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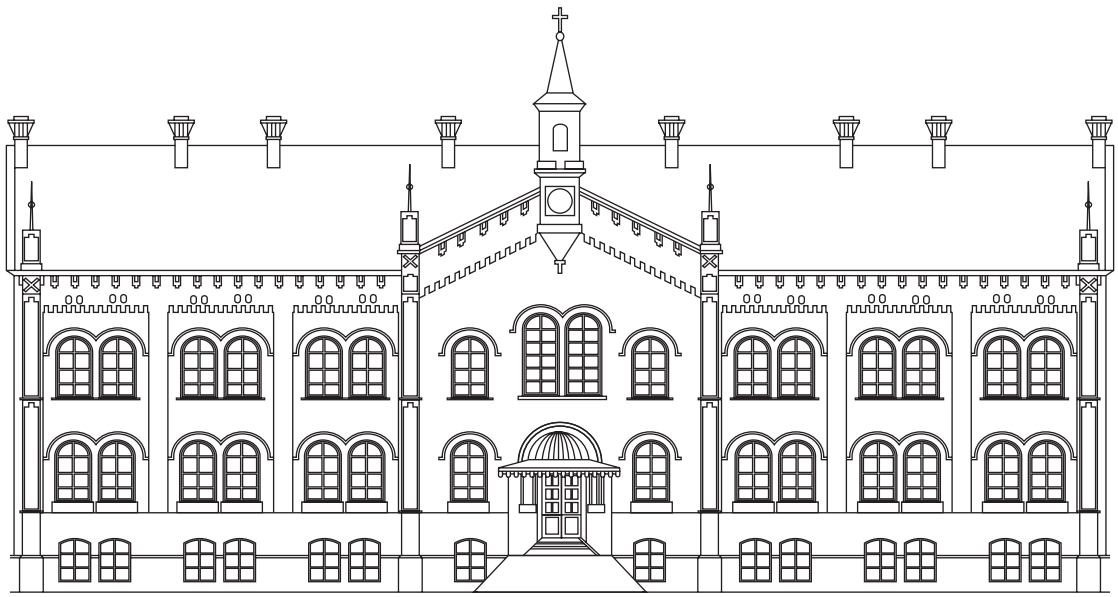

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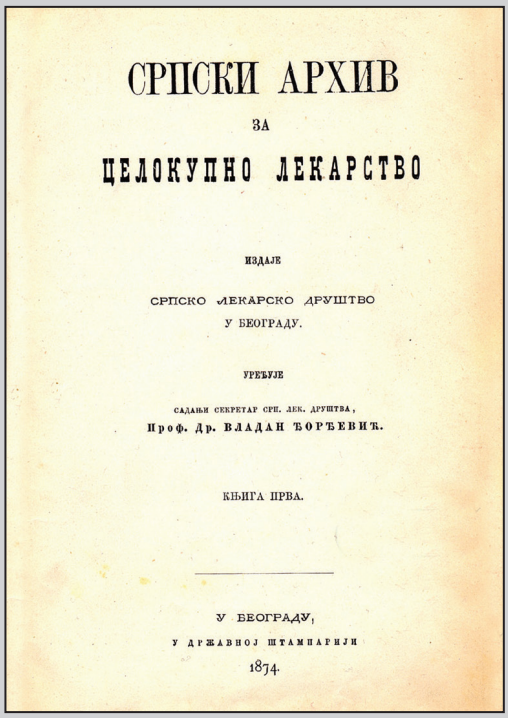

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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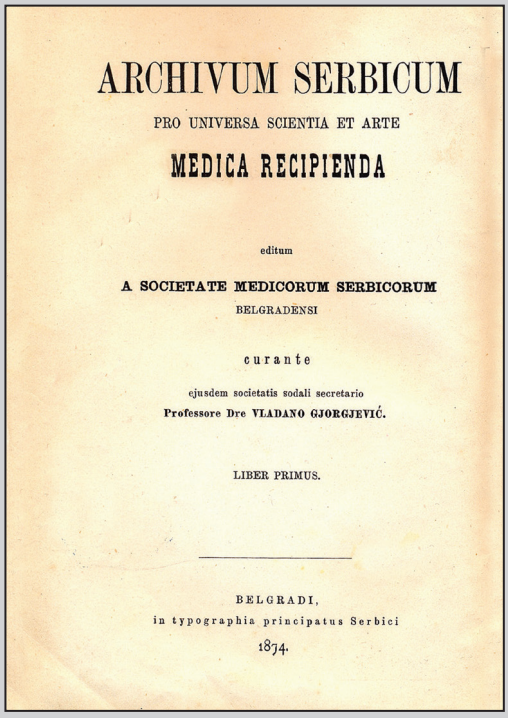
КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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BELGRADI,

in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основаног 1872. године, први пут штампан 1874. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Објављују се: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике и регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози.

