Prikazali smo neuobičajen slučaj JSRD sa unilateralnom, frontalnom polimikrogirijom i mikrocistama supratentorijalne bele mase, detektovane magnetnorezonantnom snimanjem (MRI) fetalnog mozga u 30. gestacijskoj nedelji. Prenatalno uočena morfologija je potvrđena MRI snimkom na uzrastu od šest meseci. Kliničkim praćenjem u prvih šest meseci života, uočeno je pogoršanje od rođenja prisutnih epileptičnih napada i neuroloških simptoma tipičnih za JSRD (hipotonija, razvojno zaostanje, poremećaji pokretanja očnih jabučica). **Zaključak.** Prema saznanjima autora, do sada nije objavljen slučaj prenatalno postavljene MRI dijagnoze JS udruženog sa supratentorijalnim razvojnim poremećajima, postnatalno manifestovanih cerebelookularnom simptomatologijom i epilepsijom. Smatramo da prikazani slučaj može proširiti uzak spektar neuroradioloških prezentacija JSRD u fetalnom

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i postnatalnom periodu i potvrditi mogući uticaj udruženih poremećaja neuronalne migracije na prognozu i klinički ishod u fetusa sa JSRD.

Ključne reči:
mozak, mali; anomalije; dijagnoza, diferencijalna; epilepsija; neurološke manifestacije; rast, poremećaji; prognoza.

Introduction

Joubert syndrome (JS) and related disorders (JSRD) are a group of rare developmental, multiple congenital anomalies syndromes, defined by complex midbrain-hindbrain malformation that creates the "molar tooth sign" (MTS) on brain imaging, associated with variable multiorgan pathology, mainly of the retina, kidneys, liver and skeleton [1]. MTS describes hypoplastic vermis, narrow mesencephalic isthmus, deep interpeduncular fossa and thick horizontally placed superior cerebellar peduncles, with consequent midline cerebellar cleft and dysmorphic fourth ventricle. The incidence of JSRD ranges between 1/80,000 and 1/100,000 live births, although these figures may represent an underestimate [2]. JSRD is thought to be part of ciliopathies, genetic disorders related to the disturbed development of primary cilia, organelles that play key roles in the development and functioning of retinal photoreceptors, neurons, kidney tubules and bile duct. JSRD are transmitted in autosomal recessive fashion, with the exception of rare cases following X-linked recessive inheritance [3]. JSRD are clinically heterogenous; the characteristic features include hypotonia, ataxia, oculomotor apraxia, neonatal breathing dysregulation, developmental delay associated with variable multiorgan pathology. Based on the main organ involvement and genotype-phenotype correlates, JSRD are classified in six phenotypic subgroups: pure JS and JS with ocular, renal, oculorenal, hepatic and orofaciodigital defects [1, 2]. Since the first Gleeson`s report in 2004, only five cases of JRS associated with neuronal migration anomalies have been described, but only based on postnatal imaging and/or autopsy findings [4, 5]. A single case of white matter cyst in JRSD detected by magnetic resonance imaging (MRI) was described by Senocak et al. [5]. To the best of our knowledge, prenatal imaging features of JSRD were described in the literature in only 17 fetuses, mostly examined by ultrasound (US) [6]. In all available prenatal imaging studies, MTS was noted as isolated finding, not a single case in conjunction with associated brain anomalies. We describe a unique JSRD case and fetal MRI features of the coexistence of MTS, cortical polymicrogyria (PMG) and white matter cyst.

Case report

A 37-year-old woman, (grav 2, para 1) with no relevant medical history, was referred to MRI unit, at 30 weeks of uneventful pregnancy, after previous US exam, performed two weeks earlier, suggested forth ventricle enlargement and vermian abnormality, as isolated findings. Fetal brain MRI was performed on a 1.5 T unit, with 3mm thick half Fourier single-shot fast spin-echo (760/104/1; TR/TE/excitations) T2-weighted images obtained in 3 reference planes. The fetal MRI study revealed MTS with vermian hypoplasia, bilateral horizontally oriented and slightly thicker superior cerebellar peduncles and deepened interpeduncular fossa. The forth ventricle was enlarged, with abnormally rounded fastigium. There were no abnormal MRI findings related to the PF size and cerebellar hemispheres. In the right frontal lobe, the gyral/sulcal pattern was abnormal with multiple irregular, shallow, cortical infoldings, consistent with polymicrogyria. Frontal white matter was slightly hyperintense with scattered punctiform zones, with signal intensity resembling cerebrospinal fluid (CSF). There were no abnormal MRI findings related to the cortex in the left cerebral hemisphere (Figure 1. A–D). Based on a presumptive diagnosis of JSRD, the parents were counseled that the fetus had a complex rombencephalic developmental abnormality combined with neuronal migration disorder, with expected developmental impairments and high risk of epilepsy. Due to religious beliefs, the family decided to continue the pregnancy.

A male neonate was born at 39 gestational weeks, *via* uncomplicated spontaneous vaginal delivery. At first day of life neonate manifested apnea spells and hypotonia, accompanied with oculomotor apraxia and seizures in the form of epileptic spasms. Physical examination at birth was unremarkable. Karyotype testing was not revealed chromosomal abnormalities, unfortunately; genetic testing (whole exome sequencing and/or targeted panels) was not performed. Clinical assessments in the following six months of age, revealed aggravation of cerebello-occular symptoms, an increase in number of epileptic attacks and significant developmental delay. Despite neurological deterioration, infant's respiratory abnormalities progressively improved with age, disappearing around the beginning of fourth month. An abnormal interictal EEG was recorded at six months, with electroencephalographic pattern of focal frontal spikes in the right central frontal region.

Postnatal brain MRI scan was obtained at 6 months of life, by using the same system of the prenatal studies. The MRI study confirmed prenatal MRI diagnosis; MTS was present with pathognomonic appearance, associated with midline cerebellar cleft in the place of the hypoplastic vermis, deep interpeduncular fossa and enlarged, "bat wing" shaped fourth ventricle on the axial scans. The unilateral PMG in the entire right frontal lobe was confirmed, without the gradient of severity in anteroposterior (AP) direction. Supratentorial white matter had slightly increased signal intensity on T2 sequence, suggestive of mild myelination delay. Multiple white mater microcysts were detected in frontoparietal regions bilaterally, more pronounced on the right side. Supratentorial ventricles, corpus callosum, basal ganglia and the cerebral cortex in the left hemisphere, appeared to be normal (Figure 1, E–H).

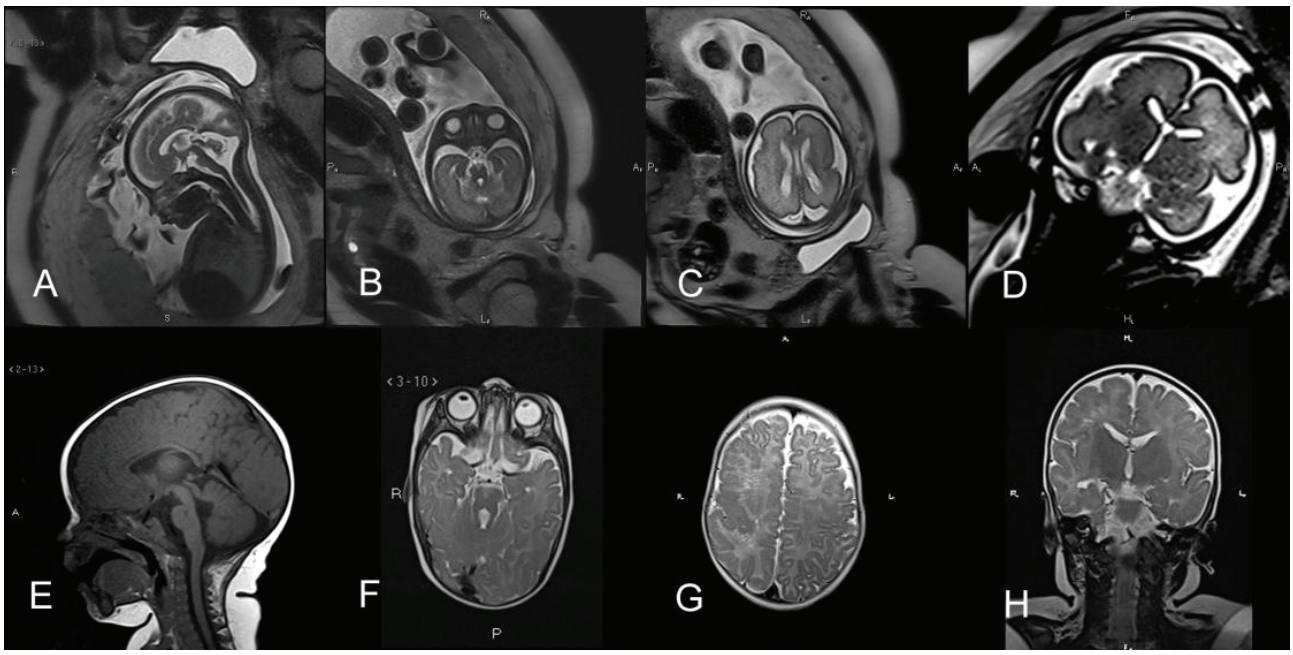

Fig. 1 – Fetal and postnatal magnetic resonance imaging features of Joubert syndrome and related disorders with unilateral polymicrogyria and white matter microcysts.