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Possible association between COVID-19-caused stress and periodontal health – a pilot study

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SUMMARY

Introduction/Objective Stress is proposed as one of the risk factors linked to periodontal disease. The COVID-19 pandemic has a significant negative impact in population on mental and somatic health. This study aimed to examine the possible association between COVID-19 resultant stress and periodontal health.

Methods An observational pilot study was conducted from March 2020 to October 2021 and included 202 participants. Participants graded their stress level using the Perceived Stress Scale (PSS). Periodontal Disease Index and Clinical Attachment Level were determined. Participants were categorized into following groups: Ia (low stress), IIa (moderate stress), IIIa (high stress) and Ib (healthy parodontium), IIb (mild periodontal disease), IIIb (severe periodontal disease). The cause/effect relationship between stress and health was measured.

Results The results indicated a statistically significant difference between the groups classified according to the stress level concerning values of all the measured parameters. The Poisson regression analysis showed that in both models, crude and adjusted, periodontal health-related covariables were higher in subjects perceiving greater stress (Periodontal Disease Index – Pradjusted = 1.042, 95% CI [1.030–1.055] and Clinical Attachment Level – PRadjusted = 1.108, 95% CI [1.094–1.122]).

Conslusion During COVID-19 pandemic increased stress has a negative impact on mental health and may result in the deterioration of the entire oral cavity's health, including the periodontium.

Keywords: COVID-19; pandemic; stress; periodontal disease

INTRODUCTION

The COVID-19 pandemic is a global health emergency that so far affected more than 290 million people worldwide, including the 5.4 million death toll [1]. Rapid transmission has called for compulsory measures such as quarantine and community containment, which led to psychological disorders like stress, anxiety, and depression [2]. Findings of the study conducted in China showed that almost one-half of the participants deemed the impact of COVID-19 on mental health as moderate or severe, with a third of them experiencing anxiety symptoms [3]. Some studies suggest that oral conditions such as periodontal disease could be a risk factor for serious form of COVID-19, considering its mutual inflammatory pathways [4].

Periodontal disease is a multifactorial disease of the supporting tissues of the teeth [5]. It is characterized by progressive destruction of epithelial attachment and resorption of alveolar bone, resulting in luxation, migration, and, eventually, tooth loss [6]. The main etiological factor is dental plaque [7]. Clinical manifestations of the disease are determined by the nature of the immune response to microorganisms clustered in biofilm [8]. In addition to oral plaque, the onset and progression of the disease are influenced by other local and systemic factors, such as tobacco consumption, viral infections, and diabetes mellitus [9]. However, more attention has been paid to the role of psychological determinants in this disease's pathogenesis [10].

In the course of the current pandemic, people worldwide are put under severe psychological stress whose extent to the mental and oral health is yet to be determined [2]. During stressful events, significant changes occur on biological, physiological, and behavioral levels [11]. It is hypothesized that chronic stress can alter the host's immune response, increasing the patient's susceptibility to disease and causing severe periodontal destruction [12]. Also, anxiety and fear of the unknown, extensively present during this pandemic, often lead to adopting detrimental behavioral changes that affect oral and general health [13]. Studies have shown that people under stress tend to neglect their oral health, which manifests in visiting their dentist less often, having a comfort diet, and brushing their teeth less frequently [14].

This study aims to establish whether a significant clinical correlation exists between stress levels and the severity of the manifestations of

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Table 1. Participants' characteristics according to the stress level

Covariables	1) Low-stress level (n = 55)	2) Moderate stress level (n = 87)	3) High-stress level (n = 60)	p
	n (%)			
Gender				
male female	19 (22.4) 36 (30.8)	36 (42.4) 51 (43.6)	30 (35.3) 30 (25.6)	[a]0.094
Age (in years)				
X ± SD; med (min–max)	28.1 ± 6.8; 26.0 (22–54)	43.3 ± 18.6; 41.0 (19–82)	56.9 ± 12.2; 60.0 (25–72)	[b]0.000* [c](1 *vs.* 2) 0.000* [c](1 *vs.* 3) 0.000* [c](2 *vs.* 3) 0.000*
Marital status				
single in a relationship married divorced widowed	14 (30.4) 21 (38.2) 14 (23.7) 5 (17.9) 1 (7.1)	18 (39.1) 21 (38.2) 30 (50) 13 (46.4) 5 (35.7)	14 (30.4) 13 (23.6) 15 (25.4) 10 (35.7) 8 (57.1)	[a]0.137
Household status				
living alone living with up to five housemates living with more than five housemates	22 (56.8) 29 (24.2) 4 (11.4)	15 (31.9) 55 (45.8) 17 (48.6)	10 (31.3) 36 (20) 14 (40)	[a]0.006* [a](1 *vs.* 2) 0.005* [a](1 *vs.* 3) 0.005* [a](2 *vs.* 3) 0.647
Property ownership				
owner tenant	38 (26.6) 17 (28.8)	59 (41.3) 28 (47.5)	46 (32.2) 14 (23.7)	[a]0.484
Employment				
student employed unemployed retired	26 (59.1) 24 (28.2) 5 (12.2) 0 (0)	18 (40.9) 44 (51.8) 14 (34.1) 11 (34.4)	0 (0) 17 (20) 22 (53.7) 21 (65.6)	[a]0.000* [a](1 *vs.* 2) 0.001* [a](1 *vs.* 3) 0.000* [a](2 *vs.* 3) 0.000*
Education				
elementary school high school degree university degree	2 (100) 14 (13.9) 39 (39.4)	0 (0) 41 (40.6) 46 (46.5)	0 (0) 46 (45.5) 14 (14.1)	[a]0.000* [a](1 *vs.* 2) 0.011* [a](1 *vs.* 3) 0.000* [a](2 *vs.* 3) 0.000*
Smoker				
yes no	22 (24.7) 33 (29.2)	36 (40.4%) 51 (45.1%)	31 (34.8) 29 (25.7)	[a]0.203
Body Mass Index				
underweight normal overweight obesity	0 (0) 39 (31.2) 14 (23.7) 2 (14.3)	0 (0) 52 (41.6) 28 (47.5) 7 (50)	4 (100) 34 (27.2) 17 (28.8) 5 (35.7)	[a]0.057

X – mean value; SD – standard deviation; Med – mediana; min – minimum; max – maximum; [a]χ^2 test; [b]Kruskal–Wallis test; [c]Mann–Whitney test; *statistically significant

periodontal disease during the COVID-19 pandemic, one of the potential major stressful factors.

METHODS

The study was designed as an observational clinical pilot study; it was conducted in accordance with the Helsinki Declaration. Approval was obtained from the Ethics committee of Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia (No. 01-2925). The study included all patients who came for a regular dental examination or intervention, starting from March 2020 to October 2021.

To estimate the sample size for the study, the following formula was used: $n = (1.96)^2 \times 4 \times SD^2/d^2$ (SD – standard deviation of the measured loss of clinical attachment and d-the desired width of the confidence interval) and data-based on the study by Coelho et al. [15]. According to the available data, the calculated sample size was 72 patients and the researchers included 202 patients in this study.

Including factors for entering the study were that person gave written consent to participate in the study and was older than 18 years. Excluding factors were less than three teeth present, pregnancy, life-threatening conditions, uncontrolled diseases, mental disorders, and refusal of patients to participate.

Before participating in the study, the procedure and aim of the research were explained to all patients. After signing the information form and signing consent to participate in the study, all patients completed the survey, thus providing socio-epidemiological data of age, marital status, housing status, employment status, education, smoking status, body weight, and height and potential systemic diseases. After that, the patients filled the Perceived Stress Scale (PSS) [16, 17], with the note of the examiner to fill the scale with special reference to the current situation, i.e., primarily considering the pandemic's impact. This scale consisted of 10 questions, each being scored from 0 to 4, depending on the answer. The score of the PSS was obtained, based on which the patients were categorized into three groups – group Ia: low stress (0–13), group IIa: moderate stress (14–26), and group IIIa: high perceived-stress (27–40).

All patients were examined by a single examiner from the Department of Periodontology and Oral Medicine. Faculty of Medical Sciences, University of Kragujevac, due to gaining objectivity and consistency of the obtained results. The doctor had all the protective equipment during the examination. The following parameters were established during the examination: Periodontal Disease Index and Clinical Attachment Level. A dental mirror and a periodontal probe (Williams probe, Hu-Friedy, Chicago, IL, USA) were used for the examination.

The Periodontal Disease Index (PDI, Ramfjord, 1959) was an instrument used to assess the entire periodontal health [18]. Numerical values 1–3 indicated the severity of gingivitis, and 4–6 indicated loss of attachment, while 0 indicated healthy periodontium [18]. The tooth's mesial and vestibular sites were examined, and the representative teeth were: first upper right molar, left upper central incisor, left