Midline sagittal T2W image (A) in a 30-week old fetus shows vermian hypoplasia, an enlarged forth ventricle with abnormally rounded fastigium, and horizontalized and thickened superior cerebellar peduncles. Axial T2W image (B) at the level of pontomesencephalic junction reveals deep interpeduncular fossa and dysmorphic fourth ventricle suggestive of MTS. Axial T2W scan, at a supraventricular level (C), shows the abnormal gyral and sulcal pattern in the right frontal lobe, consistent with polymicrogyria. Note the hyperintense signal of the supratentorial white matter, with scattered punctiform zones, resembling cerebrospinal fluid signal intensity (F). Postnatal brain MRI in sagittal (E), axial (F, G) and coronal planes (D), obtained at 6 months of life show presence of dysmorphic rhombencephalon, unilateral, frontal polymicrogyria, white matter microcysts and delayed myelination.

Discussion

Prenatal diagnosis of JSRD has proved difficult because of the rarity of the condition, the US limitations in evaluation of the fetal posterior fossa and the relatively nonspecific US features reported in most affected fetuses [6, 7]. The prenatal diagnosis of JSRB could be suspected in all fetuses with US findings of abnormal cerebellar vermis, either as isolated findings or associated with additional central nervous system or multisystem malformations. On the US scans of the fetal posterior fossa, MTS frequently remains unrecognized or overlooked. In our opinion, low sensitivity of US in detection of MTS could be caused by technique's inability to obtain clear transversal posterior fossa scans, perpendicular to clival line at the level of the pontomesencephalic junction.

Although MRI overcomes disadvantages of US in evaluation of the fetal rhombencephalon, its sensitivity in JSRD has not been systematically evaluated. Fetal MRI reports of JSRD are scarce and they do not reflect disease heterogeneity, describing only posterior fossa abnormalities. MTS was not detected in two of eight reported cases. Doherty et al. [8] reported only vermian hypoplasia, without being able to demonstrate MTS. We agree with current opinions that for fetal diagnosis of MTS, all pathological elements should be visualized on MRI scans and that coronal planes are dedicated for visualization of cerebellar cleft, axial planes for midbrain isthmus and fourth ventricle [9]. But, according to our study, fetal morphology of horizontalized and thickened superior cerebellar peduncle is better displayed in sagittal rather than in recommended axial plane.

It is not expected that MTS, as the neuroradiological hallmark of JSRD, changes its appearance, but according to Porreti et al. [10], rhombencephalic abnormalities could exhibit different degrees of hypoplasia or dysplasia (mild, moderate, severe). This can partially explain why mild forms of JSRD could be overlooked or misdiagnosed as isolated vermian hypoplasia or malformation from the Dandy-Walker spectrum. In order to enhance fetal MRI accuracy in diagnosing of JSRD, Saleem and Zaki [11] proposed quantification of morphologic changes at pontomesencephalic junction by measurements of AP diameters of interpeduncular fossa, isthmus and forth ventricle and calculation of the ratio of AP diameters. The reliability of the obtained parameters is not statistically verified. It is calculated in three cases, and we still do not know the normative values for healthy fetuses at different gestational age.

Although prenatally diagnosed infratentorial malformations in JSRD have been described sporadically in the radiological literature, coexisting supratentorial structural brain abnormalities were not reported at all. [8, 11]. It is possible that lack of data on supratentorial abnormalities is caused by moderate sensitivity of fetal MRI in detecting disorders at an early gestational age (less than 26 gestational weeks) and low conspicuity of the supratentorial abnormalities in JSRD

affected fetuses (e.g. hippocampal malrotation, mild midline defects, hypothalamic hamartomas and malformations of cortical development). According to our best knowledge, this is the first reported case of fetal JSRD with neuronal migration anomalies as associated supratentorial malformation [12]. Even though, our case is specific not only due to PMG being unilateral and exclusively localized in the frontal lobe, which sets it apart from two previously published pediatric cases of JS with bilateral, mostly perysilvian malformation, but due to the fact, that it is associated with delayed myelination and white matter cysts [13]. It is also of note, that the prenatally diagnosed right frontal lobe lesion represents a focal epileptic zone in concordance with postnatal seizure semiology and EEG findings. Unfortunately, clinical and imaging diagnosis in our patient was not confirmed by genetic/molecular data. According to Vilboux et al. [14], causative gene could be identified in 94% of JSRD patients, with significant genotype/phenotype correlation and high percent of unique potentially pathogenic gene variants linked to unusual clinical/imaging JSRD cases.

Conclusion

Presented case supports the claim that JSRD should be considered as a highly heterogenous malformation, with a variable degree of severity and clinical outcome. The prenatal MRI has to be mandatory part of prenatal diagnostic algorithm in fetuses with suspected JSRD, to confirm posterior fossa anomalies and detect the presence of associated supratentorial abnormalities. Presented MRI features contribute to the broadening of the prenatal neuroimaging spectrum, and could aid in prenatal prediction as to which JSRD affected fetus could develop epileptic seizures in the postnatal period.

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CASE REPORT

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Surgical treatment of acquired tracheoesophageal fistula caused by balloon dilatation of corrosive esophageal stricture in a child

Hirurško lečenje stečene traheoezofagusne fistule prouzrokovane balon dilatacijom korozivne stenoze jednjaka kod deteta

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Abstract

Introduction. Tracheoesophageal fistula (TEF) as a complication of balloon dilatation (BD) of corrosive esophageal stricture is a very rare and serious condition. Life threatening aspiration pneumonia requests urgent lungs' protection, but overall treatment strategy is not clearly defined. **Case report.** Twenty-month-old female child accidentally ingested a household bleach. Caustic injury of esophagus was healing with development of strictures of cervical and proximal thoracic esophagus. TEF was developed during the third BD. Healing of TEF and pulmonary infection was achieved by exclusion of the esophagus (pharyngostoma and feeding gastrostomy together) with prolonged tracheobronchial intubation and toilette. Retrosternal colon interposition was performed a year later, with excellent functional results over four-year follow-up. **Conclusion.** Esophageal exclusion in the first stage, and pharyngoesophageal reconstruction in the second stage, is a useful therapeutic option in the treatment of TEF caused by balloon dilatation of corrosive esophageal stricture in children.

Key words:
esophageal stenosis; burns, chemicals; dilatation; tracheoesophageal fistula; digestive system surgical procedures; child preschool; treatment outcome.

Apstrakt

Uvod. Traheoezofagusna fistula (TEF), kao komplikacija balon dilatacije (BD) korozivne stenoze jednjaka, je vrlo retko i teško stanje. Po život opasna aspiraciona pneumonija zahteva urgentnu zaštitu pluća, ali opšta strategija lečenja nije jasno definisana. **Prikaz bolesnika.** Dvadesetomesečna devojčica je zadesno progutala kućni izbeljivač. Stenoza vratnog i proksimalnog grudnog jednjaka razvila se kao posledica kaustične povrede jednjaka. Do razvoja TEF došlo je tokom treće procedure BD. Sanacija TEF i plućne infekcije postignuta je ekskluzijom jednjaka (faringostoma i nutrutivna gastrostoma) uz prolongiranu traheobronhijalnu intubaciju i toaletu. Retrosternalna interpozicija dugog segmenta kolona je urađena nakon godinu dana, sa odličnim funkcionalnim rezultatom tokom četvorogodišnjeg praćenja. **Zaključak.** Ekskluzija jednjaka u prvom, i faringoezofagealna rekonstrukcija u drugom stadijumu, predstavlja efikasan način lečenjaTEF nastale prilikom BD korozivne stenoze jednjaka.