Table 2. Participants' characteristics according to the periodontal health

Covariables	1) Healthy periodontium (n = 41)	2) Mild periodontal disease (n = 83)	3) Severe periodontal disease (n = 78)	p
	n (%)			
Gender				
male female	14 (16.5) 27 (23.1)	28 (32.9) 55 (47)	43 (50.6) 35 (29.9)	[a]0.012* [a](1 *vs.* 2) 0.964 [a](1 *vs.* 3) 0.030* [a](2 *vs.* 3) 0.006*
Age (in years)				
X ± SD; med (min–max)	46.6 ± 19.6; 54 (19–72)	41.8 ± 17.1; 39 (20–77)	42.9 ± 18; 40 (20–82)	[b]0.348
Marital status				
single in a relationship married divorced widowed	6 (13) 9 (16.4) 15 (25.4) 6 (21.4) 5 (35.7)	15 (32.6) 23 (41.8) 31 (52.5) 9 (32.1) 7 (35.7)	25 (54.3) 23 (41.8) 13 (22) 13 (46.4) 4 (28.6)	[a]0.016* [a](1 *vs.* 2) 0.185 [a](1 *vs.* 3) 0.024* [a](2 *vs.* 3)0.163
Household status				
living alone living with up to 5 housemates living with more than 5 housemates	7 (14.9) 28 (23.3) 6 (17.1)	13 (27.7) 54 (45) 16 (45.7)	27 (57.4) 38 (31.7) 13 (37.1)	[a]0.042* [a](1 *vs.* 2) 0.587 [a](1 *vs.* 3) 0.222* [a](2 *vs.* 3) 0.036*
Property ownership				
owner tenant	37 (25.9) 4 (6.8)	60 (42) 23 (39)	46 (32.2) 32 (54.2)	[a]0.000* [a](1 *vs.* 2) 0.023* [a](1 *vs.* 3) 0.000* [a](2 *vs.* 3) 0.076
Employment				
student employed unemployed retired	13 (29.5) 16 (18.8) 2 (4.9) 10 (31.3)	19 (43.2) 42 (49.4) 15 (36.6) 7 (21.9)	12 (27.3) 27 (31.28) 24 (58.5) 15 (46.9)	[a]0.002* [a](1 *vs.* 2) 0.556 [a](1 *vs.* 3) 0.937 [a](2 *vs.* 3) 0.021*
Education				
elementary school high school degree university degree	2 (100) 19 (18.8) 20 (20.2)	0 (0) 43 (42.6) 40 (40.4)	0 (0) 39 (38.6) 39 (39.4)	[a]0.090
Smoker				
yes no	8 (9) 33 (29.2)	49 (55.1) 34 (30.1)	32 (36) 46 (40.7)	[a] 0.000* [a](1 *vs.* 2) 0.000* [a](1 *vs.* 3) 0.019* [a](2 *vs.* 3) 0.023*
Body Mass Index				
underweight normal overweight obesity	0 (0) 26 (20.8) 12 (20.3) 3 (21.4)	2 (50) 50 (40.4) 24 (40.7) 7 (50)	2 (50) 49 (39.2) 23 (39) 4 (28.6)	[a]0.790

X – mean value; SD – standard deviation; Med – mediana; min – minimum; max – maximum; [a]χ^2 test; [b]Kruskal–Wallis test; [c]Mann–Whitney test; *statistically significant

Table 3. Periodontal health-related covariables according to the stress level

Oral parameters	Low-stress level (n = 55)	Moderate stress level (n = 87)	High-stress level (n = 60)	[a]p
	n (%)			
PDI				
healthy mild severe	37 (63.8) 17 (31.5) 1 (1.1)	21 (36.2) 30 (55.6) 36 (40)	0 (0) 17 (11.7) 53 (58.9)	0.000* (1 *vs.* 2) 0.000* (1 *vs.* 3) 0.000* (2 *vs.* 3) 0.000*
CAL				
healthy mild severe	52 (58.4) 0 (0) 3 (3.8)	37 (41.6) 17 (51.5) 33 (41.3)	0 (0) 16 (48.5) 44 (55)	0.000* (1 *vs.* 2) 0.000* (1 *vs.* 3) 0.000* (2 *vs.* 3) 0.000*

PDI – Periodontal Disease Index; CAL – Clinical Attachment Level

upper first premolar, left lower first molar, right lower central incisor, and right lower first premolar [18]. Based on the PDI, patients were categorized into the following groups: patients with healthy periodontium (PDI = 0–3; no periodontal involvement), patients with mild periodontal disease (PDI = 4; the loss of attachment is 3 mm or less), and patients with severe periodontal disease (PDI = 5–6; the loss of attachment is more than 3 mm) [18].

Clinical Attachment Level (CAL) represented the distance from the enamel-cement border to the bottom of the periodontal sulcus/pocket [18]. Measurement of this value was performed with a Williams-graded periodontal probe (Hu-Friedy) at four points on each tooth present, the middle of the oral tooth surface, mesial, distal, and vestibular. Values were expressed in millimeters [18]. The mean values of each subject's clinical attachment levels were obtained by summing the measured values and dividing the obtained sum by the number of examined teeth and the number of examined surfaces [18]. Based on the size of the loss of clinical attachment, patients were divided into the following groups: patients with healthy periodontium (who do not have a loss of clinical attachment), patients with mild periodontal disease (loss of the clinical attachment leveled up to 3 mm) and patients with severe periodontal disease (loss of the clinical attachment higher than 3 mm) [18].

After determining all oral clinical parameters and based on the groups assigned to participants within these parameters, patients were cathegorized into final groups that reflected the health of the entire periodontal tissue (Group Ib: patients with healthy periodontium, Group IIb: patients with mild periodontal disease, and Group IIIb: patients with severe periodontal disease).

ll data were processed in the SPSS statistical program, version 21. Descriptive methods were used for statistical data processing. Differences in values of a categorical variable among the groups were tested for significance by χ^2 test or by Fisher's exact test if assumptions for the χ^2 test were not met. The measurement of association between periodontal health covariables and stress was performed by univariate and multivariate Poisson regression analysis. In the multivariate model, socio-epidemiological variables entered the model. A p-value < 0.05 was considered to be a measure of statistical significance for all statistical tests used.

Table 4. Prevalence ratio for the association of periodontal health covariables and stress

Covariables	Periodontal Disease Index		Clinical Attachment Level	
	Crude (95% CI)	Adjusted (95% CI)	Crude (95% CI)	Adjusted (95% CI)
Stress score	**1.052** (1.043–1.061)	**1.042** (1.030–1.055)	**1.104** (1.093–1.116)	**1.108** (1.094–1.122)
Gender				
male	**1.269** (1.076–1.496)	1.125 (0.942–1.345)	1.104 (0.926–1.317)	-
female	Ref.	Ref.		
Marital status				
single	Ref.	Ref.	Ref	Ref.
in a relationship	0.769 (0.589–1.005)	0.791 (0.592–1.055)	1.042 (0.744–1.460)	0.865 (0.604–1.239)
married	**1.337** (1.059–1.687)	1.151 (0.901–1.471)	**2.403** (1.800–3.207)	**2.074** (1.548–2.778)
divorced	**1.408** (1.072–1.849)	1.069 (0.799–1.431)	**2.532** (1.834–3.494)	**1.747** (1.257–2.429)
widowed	**1.819** (1.334–2.481)	1.016 (0.923–1.325)	**4.417** (3.171–6.152)	**1.565** (1.101–2.224)
Household status				
living alone	Ref.	Ref.	Ref.	Ref.
living with up to 5 housemates	1.218 (0.982–1.510)	0.958 (0.712–1.273)	**1.820** (1.401–2.365)	1.331 (1.007–1.760)
living with more than 5 housemates	**1.379** (1.061–1.794)	0.952 (0.712–1.273)	**2.311** (1.714–3.115)	**1.444** (1.047–1.990)
Property ownership				
owner	1.044 (0.870–1.254)	-	1.033 (0.851–1.254)	-
tenant	Ref.			
Employment				
student	Ref.	Ref.	N/A	N/A
employed	**2.865** (2.071–3.965)	**1.720** (1.202–2.462)		
unemployed	**3.494** (2.483–4.917)	**1.688** (1.117–2.549)		
retired	**4.605** (3.275–6.473)	**1.795** (1.172–2.748)		
Education				
elementary school	Ref.	-	Ref.	-
high school degree	3.574 (0.890–14.346		3.443 (0.990–9.575)	
university degree	2.040 (0.507–8.214)		4.834 (0.157–14.832)	
Smoker				
yes	1.162 (0.958–1.371)	-	1.186 (0.996–1.414)	-
no	Ref.		Ref.	
Body Mass Index				
normal	Ref.	Ref.	Ref.	Ref.
underweight	**1.725** (1.074–2.773)	1.325 (0.779–2.253)	**2.429** (1.574–3.749)	1.398 (0.870–2.246)
overweight	1.170 (0.975–1.403)	1.101 (0.905–1.340)	**1.228** (1.013–1.488)	1.222 (0.992–1.504)
obesity	1.123 (0.812–1.1554)	0.961 (0.672–1.374)	1.010 (0.700–1.455)	1.051 (0.710–1.554)

RESULTS

The study included 202 participants (85 males and 117 females, aged 19–82 years; mean age 43.19 years). Analyzing the subjects in relation to the stress, a statistically significant difference was registered between the observed groups in terms of age (between all three groups), household status (between group with low-stress level and group with moderate stress level, and between group with low-stress level and group with high-stress level), employment (between all three groups) and education (between all three groups) shown in Table 1. In terms of age, the youngest group was group with low-stress level (28.1 ± 6.8 years), while the oldest group was the group with high-stress level (56.9 ± 12.2 years) as shown in Table 1.

Similar results were registered between the groups according to the periodontal health (Table 2). A statistically significant difference was registered between the observed groups in terms of gender (between group with low-stress level and group with high-stress level, and between group with moderate stress level and group with high-stress level), marital status (between group with low-stress level and group with high-stress level), household status (between the same groups as for gender), property ownership (between group with low-stress level and group with moderate stress level, and between group with low-stress level and group with high-stress level), employment (between group with moderate stress level and group with high-stress level) and smoking status (between all three observed groups) shown in Table 2.

Table 3 represents periodontal health-related covariables in relation to the level of stress of the subjects. A statistical significance was registered between all three groups of patients according to the stress level in terms of periodontal health parameters. The participants from the healthy group experienced the lowest levels of stress, while subjects with severe form of periodontal disease, according to the measured indices, encountered the highest stress.

The results of the univariate and multivariate Poisson regression analysis with adjustment for potential confounders are shown in Table 4. The Poisson regression analysis showed that in both models, crude and adjusted, periodontal health-related covariables were higher in subjects perceiving greater stress (Table 4).