Ključne reči:
jednjak, stenoza; opekotine hemijskim sredstvima; dilatacija; fistula, traheoezofagusna; hirurgija, digestivnog sistema, procedure; deca, predškolska, lečenje, ishod.

Introduction

Corrosive agents ingestion in children is accidental and usually caused by alkalies. Deep tissue involvement with inflammatory process often causes esophageal stricture which requires dilatation or even esophageal replacement. Esophageal perforation with formation of tracheoesophageal fistula (TEF) during dilatation of corrosive esophageal stricture is a very rare complication, and may lead to fatal aspiration pneumonia [1–4].

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There is no consensus about the treatment, and we believe that each case report can help in outlining the optimal strategy for distinct clinical presentations.

Case report

Twenty-month-old female child accidentally ingested a household bleach containing sodium hydroxide. Caustic injury of esophagus was healing with development of strictures of cervical and proximal thoracic esophagus. Barium swallow showed stenosis of the cervical and proximal thoracic part of the esophagus without stenosis of infracarinal part of esophagus and stomach, and without gastroesophageal reflux. There were no clinical signs of inhalation injury. Ballon dilatations started 15 days after the injury, in ten-day intervals. First two dilatations were performed without complications and sufficient peroral feeding was possible between them. Hypersalivation and coughing appeared immediately after the third dilatation. The next day there was a clinical deterioration manifested by tachypnea, tachycardia and high body temperature, accompanied by leukocytosis. A chest X-ray showed bilateral infiltrates in basal lung zones without mediastinal widening. Fiberoptic tracheobronchoscopy showed a 15 mm long laceration of the membranous tracheal wall, located about 1 cm above the carina (Figure 1a). Cervicothoracic computed tomography (CT) scan, after pulling upwards of endotracheal tube, confirmed the 15 mm long fistula between the strictured esophagus and the membranous tracheal wall (Figure 2). There were bilateral lung infiltrates, without gas and liquid collection in the mediastinum.

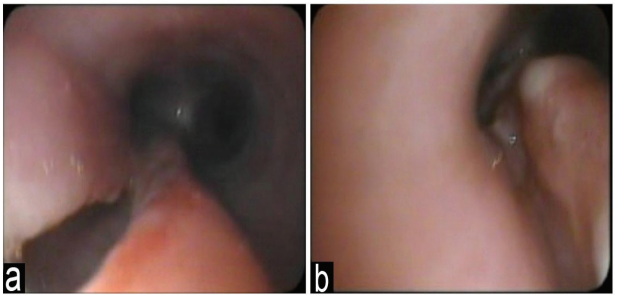

Fig. 1 – Bronchoscopic appearance of tracheoesophageal fistula (TEF) initially (a) and ten days after surgery (b).

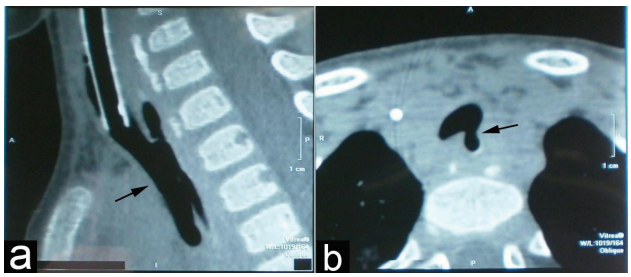

Fig. 2 – Computed tomography (CT) findings after pulling upwards endotracheal tube, sagittal (a) and axial plane (b) (TEF – black arrow).

We opted for two-stage surgery. First stage consisted of esophageal exclusion with forming of salivary fistula and gastrostomy (Figure 3).

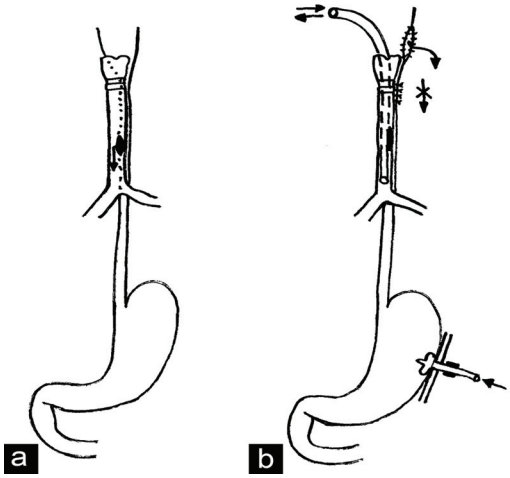

Fig. 3 – Schematic presentation of tracheoesophageal fistula (TEF) (a) and surgical procedure (b).

Prolonged tracheal intubation with tracheobronchial toilet was planned in the postoperative period, expecting spontaneous healing of the TEF (Figure 4). During the surgery through the left cervicotomy, the cervical esophagus firmly attached to the trachea was found, without possibility open circling it.

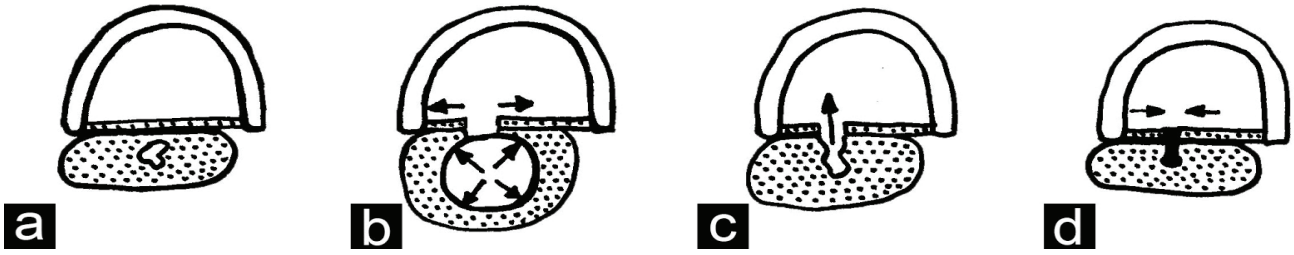

Fig. 4 – Schematic presentation of the mechanism of tracheoesophageal fistula (TEF) development caused by pneumatic dilatation (a, b, c) and spontaneous healing of TEF (d).

Lateral pharyngotomy was performed, stenotic cervical esophagus was completely obliterated by several individual stitches, and pharyngostomy was created (Figure 5).

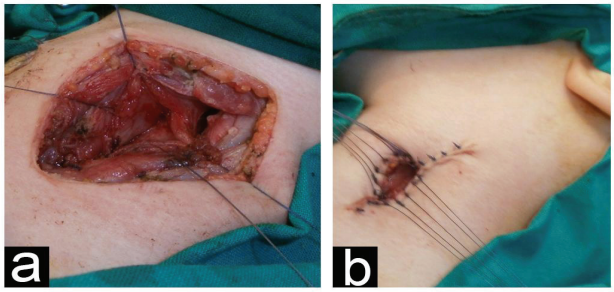

Fig. 5 – Exclusion of the stenotic cervical esophagus (a) and lateral pharyngostomy (b).

The pulmonary infection was gradually cured postoperatively. Tracheobronchial fiberoptic endoscopy was used to treat atelectasis of the left lung. On the 10th postoperative day, since the bronchoscopy showed healed laceration on membranous tracheal wall, the child was extubated (Figure 1b). The child was fed through gastrostomy, without signs of gastroesophageal reflux.

The second stage surgery, bypass retrosternal colon interposition with pharyngocolic anastomosis, was performed a year later. Before reconstruction, we confirmed normal vocal cord function, good pulmonary function, and normal nutritive status. We used long peristaltic colonic segment, vascularized by left colic vessels (Figure 6).

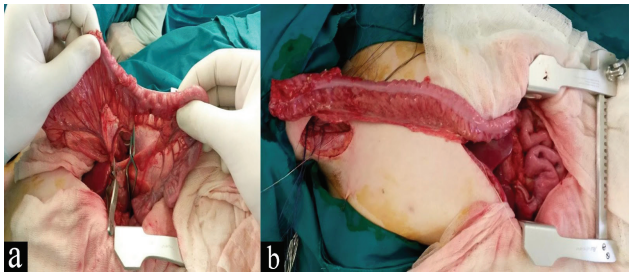

Fig. 6 – Temporary clamping of middle colic vessels and marginal vascular arcade (a) and isoperistaltic colonic conduit vascularized by left colic artery (b).