DISCUSSION

This study aimed to examine the possible impact of stress during the COVID-19 pandemic on periodontal health. Given to the study's findings, it was shown that there was a statistically significant correlation between the level of stress during the COVID-19 pandemic and the status of the health of the entire periodontium. Patients with a higher level of stress had a more severe form of

periodontal disease, in contrast to patients whose scores on the Perceived Stress Scale were lower, which was in agreement with previous studies that also researched this topic [12, 19]. Since the COVID-19 outbreak represents a major stressful event, the impact on mental health in the form of elevated levels of stress, anxiety, and depression was noted among the global population [3]. Periodontal disease is a chronic condition that shares similar pathogenesis like stress, so it could be said that the COVID-19 pandemic has an indirect impact on the periodontial overall health [20].

The study results showed that there was a statistically significant difference in the status of periodontal health between the sexes. Possible explication for worse periodontal health in men could be due to the association of sex hormones, specifically testosterone, with periodontal disease [21]. The percentage of unemployed participants was the highest in the group with severely impaired periodontal health, and the employment variable had a statistically significant effect on periodontal health, in terms of PDI, especially in the case of unemployed subjects. However, other similar studies that were conducted before the current pandemic, did not find a significant association [22, 23]. Subjects who were single had the most severe periodontal disease, which was statistically significant and was also consistent with a previous study, performed in 2018 [15]. Nevertheless, regarding each measured periodontal disease indicator, a marital status significantly affected only the clinical attachment level. This study did also show an association between smoking status and periodontal health, confirming the results from earlier studies and the most common clinical expectations [15, 22, 23].

According to the results of this study, and bearing in mind the confounding covariables, the impact of stress on periodontal health was statistically significant. This analysis indicated that if the stress level increased by one unit, on the PDI would deteriorate by 4.2% (PRadjusted = 1.042) and CAL by 10.8% (PRadjusted = 1.108). These results coincided with a previous similar study [15].

The limitations of this study are reflected in relatively short time span of the research, the lack of radiographic monitoring of the periodontal health, and a smaller number of patients who attended the dental examination due to fear of COVID-19 infection. Since there were no other studies researching this topic during the COVID-19 pandemic, researchers could only compare their results with similar studies, performed before the current pandemic, which examined the effects of stress in general. However, these limitations do not affect the relevance of the obtained data.

CONCLUSION

Having in mind all the obtained results of this research, etiological factors and pathogenesis of periodontal disease, multifactorial and various variables considered in this study, and above all, the current global situation, it may be concluded that the increased stress during the COVID-19 pandemic may result in deterioration of the entire oral cavity's health, including the periodontium. However, more studies are necessary to further investigate this relationship and its' long-term implications.

Conflict of interest: None declared.

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Могућа повезаност стреса изазваног пандемијом ковида 19 и пародонталног здравља – пилот-студија

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САЖЕТАК

Увод/Циљ Стрес се наводи као један од фактора ризика повезаних са пародонталним здрављем. Пандемија ковида 19 има значајан негативан утицај на ментално и телесно здравље популације.

Циљ овог истраживања био је да се испита могућа повезаност између стреса изазваног пандемијом ковида 19 и пародонталног здравља.

Методе Опсервационо пилот-истраживање спроведено је од марта 2020. до октобра 2021. године и обухватило је 202 испитаника. Испитаници су оцењививали свој ниво стреса користећи српску верзију упитника Скала перципираног стреса. Индекс пародонталног обољења, индекс крварења гингиве и ниво припојног епитела одређивани су клиничким прегледом. Испитаници су подељени у следеће групе: Ia (низак ниво стреса), IIa (умерен ниво стреса), IIIa (висок ниво стреса), Ib (здрав пародонцијум), IIb (умерена форма обољења) и IIIb (тешка форма обољења). Процењивана је узрочно-последична веза између стреса и пародонталног здравља.

Резултати Резултати указују на статистички значајну разлику између испитиваних група на основу стреса у свим посматраним параметрима. Поасонова регресија је у оба модела показала да су варијабле повезане са пародонталним здрављем биле више код испитаника код којих је регистрован виши ниво стреса (индекс пародонталног обољења – *PRadjusted* = 1,042, 95% *CI* [1,030–1,055] и ниво припојног епитела – *PRadjusted* = 1,108, 95% *CI* [1,094–1,122]).

Закључак Појачан стрес током пандемије ковида 19 негативно утиче на ментално здравље и може довести до погоршања здравља целе усне дупље, укључујући и пародонцијум.

Кључне речи: ковид 19; пандемија; стрес; пародонтитис

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The impact of COVID-19 pandemic and national lockdown on the surgical care of ophthalmic patients in a tertiary health care institution

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SUMMARY

Introduction/Objective World Health Organization in January 2020 declared a pandemic of the coronavirus disease named COVID-19. The state of emergency in the Republic of Serbia began on March 15 2020, which greatly influenced the treatment of those patients who were not affected by COVID-19. The aim of this paper is to compare the most common ophthalmic surgeries during quarantine with those performed in 2019 in the same period.

Methods This is a retrospective study. We collected data from the operating protocol of the main ophthalmic operating room. We have followed the changes related to surgical procedures during these two years.