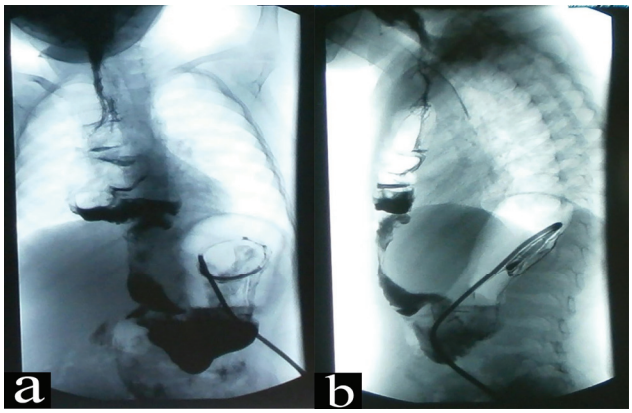

Fig. 7 – Plain (a) and lateral (b) contrast radiographies two weeks after pharyngoesophageal reconstruction with retrosternal colon interposition.

Over four-year follow-up, the girl was normally fed perorally, with normal development and without occurrence of pulmonary infections (Figure 7).

Discussion

Tracheoesophageal fistula occurring during pneumatic dilatation of esophageal corrosive stricture is a very rare complication and a great challenge for clinicians [1–4]. According to our best knowledge, only 10 similar cases were reported until now [1–3]. With no clearly defined treatment strategy, the approach should be individually tailored, taking into account all relevant clinical features. Anatomy of esophageal stenosis, localization and appearance of TEF, the presence of pulmonary, mediastinal and pleural infection and signs of sepsis, age, nutritional status and general condition of a patient, together with operative findings are essential for treatment strategy [3, 5, 6].

Symptoms of TEF may be different. The slightest suspicion of TEF requires timely and adequate diagnostic procedure. Esophagography and esophagoscopy can show anatomy of the TEF [7]. Bronchoscopy allows more accurate localization and assessment of TEF morphology [5]. CT scan gives information about inflammatory changes in the mediastinum, pleural cavity and lungs, but details of TEF anatomy can remain undetected in intubated patients [5, 8]. According to our experience in this case, we believe that CT examination may be more useful when endotracheal tube is slightly pulled upwards to the larynx to express TEF (Figure 2). CT may show localization and size of TEF, and allow precise surgical planning, too.

The violation of the membranous wall of the trachea during BD of corrosive esophageal stricture is the result of transmural inflammation. Adhesions between the wall of the esophagus and the membranous wall of the trachea essentially make the walls of these organs behave as a single rigid structure. Fibrosis involving both esophageal and membranous tracheal wall complicates the primary surgical reparation of TEF, but may help in its spontaneous healing (Figure 4). Lungs protection from aspirated saliva and refluxed gastric content contamination is crucial for treatment of TEF. Treatment with esophageal stenting as bridging procedure before definitive surgical treatment may be useful, but can be controversial since esophageal stent covers TEF, but separates the edges of TEF and does not support the healing [3, 6]. Surgical exclusion of the esophagus is another way to protect lungs. In both cases, delayed reconstruction of the esophagus is the second stage of the treatment [3, 5]. Various endoscopic options such as glue, laser and cauterization are in use for smaller TEF of some other etiology, while primary surgical TEF repair is adequate only when it is not too risky for airway safety [5].

The staged surgical treatment is an optimal solution for TEF caused by dilatation of a corrosive esophageal stenosis. Our patient had a similar treatment as a young adult with caustic ingestion reported by Crema et al [9]. In our case, the primary repair of TEF was risky. Effective protection of the lungs and the spontaneous healing of TEF was achieved by

exclusion of the esophagus, together with repeated tracheobronchial toilette through an endotracheal tube. Delayed retrosternal colonic interposition with pharyngocolic anastomosis was performed according to our previous experience [10]. Over four-year follow-up, excellent functional results were achieved.

Conclusion

Two-stage surgical treatment consisting of esophageal exclusion, esophagostomy or pharyngostomy and gastrostomy in the first stage, and pharyngoesophageal reconstruction with retrosternal colonic interposition in the second stage, is a useful therapeutic option in the treatment of tracheoesophageal fistula caused by pneumatic dilatation of corrosive esophageal stricture in children.

Acknowledgement

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CASE REPORT

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Adenocarcinoma of the prostate with small cell component and low levels of prostate specific antigen

Adenokarcinom prostate sa mikrocelularnom komponentom i niskom vrednosti prostata specifičnog antigena

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Abstract

Introduction. Prostate cancer is one of the most common malignancies in men. The most common type is acinar adenocarcinoma. Small cell prostate cancer (SCPC) usually occurs together with coexisting prostate adenocarcinoma. **Case report.** A 72-years-old patient with voiding simptoms is presented. Initial level of prostate specific antigen (PSA) was 2.87 ng/mL. Twelve prostate biopsies were taken and in six of them neoplastic tissue was detected. The viewed tissue was most convenient to "small cell carcinoma". Bone scintigraphy did not demonstrate dissemination of the cancer into the skeletal system. Multislice computed tomography (MSCT) of the pelvis did not reveal any special pathological changes. The patient underwent surgery – radical retropubical prostatectomy. Histopathological analysis revealed a poorly differentiated adenocarcinoma of the prostate with small cell carcinoma zones [Gleason score 5+5 (10), grade III, pT3bN1, stage IV]. **Conclusion.** Poorly differentiated adenocarcinoma of the prostate, especially in combination with SCPC, is an aggressive malignancy with most cases presenting with the extensive disease dissemination on diagnosis and poor prognosis. Small cell carcinomas of the prostate are extremely rare tumors of the neuroendocrine origin. Patients with mixed prostate cancer, compared to pure SCPC, have a better prognosis and greater survival rate. There is a lack of the evidence guiding treatment for SCPC.

Key words:
prostatic neoplasms; prostate-specific antigen; diagnostic techniques and procedures; prostatectomy; drug therapy; prognosis.

Apstrakt

Uvod. Karcinom prostate je jedan od najčešćih malignih oboljenja kod muškaraca. Najčešći tip je adenocarcinom prostate. Karcinom malih ćelija prostate (KMĆP) obično se javlja u kombinaciji sa adenokarcinom prostate. **Prikaz bolesnika.** Prikazan je 72-godišnji bolesnik sa simptomima otežanog pražnjenja mokraćne bešike. Inicijalni nivo prostata specifičnog antigena (PSA) bio je 2,87 ng/mL. Uzeto je dvanaest bioptata prostate i u šest je otkriveno maligno tkivo. Analizirano tkivo najviše je odgovaralo "karcinomu malih ćelija". Scintigrafija skeleta nije otkrila širenje karcinoma u skeletnom sistemu. Kompjuterizovana tomografija (KT) male karlice nije otkrila infiltraciju okolnog tkiva tumorom. Bolesnik je operisan – urađena je radikalna retropubična prostatektomija. Patohistološka analiza pokazala je slabo diferentovani adenokarcinom prostate sa zonama karcinoma malih ćelija [Gleason skor 5 + 5 (10), razred II, pT3bN1, stadijum IV]. **Zaključak**. Slabo diferentovan adenokarcinom prostate, posebno u kombinaciji sa karcinomom malih ćelija, jeste agresivan maligni tumor koji je u većini slučajeva povezan sa opsežnim širenjem bolesti u trenutku postavljanja dijagnoze i ima lošu prognozu. KMĆP izuzetno su retki tumori neuroendokrinog porekla. Bolesnici sa mešovitim karcinomom prostate imaju bolju prognozu i veću stopu preživljavanja. Trenutno ne postoje vodiči zasnovani na dokazima za lečenje ove vrste karcinoma prostate.

Ključne reči:
prostata, neoplazme; prostata, specifični antigen; dijagnostičke tehnike i procedure; prostatektomija; lečenje lekovima; prognoza.

Introduction

Prostate cancer is one of the most common malignancies in men. In the United States of America it is on the second place, immediately after lung cancer [1]. It is also the leading cause of mortality in males. The most common type is acinar adenocarcinoma [2–6]. Small cell prostate cancers (SCPC) usually occur together with coexisting prostate ade-

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nocarcinoma [3–6]. Carcinomas of the prostate can be divided into two groups: acinar and non-acinar ones. According to certain data, non-acinar prostate tumors can be found in 5–10% of patients with prostate malignancy [2–7]. Small cell prostate cancer is one of the rarest type of prostate cancers and makes 0.3–1% of all prostatic tumors [6]. Incidence of prostate adenocarcinoma with a small cell component in Serbia is not known as well as in Southeast Europe. In the available literature, reported cases of this type of prostate cancers in Southeast Europe are not found.