Results During the state of emergency, significantly more operations were performed on male patients than on female patients compared to the same period in 2019 ($p = 0.043$). In the observed period, in 2019 significantly more patients older than 65 were surgically treated ($p < 0.001$). During 2019, there were 397 (64.3%) elective and 220 (35.7%) urgent procedures, while for the same period next year there were 9 (9.1%) elective and 90 (90.9%) urgent procedures. Significantly more urgent interventions were performed during 2020 compared to 2019 ($p < 0.001$).

Conclusion The coronavirus pandemic has led to numerous changes in the treatment of ophthalmic patients. Many patients did not have access to adequate treatment, which certainly led to the impairment of many ophthalmic diseases.

Keywords: COVID-19; quarantine; operations; ophthalmology

INTRODUCTION

In the last days of 2019, the Chinese health authorities reported to the World Health Organization the occurrence of few cases of respiratory infections in people in Wuhan. The coronavirus (SARS-Cov-2) was identified as the causative agent of the disease, while the disease was named COVID-19 [1, 2]. Rapid spread of the virus around the world resulted in a global public health threat, and COVID-19 reached the level of a pandemic in March 2020 [1, 2]. In Serbia, the first case of coronavirus was recorded on March 6, 2020 [3], while on March 15, 2020, a state of emergency was declared. In an attempt to limit the spread of this severe respiratory disease, the movement of citizens, especially the vulnerable part of the population (citizens over 65), is limited, online teaching in schools and colleges is introduced, work from home is proposed and many social, service and cultural facilities are closed. These measures were abolished on May 6, 2020 [3].

In addition to these restrictions, the state of emergency distressed the functioning of health facilities and the treatment of a large number of patients who were not primarily affected by COVID-19. Lack of resources and health staff, limited movement of patients and potential fear of infection, have led to the progression of many ophthalmic diseases [4]. Also, during the first few months of the pandemic, most ophthalmology departments postponed their elective activities and limited their practice to emergencies only [5]. The American Academy of Ophthalmology (AAO), the world's largest organization of ophthalmologists, recommended on March 18, 2020, that ophthalmologists stop performing all interventions that are not urgent. In addition, the AAO published a list of emergency ophthalmic surgeries [6]. These recommendations have led to a decrease in the number of patients visited and surgically treated compared to the period before the pandemic [7].

The aim of our research is to present the most common ophthalmic interventions performed during the state of emergency in the Republic of Serbia, declared due to the COVID-19 pandemic, as well as to compare them with procedures performed in the same period last year. With the help of this study, we want to provide a preliminary insight into the consequences of the COVID-19 pandemic and its impact on the treatment of ophthalmic patients.

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METHODS

This retrospective study was conducted at the Eye Clinic, University Clinical Center of Serbia in Belgrade. The period of monitoring ophthalmological patients was from March 15 to May 6, 2020, during the state of emergency in the Republic of Serbia, as well as in the same period in 2019. Surgical protocols from the main ophthalmologic operating room were used as a data source. The collected data include the age and sex of the patients, diagnosis, type of surgery, urgency of their condition and the correlation of these parameters. We divided the patients into four age groups (18 and younger, 18–44, 45–64 years and 65 and older). We divided surgical procedures into urgent and elective according to AAO criteria [6]. The procedure was considered urgent if postponing the operation for 10 days or more would lead to significant loss of vision or reduced chances of recovery. Diagnoses are divided into the following groups: vitreoretinal diseases, trauma, eye infections, eye tumors, cataracts, changes in the eyelids, conjunctiva, tear ducts and cornea, exudative maculopathies, strabismus and other diagnoses. Similarly, we divided operations into the following categories: vitreoretinal surgery, trauma, eye infections, eye tumors, cataract surgery, eyelid, conjunctival and tear duct surgery, corneal surgery, intravitreal injections, strabismus surgery and others [4]. All vitreoretinal surgeries were classified in one category, except those performed due to trauma, which we classified in the category of the same name. This study was undertaken according to the tenets of Helsinki Declaration and approved by the hospital's Ethical Committee.

The outcomes we followed are the differences we found comparing the results from the same period of these two years, which are related to surgical treatment.

Statistical analysis

The description of the categorical variables was performed by using an absolute and relative number in the form n (%). The categorical variables were compared using the χ^2 test – contingency table. Continuous variables are displayed as mean value and standard deviation and were compared using the Mann–Whitney test (with the assessment of the distribution normality). The result was considered statistically significant for the selected level of significance from 0.05. Statistical analysis was performed using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The total number of surgically treated patients from March 15 to May 6, 2019 was 617, while for the same period in 2020, during the state of emergency declared due to the COVID-19 pandemic, that number was 99 patients. The average age of treated patients in 2019 was 57.5 ± 22.4 years, while in 2020 it was 39.3 ± 26.6 years, which showed a statistically significant difference ($p < 0.001$). In the observed period in 2019, 338 (54.8%) male patients and 279 (45.2%) female patients were operated, while in the same period next year, 65 (65.7%) male patients and 34 (34, 3%) female patients underwent surgery. A statistically significant difference was found in the number of surgically treated male and female patients in the observed period in 2019 compared to the same period in 2020 ($p = 0.043$). During the state of emergency in 2020, significantly more operations were performed on male patients than on female patients compared to the same period in 2019 (Table 1). There was a statistically significant difference in the age distribution of patients during the same period in 2019 and 2020 ($p < 0.001$). In the observed period, in 2019 significantly more patients older than 65 were surgically treated, while in 2020, more patients younger than 18 were operated on (Table 2).