Case report

A 72-years-old patient was with voiding simptoms which started six months before his first visit to an urology specialist. The patient suffered from arterial hypertension, which was controlled by drugs. Diagnostics including digitorectal examination, the value of prostate specific antigen (PSA) in the blood and transrectal ultrasound of prostate was firstly conducted. An initial value of PSA was 2.87 ng/mL. Other laboratory findings (urinanalysis, white blood cells, erythocyte sedimentation rate) were unremarkable. A transrectal prostate biopsy with histopathological examination was indicated, because during digitorectal examination at the left lobe of the prostate one nodule of stiffer consistency was found. Twelve prostate biopsies were taken and in six of them neoplastic tissue was detected. The tumor tissue was built of round atypical cells with hyperchromatic vesicular nuclei, focally visible nucleus and sparing cytoplasm. Tumor cells were of lesser extent, with short sequences and less solid beach, and a large part in the non cohesive schedule. Viewed tissue was most convenient to "small cell carcinoma". An immunohistochemistry analysis of prostate samples revealed following immunofenotypes: TTF-1+, Ckae 1/ae3+, Ki-67~65%, CD117+, CD56-, Chromogranin A-, Synaptophysin -, NSE-, CK7-, CK20-, BCL2-, LCA-, CD99. These findings were substantially compatible with SPCP. Bone scintigraphy with technetium-99m (^{99m}Tc) and 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) did not reveal dissemination of the cancer into the skeletal system. Multislice computed tomography (MSCT) of the pelvis did not demonstrate any special pathological changes in the prostate anatomy – the prostate dimension was 40 × 54 mm, there were parenchymal calcifications and capsule of the prostate was clearly limited. Also, there was not present any sign of the retroperitoneal lymphadenomegaly. The patient underwent surgery – radical retropubical prostatectomy. Histopathological analysis of surgically removed tissues and organs revealed that it was a poorly differentiated adenocarcinoma of the prostate with small cell carcinoma zones in poorly differentiated areas. The tumor invaded both lobes of the prostate and prostatic capsule penetrating to both seminal vesicles. Twenty-two lymph nodes were surgically removed and in two of them metastases were present. There was also present prostatic intraepithelial neoplasia (PIN) of low and high grade. Gleason score was 5 + 5 (10), grade III, pT3bN1, stage IV. The prostate size was 5 × 4.5 × 3.5 cm. (Figure 1).

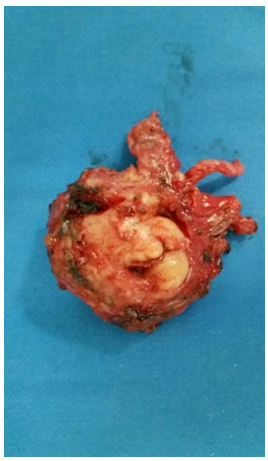

Fig. 1 – Surgically removed prostate gland with macroscopically visible tumor.

By an oncologist indication, the patient postoperatively received four cycles of chemotherapy, according to the protocol including etoposide and cisplatin, at the Oncology Institute of Vojvodina. The patient also started therapy with luteinizing hormone-releasing hormone (LHRH) antagonists. The PSA value during hormone therapy was 0.7 ng/mL.

The patient's general condition was gradually worsening one year after the chemotherapy performed. A complete body skeleton scintigraphy in the anteroposterior (AP) and posteroanterior (PA) with ^{99m}Tc-DPD was performed showing a diffusely pronounced pathological hyperfixation of the radiolabel in the axial and apendicular part of the skeleton. This finding suggested the diffusion of the basic pathological process into the bone and joint system (Figure 2).

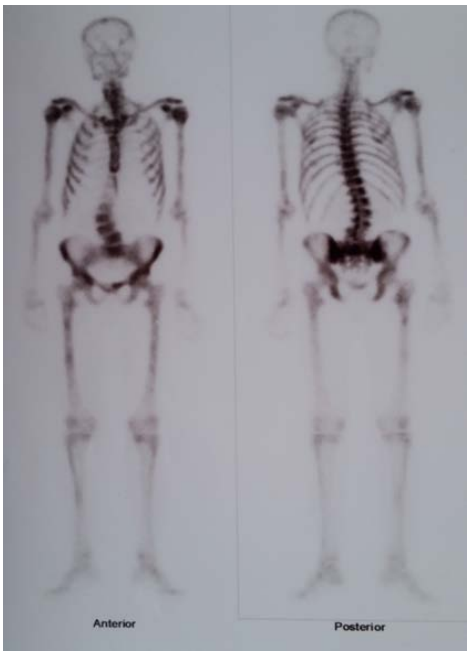

Fig. 2 – A complete body skeleton scintigraphy in the anteroposterior (AP) and posteroanterior (PA) projections with a technetium-99m (^{99m}Tc) and 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), one year after the ending of chemotherapy.

Discussion

The most frequent presentation of neuroendocrine tumors in humans are in the prostate, lungs, and pancreas [8]. One third of patients with SCPC already suffered from prostate adenocarcinoma. The average age of these patients is between fifty and seventy years [9]. Metastasis appears in 60% of cases with rate between five to eighteen months. All patients observed with mixed prostate cancer have better survival rate [9]. Clinical presentation of SCPC and adenocarcinoma is quite different; obstructive uropathy as well as dissemination of the disease dominate in patients suffering from SCPC [10]. Aggressive clinical course takes place in most of the cases with small cell adenocarcinoma [11]. Most of the patients, when diagnosed, already had advanced stage of the disease [12]. The lungs are the most frequently included. The bladder, liver, and bones were also targeted. Almost all of the patients have symptoms typically related to enlarged prostate gland. A low grade fever also appears in some of the patients who are attributed to the underlying malignancy. In patients with SCPC, PSA level may not be elevated, or PSA level is not in proportion with the tumor size. Neuroendocrine markers, including chromogranin A, CD 56, synaptophysin, and neuron specific enolase are usually positive when SCPC is diagnosed [13, 14]. In at least 90% of the cases with SCPC, these markers are positive. It is well known that serum PSA level never correlates with burden of the disease, although prostatic adenocarcinoma and SCPC can occur concomitantly [14]. PSA levels can be elevated in patients with mixed prostatic adenocarcinoma and SCPC. Since that condition occurs very seldom, there is a lack of evidence guiding treatment for SCPC [11–14]. There are few possibilities of treatment such as surgery, chemotherapy, and radiotherapy. The course of therapy is manly defined depending on the disease stage [14]. Prospective randomized trials are precluded due to rarity of the disease. The therapy is mainly modeled after those in small cell carcinoma of the lungs. Chemotherapy is usually used (cisplatin and etoposide) as the main treatment, but causes the aggressiveness of the disease. Response to the treatment is the most important thing when we estimate patients' survival. An increased survival rate is noticed in patients who underwent radical surgical resection in combination with other treatment modalities. Metastatic symptoms as well as the local disease status can be treated with radiotherapy [15]. Hormonal therapy is not recommended in a pure SCPC, and is still controversial in a mixed histology. The neuroendocrine differentiation development could be associated with it in other forms of prostate cancer. Poor prognosis is observed in patients having Gleasone score 8 or greater after radical prostatectomy, especially if nodal metastases are present as the most important prognostic factor [12–15].

Conclusion

Poorly differentiated prostate adenocarcinoma, especially in combination with SCPC, is an aggressive malignancy, in most cases presenting with the extensive disease dissemination and has poor prognosis. Early detection is of the key importance for improving prognosis. There is a lack of the evidence guiding treatment for SCPC, and due to this, further research is required to establish the standard treatment protocol, in order to reduce mortality rate and extend patients survival.

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CASE REPORT

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Accidental colchicine poisoning with fatal outcome after ingestion of meadow saffron (*Colchicum autumnale* L.)

Zadesno trovanje kolhicinom sa smrtnim ishodom nakon ingestije biljke mrazovac (*Colchicum autumnale* L.)

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Abstract

Introduction. Meadow saffron (*Colchicum autumnale* L.) is a perennial herbaceous plant belonging to the Lily family (*Liliacea*). It is similar to the edible wild garlic (*Allium ursinum* L.). Toxic substance in meadow saffron is alkaloid colchicine. Colchicine poisoning is a very dangerous condition which can lead to a fatal outcome. **Case report.** A 50-years-old male was addmited to the hospital complaining of weakness, abdominal pain, nausea, vomiting and diarrhea without blood. The day before, the patient ate two plants thinking they were wild garlic and seven hours after ingestion he felt first symptoms. During the course of the hospital stay, he had gastroenterocolitis, acute renal faliure, hepatic lesions and cardiorespiratory insufficiency with a fatal outcome. Post-mortem examination revealed: brain oedema, lung oedema and congestion, heart weighing 700 g with ventricular hypertrophy, myocardial fibrosis, liver congestion and steatosis, spleen congestion, pancreatic fibrosis. Organs sections were taken for histopathological analysis. Body fluids and parts of organs were toxicologically analyzed. Histopathological findings were: brain oedema, diffuse perivascular and interstitial myocardial fibrosis, myocardial haemorrhage, lungs congestion and oedema, microvesicular and macrovesicular liver steatosis, centrilobular liver necrosis, lymphocytic inflammatory infiltrate in liver portions, red pulp congestion of the spleen, kidney congestion and interstitial bleeding, coagulation necrosis of the proximal tubules of the kidney. Toxicological analysis showed colchicine in the blood – 0.011 mg/L, urine – 0.051 mg/L, liver with gallbladder – 0.007 mg/kg, kidney – 0.008 mg/kg. **Conclusion.** Ingestion of meadow saffron can lead to poisoning with a fatal outcome due to the presence of the alkaloid colchicine. Colchicine intoxication should be suspected in patients with gastrointestinal symptoms after consuming wild plants.

Key words:
poisoning; colchicine; plants, toxic; multiple organ failure; death.

Apstrakt

Uvod. Mrazovac (*Colchicum autumnale* L.) je višegodišnja zeljasta biljka iz familije ljiljana (*Liliacea*), sličan jestivoj biljci sremuš (*Allium ursinum* L.). Toksična supstanca u mrazovcu je alkaloid kolhicin. Trovanje kolhicinom je veoma opasno stanje, koje se može završiti smrtnim ishodom. **Prikaz bolesnika.** Muškarac starosti 50 godina primljen je u bolnicu zbog sumnje na zadesno trovanje biljkom mrazovac. Bolesnik je dan pre pojeo dve biljke misleći da su sremuš. Sedam sati nakon ingestije počele su tegobe: malaksalost, bolovi u abdomenu, mučnina, povraćanje i prolivaste stolice bez krvi sa kliničkom slikom gastroenterokolitisa, akutnom bubrežnom insuficijencijom, lezijom jetre, kardiorespiratornom insuficijencijom i smrtnim ishodom. Obdukcijom je makroskopski ustanovljeno: edem mozga, edem i kongestija pluća, srce mase 700 g sa hipertrofijom leve i desne komore, fibroza miokarda, kongestija i steatoza jetre, kongestija slezine, fibroza pankreasa. Uzeti su isečci organa za patohistološku analizu i telesne tečnosti i delovi organa za toksikološko-hemijsku analizu. Patohistološki nalaz je bio: edem mozga, difuzna perivaskularna i intersticijalna fibroza miokarda, intersticijalno krvarenje u miokardu, kongestija i edem pluća, mikrovezikularna i makrovezikularna steatoza jetre i centrilobularna nekroza jetre, limfocitni zapaljenski infiltrat u portnim prostorima jetre, kongestija crvene pulpe slezine, kongestija i intersticijalno krvarenje u bubregu, koagulaciona nekroza proksimalnih tubula bubrega. Toksikološko-hemijskom analizom potvrđeno je prisustvo kolhicina u:

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krvi – 0,011 mg/L, urinu – 0,051 mg/L, jetri sa žučnom kesom – 0,007 mg/kg, bubregu – 0,008 mg/kg. Zaključeno je da je bolesnik zadesno otrovan alkaloidom kolhicinom sa smrtnim ishodom. **Zaključak.** Ingestija biljke mrazovac, zbog prisustva toksičnog alkaloida kolhicina, može dovesti do trovanja sa smrtnim ishodom. Na trovanje kolhicinom treba posumnjati kod bolesnika sa gastrointestinalnim simptomima posle konzumiranja divljih biljaka.

Ključne reči:
trovanje; kolhicin; biljke, otrovne; insuficijencija više organa; smrt.

Introduction

Meadow saffron (*Colchicum autumnale L.*), (Figure 1) is a perennial herbaceous plant from the Lily family (*Liliacea*). It is commonly known as autumn crocus, wild saffron, naked lady, son before the father. Its flower is very similar to the flower of saffron (*Crocus sativus L.*), it has no special smell and is spread over mountain meadows and pastures. Meadow saffron seems like the edible wild garlic (*Allium ursinum L.*) (Figure 2), commonly called "sremush" in Serbian, which has specific garlic like smell [1]. The toxic substance found in meadow saffron is alkaloid colchicine. Colchicine poisoning is a very dangerous condition and a fatal outcome is one of possible consequences. An antidote for this substance does not exist yet, but there are possibility of colchicine specific monoclonal antibodies to be used in future [2]. All parts of the plant are poisonous and contain colchicine, but the highest concentration of this alkaloid is in seeds (0.2–0.8 %) and bulbs (0.4–0.6 %), while it is low in leaves. There are some other toxins present in meadow saffron, but they are less dangerous to humans [1]. Colchicine is also used in medicine for the treatment of gout [3], Mediterranean fever [4], sarcoidosis [5], scleroderma [6], amyloidosis [7], Behcet's disease [8], Paget's disease [9], psoriasis [10], cutaneous vasculitis [10], alcoholic cirrhosis of the liver [11] and primary biliary cirrhosis [12].

Fig. 1 – Meadow saffron (*Colchicum autumnale* L.).

Fig. 2 – Wild garlic (*Allium ursinum* L.).

Case report

A 50-year-old man was admitted to the General Hospital in Loznica because of a suspicion of herbal poisoning. One day before the admission to the hospital at 03:00 p.m., the patient had eaten two whole herbs regarded as wild garlic. Seven hours after consumption the first symptoms appeared. He had diffuse abdominal pain, nausea, vomiting, and diarrhea without blood (at least ten watery stools). The patient had fifteen years history of arterial hypertension and diabetes mellitus type 2, treated with insulin. On examination he was conscious, well oriented to the place, time and person with slowed communication and euphoric. Auscultation of the heart and lungs showed no abnormalities. Patient had an oxygen saturation level of 96%, a blood pressure of 140/90 mmHg and a heart rate of 103 beats/min. The electrocardiogram (ECG) showed sinus rhythm without change in ST and T segments. Abdomen was soft in the chest level, diffusely painfully sensitive to deep palpation, with no muscular defense. The extremities were with normal colour and without oedema. With the worsening health condition, the patient was shifted to a referral tertiary health care institution (Clinic for Emergency and Clinical Toxicology, National Poison Control Center, Military Medical Academy in Belgrade) on the same day at 03:45 p.m. On the arrival, the patient was in the same condition as in the previous health care institution. ECG revealed sinus rhythm with occasional extrasystoles and no significant conduction and repolarization defects. The chest X-ray was normal. Laboratory data out of reference range are shown in Table 1. Coagulation factors (F) were: FII 0.33; FV 0.1; FVII 0.13; FIX 0.61; activated partial thromboplastin time (APTT) 117 sec; international normalized ratio (INR) 5.21; arterial blood gases were: pH 7.302; pCO2 28.3 mmHg; pO2 68.9 mmHg; base excess (BE) 11.6 mmol/L, bicarbonates 15.9 mmol/L, lactates 5.4 mmol/L. Oxygen saturation level was 90%. Colchicine was proven in

the urine by toxicological analysis. The patient developed gastroenterocolitis, acute renal faliure, hepatic lesions and cardiorespiratory insufficiency, and was treated with rehydration and supportive therapy (oxygen, activated carbon, proton-pump inhibitors and diuretics). The next day, the patient went into cardiac arrest and cardiopulmonary resuscitation was unsuccessful. Death was pronounced at 10:55 a.m. Medicolegal autopsy was conducted at the Institute for Pathology and Forensic Medicine, Military Medical Academy in Belgrade.

Table 1
Laboratory analyses of blood in the patient, one day after ingestion of meadow saffron

Test parameters	Results	Reference range
WBC (× 10^9/L)	19.76	4.00–11.00
RDW (%)	14.80	11.5–14.50
Neutrophils (× 10^9/L)	13.3	1.9–1.8
Monocytes (× 10^9/L)	2.3	0.16–1.20
Eosinophils (× 10^9/L)	1.8	0.00–0.80
Basophils (× 10^9/L)	2.80	0.00–0.40
Glucose (mmol/L)	8.0	4.1–5.9
Urea (mmol/L)	12.8	2.5–7.5
Creatinine (μmol/L)	124	62–115
AST (U/L)	280	0–37
ALT (U/L)	84	7–49
CK (U/L)	969	32–300

WBC – white blood cells; RDW – red cell distribution width; AST – aspartate aminotransferase; ALT – alanine aminotransferase; CK – creatine kinase.

The external examination of the body was unremarkable, but medicolegal autopsy revealed oedematous brain weighing 1,500 g, congested and oedematous lungs with left and right lung weighing 650 g and 800 g, respectively, the heart weighing 700 g with left and right ventricular hypertrophy (19 mm and 7 mm, respectively), cardiac muscle fibrosis, coronary arteries atherosclerosis, aortic atherosclerosis, liver congestion and steatosis, spleen congestion, and pancreatic fibrosis. During the autopsy, samples of organs were taken for histopathological analysis and body fluids (blood, urine), gastric contents and parts of organs (liver with gallbladder, kidney, brain) for toxicological analyses.

Histopathological examination of formalin-fixed, paraffin-embedded and hematoxylin-eosin (H&E) stained slides was done using light microscope (Olympus BX 43, Germany) with a digital camera connected to CellSense computer software. Histopathological findings revealed brain oedema, diffuse perivascular and interstitial myocardial fibrosis, interstitial myocardial hemorrhage, congestion and oedema of lungs, microvesicular and macrovesicular liver steatosis, centrilobular liver necrosis (Figure 3), scant, mononuclear, mostly lymphocytic inflammatory infiltrate in portal spaces, spleen congestion, renal congestion, acute coagulative tubular necrosis (Figure 4) and interstitial renal hemorrhage.

Toxicological analyses were done at the Institute of Toxicology and Pharmacology, the Department of Toxicological Chemistry (National Poison Control Center, Military Medical Academy in Belgrade). The presence of alkaloid colchicine was analyzed using method of liquid chromatography-mass spectrometry (LC-MS), comparing the mass spectra of examined samples (blood, urine, liver with gallbladder, kidney and brain) with the spectra of standards (Table 2). The presence of ethanol was established using method of gas chromatography (GC) with flame ionization detector (FID) – "head space" technique in the blood – 0.23 ‰ (4.9924 mmol/L), urine – 0.58 ‰ (12.5895 mmol/L) and gastric contents – 0.37 ‰ (8.0313 mmol/L).

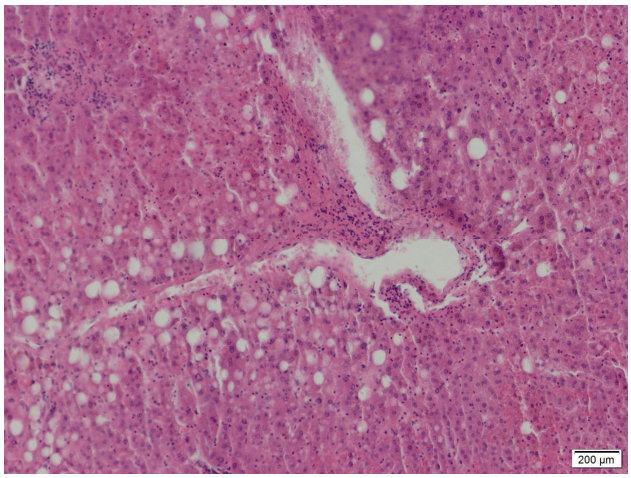

Fig. 3 – Macrovesicular and microvesicular steatosis and centrilobular liver necrosis [hematoxylin-eosin (H&E) staining, ×40 magnification].

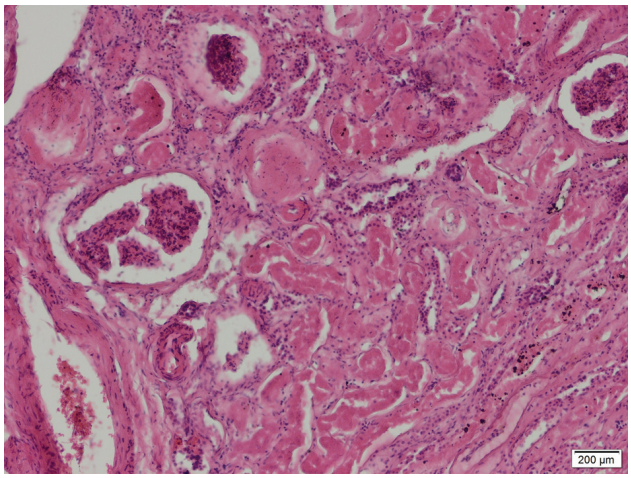

Fig. 4 – Acute tubular coagulative necrosis of the kidney [hematoxylin-eosin (H&E) staining, ×40 magnification].

Table 2
Colchicine concentrations in body fluids and organs

Sample	Colchicine
Blood	0.011 mg/L
Urine	0.051 mg/L
Gastric content	not found
Brain	not found
Kidney	0.008 mg/kg
Liver with gallbladder	0.007 mg/kg

Discussion

The case of the middle-aged male accidental fatal poisoning caused by colchicine from meadow saffron was shown. One of the most common causes of meadow saffron ingestion and colchicine poisoning is its great similarity to wild garlic, which is an edible plant and has a distinctive garlic odor. In addition to an accidental meadow saffron poisoning, suicide cases by meadow saffron ingestion were also reported [13]. Due to the extreme health risk, it is important to identify symptoms and signs of colchicine poisoning and obtain anamnestic data in order to provide adequate immediate medical care, even though final confirmation of the poisoning is provided by toxicological analysis. Symptoms and signs of colchicine poisoning are most often manifested by gastrointestinal disorders such as nausea, vomiting, abdominal pain and diarrhea [14, 15] as it was at the onset of symptoms in our case. Clinical manifestations of poisoning, according to Stapczynski et al. [16] develop through three phases. The first phase of poisoning begins 4 to 12 hours after ingestion and is characterized by vomiting and diarrhea. The second phase usually begins on the second day after ingestion and is followed by life-threatening conditions, such as arrhythmia, heart failure, kidney and liver failure, bone marrow damage and coagulopathy. The third phase begins 5 to 7 days after ingestion, and it leads to leukocytosis and then very rapidly to pancytopenia (leukocytopenia and thrombocytopenia) which indicates bone marrow aplasia [17–19]. In the third phase, poisoning may result in alopecia. This alopecia is transient, although cases in which there was no recurrence of hair have also been reported [20–23]. If patient survives poisoning, peripheral neuropathy may appear as long-term consequence [24].

Colchicine is a cytostatic agent that interrupts cell mitosis by interfering with the formation of microtubules and the mitosis spindle. It reversibly binds to tubulin and prevents its polymerization [25]. The half-life of this linked complex is 36 hours [1]. Colchicine primarily blocks cell mitosis in organs and tissues with high intensity of cell division. This is particularly expressed in the gastrointestinal system and bone marrow, which is manifested by a characteristic clinical presentation that was also seen in this case [26]. Colchicine shows its toxicity to other organs, and in a number of cases, arrhythmias, pancreatitis, acute liver failure occur [27–29]. Colchicine is accumulated in leukocytes and exhibits an inhibitory effect on leukocyte adhesion, mobility, mobilization, phagocytosis, degranulation of lysosomes and chemotaxis. Applied in therapeutic doses, it blocks the release of chemotaxis factors by neutrophils and synoviocytes and thus reduces inflammation.[1] Colchicin is predominantly absorbed in the small intestine. Due to its liposolubility it is largely bound to plasma proteins, primarily albumin. Colhicin is mostly metabolised in the liver, while one fifth remains unchanged and is eliminated by kidneys [30]. Colchicine is subjected to enterohepatic circulation. It is reported in the literature that when colchicine is administered in doses less than 0.5 mg/kg body weight, all poisoned persons survive, while when administered in amount higher than 0.8 mg/kg, all poisoned ones die. Toxic effects are rarely reported if its plasma concentration is less than 3 ng/mL. Consumption of 5 g of meadow saffron leads to fatal outcome [1, 31, 32]. Lethal concentrations of colchicine in the blood are 0.009–0.024 mg/L [33]. It is found in higher concentration in the kidney, liver and spleen than in the heart, skeletal muscles and brain. Hepatic and biliary excretion are possible reasons for the onset of gastrointestinal symptoms [34]. Due to liver and kidney failure, values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) plasma levels, as well as levels of bilirubin and blood urea nitrogen are elevated [17]. Extreme damage of central lobular regions of the liver is typical and can be explained by characteristic liver perfusion and by increasing concentrations of the poison in the liver and bile as a result of conspicuous enterohepatic circulation [35]. Myoglobinuria, due to effects of colchicine such as rhabdomyolysis and hypoxia, may impair renal function and lead to renal failure [36]. Elevated blood creatine kinase (CK) and lactate dehydrogenase (LDH) levels are noted in colchicine poisoning. Increased blood CK concentration is associated with hypoxic damage of the brain and heart. The brain damage (pericellular and perivascular edema) is probably due to multiple system organs failure.

Autopsy findings in colchicine poisoning may include inflammation of gastrointestinal mucosa, lung and brain oedema, centrilobular necrosis of the liver, proximal tubule necrosis in the kidney, petechial haemorrhage in the fatty tissue [37, 38]. Disseminated petechial bleeding in the fatty tissue, described by some authors, are caused by thrombocytopenia and liver failure [17]. There is still no an antidote for colchicine poisoning, therefore treatment is symptomatic. In the future, monoclonal antibodies to colchicine will improve chance for survival [2].

Although body fluids (blood, urine) and parts of organs can be taken for toxicological analysis in fatal colchicine poisoning, the liver with gallbladder is the best sample for analysis, because concentration of the substance is the highest in these organs [39]. In our case, samples of body fluids (blood and urine), gastric content, liver with gallbladder, kidney and brain were taken for toxicological analyses during the autopsy. Presence of colchicine was proven in the blood, urine, liver with gallbladder and kidney. Blood concentration of colchicin was 0.011 mg/L, which is the lethal one [33].

Conclusion

Ingestion of meadow saffron is rare, but it can cause life threatening condition, due to the presence of highly toxic alkaloid colchicine. Colchicine concentration determination in body fluids and organs is of particular importance in clinical toxicology due to more effective treatment of patients, as well as in forensic medicine in cases of accidental or rarely suicidal fatal poisoning. At the moment, colchicine poisoning is recommended to be treated supportively and symptomatically as soon as possible after ingestion of meadow saffron.

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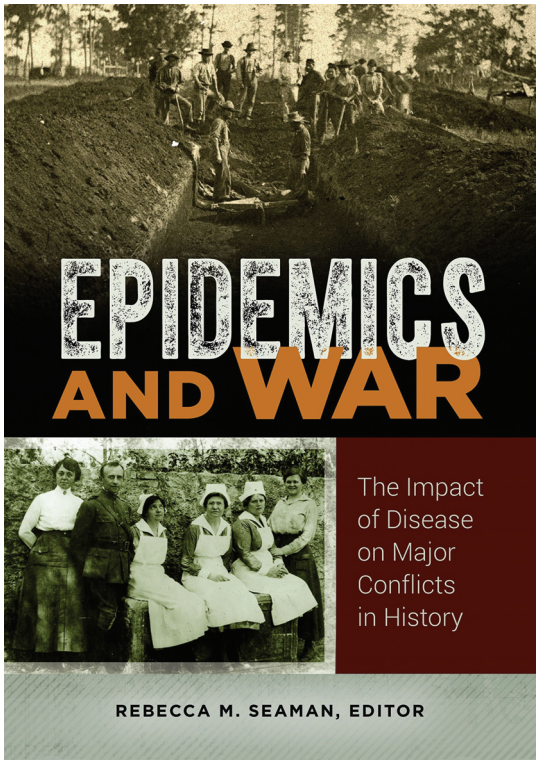
BOOK REVIEW

Epidemics and War: The Impact of Disease on Major Conflicts in History



Title: Epidemics and War: The Impact of Disease on Major Conflicts in History
Editor: Rebecca M. Seaman
Publisher: ABC-CLIO, Santa Barbara, California, USA
Published: 2018
ISBN: 978-1-4408-5224-4 (print); 978-1-4408-5225-1 (eBook)

The book "Epidemics and War: The Impact of Disease on Major Conflicts in History" was published in April 2018 by a renowned American publisher of academic and scientific literature ABC-CLIO. The editor of the book is Rebecca M. Seaman, director of the Social Sciences and Humanities Division at the Olympic College in Bremerton, USA, while the authors of chapters in the book are experts in the fields of medical, historical and military sciences. A total of 340 pages, written in a clear and understandable style, show the effects of infectious diseases on military conflicts and battles throughout history, with special emphasis on sources of infection, modes of transmission, consequences for the warring parties, but also the consequences of the epidemic after the conflict. Each chapter in this book deals with one specific disease and focuses on a specific war conflict.

The book is divided into four parts. The first part covers three infectious diseases that are still a great unknown. The Athenian plague is the name for the epidemic of an infectious disease that appeared in Athens in 430 BC during the Peloponnesian War. The second epidemic of an infectious disease of unknown origin broke out in the Roman Empire in 165 AD during the war with the Parthians and lasted intermittently for the next 15 years. In the history of medicine, it has been recorded as Antonine Plague. The third mysterious contagious disease presented in this part of the book influenced the outcome of the civil wars in medieval England, known as the Wars of the Roses, where during the decisive battle of Bosworth in 1485, soldiers from both warring sides, the Lancaster and York dynasties, suddenly fell ill. This disease, which has appeared on several occasions since then, is called the English sweating sickness.

The second part of the book deals with epidemics of infectious diseases caused by bacteria and their impact on war conflicts. This section presents consequences of the Black

Death on the wars fought in the 14th century, the typhus epidemic during Napoleon's invasion of Russia in 1812, and the cholera epidemic during the Crimean War (1854–1855), the epidemic of typhoid fever during the Spanish-American War in1898, concluding with the epidemic of diphtheria during the Tajik War in the first half of the 1990s.

Virus epidemics are the subject of the third part of the book. The smallpox epidemic decimated armies of the Spanish colonies in America during 16th and 17th centuries, but also the conflicting parties during the American War of Independence in the period 1775–1783. The epidemic of yellow fever literally destroyed the French army sent to quell the slave revolt in the Caribbean colony, which led to the success of the Haitian revolution in 1803. The First World War was marked by epidemics of chickenpox, but also by one of the deadliest pandemics in the history of mankind, known as the Spanish flu. War conflicts on the African continent over the past century have affected the spread of the human immunodeficiency virus (HIV), while in Bosnia, 15 years after the end of the conflict, a mumps epidemic broke out as a direct result of the lack of vaccination due to war.

The fourth part of the book discusses epidemics of infectious diseases of mixed origin with an emphasis on epidemics of dysentery and pneumonia during the American Civil War, as well as malaria in the Vietnam War.

At the end, biographies of the authors of the chapters, bibliography, as well as an index of terms are given.

In the midst of the COVID-19 coronavirus disease pandemic and the continuing armed conflict in the Middle East, the book "Epidemics and War: The Impact of Disease on Major Conflicts in History" is a significant work that introduces readers to the consequences of infectious disease epidemics on the battlefield.

The book is intended for epidemiologists, infectologists, medical historians, military historians, medical service officers, as well as all other readers interested in this field.

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C O R R I G E N D U M

https://doi.org/10.2298/VSP2010111E

In the original article by **Aleksandra Fejsa Levakov, Jelena Amidžić, Jelena Ilić Sabo, Tanja Lakić, Saša Vojinov, Dragan Grbić. Unusual site for metastatic renal cell carcinoma – A case report** [Neuobičajena lokalizacija metastaza karcinoma renalnih ćelija]. **Vojnosanit Pregl 2020; 77(2): 233–236. (https://doi.org/10.2298/VSP180126057F),**

there should be cited new information on the corresponding author, Aleksandra Fejsa Levakov, instead of the existing ones. The new address and e-mail are as follows:

Correspondence to: Aleksandra Fejsa Levakov, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia; E-mail: aleksandra.fejsa-levakov@mf.uns.ac.rs.

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Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

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Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

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a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ||, ¶, **, ††, ...** .

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The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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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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Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

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a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

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Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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