Table 1. Sex distribution of surgically treated patients during the examined periods in 2019 and 2020

Sex, n (%)	The year of the surgical treatment		p*
	2019	2020	
Male	338 (54.8)	65 (65.7)	0.043
Female	279 (45.2)	34 (34.3)	

*for the level of significance of 0.05

Table 2. Age distribution surgically treated patients during the examined periods in 2019 and 2020

Age groups, n (%)	The year of the surgical treatment		p*
	2019	2020	
18 and younger	62 (10)	32 (32.3)	< 0.001
18–44 years	68 (11)	11 (11.1)	
45–64 years	183 (29.7)	39 (39.4)	
65+ years	304 (49.3)	1 (17.2)	

*for the level of significance of 0.05

During 2019, there were 397 (64.3%) elective and 220 (35.7%) urgent procedures, while for the same period next year there were nine (9.1%) elective and 90 (90.9%) urgent procedures. There was a statistically significant difference in the type of surgeries performed during 2019 and 2020. Significantly more urgent interventions were performed during 2020, and more elective interventions were performed during 2019 ($p < 0.001$) (Figure 1).

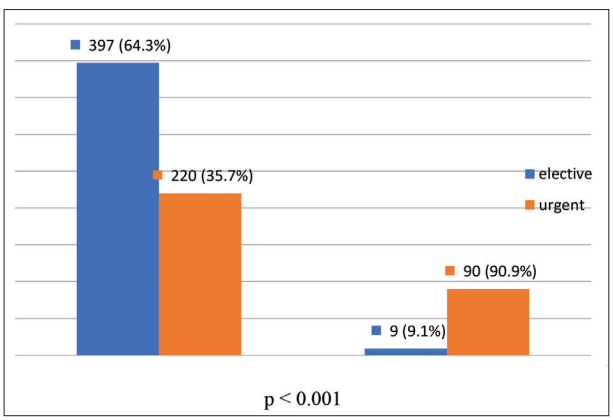

Figure 1. Types of surgeries performed during 2019 and 2020

In the study period in 2019, the patients were most often referred for surgical treatment of cataracts, 148 (24%), followed by 129 (21%) for the treatment of changes on the eyelids and 87 (14%) due to eye tumors, while in the same period in 2020, the patients were most often referred for the trauma treatment, 39 (39%) of them, followed by 23 (23%) due to eye tumors and 19 (19%) due to vitreoretinal diagnoses. In the observed period in 2020, no patient was referred for treatment of changes on the eyelids, conjunctiva and tear ducts, cornea, exudative maculopathy or strabismus. During 2020, there was a statistically significant increase in vitreoretinal diagnoses, trauma and eye tumor surgeries, while in 2019 there were significantly more cataracts. In both years, vitreoretinal diagnoses, eye trauma, eye infections, eye tumors, cataracts and glaucoma were present in the study period (Table 3).

Table 3. Refferal diagnoses that led to surgical intervention during the examined periods in 2019 and 2020

Refferal diagnoses	Year		p*
	2019	2020	
Vitroretinal diagnoses	69 (11.2)	19 (19.2)	**0.024**
Traumas	36 (5.8)	39 (39.4)	**< 0.001**
Eye infections	12 (1.9)	5 (5.1)	0.060
Eye tumors	87 (14.1)	23 (23.2)	**0.014**
Cataracts	148 (24)	7 (7.1)	**< 0.001**
Glaucoma	23 (3.7)	3 (3)	0.731
Eye lids	129 (20.9)	0	**< 0.001**
Conjuctivas and tear ducts	20 (3.2)	0	0.069
Corneas	18 (2.9)	0	0.085
Exudative maculopathy	48 (7.8)	0	**0.004**
Strabismus	20 (3.2)	0	0.069
Other	7 (1.1)	3 (3)	0.136

*For the level of significance of 0.05

Table 4. Ophthalmological surgeries performed during the examined periods in 2019 and 2020

Ophthalmological surgeries	Year		p*
	2019	2020	
Vitroretinal surgeries	68 (11)	19 (19.2)	**0.021**
Eye trauma surgeries	36 (5.8)	39 (39.4)	**< 0.001**
Eye infection surgeries	6 (1)	5 (5.1)	**0.002**
Eye tumor surgeries	87 (14.1)	14 (14.1)	0.991
Cataract surgeries	149 (24.1)	7 (7.1)	**< 0.001**
Glaucoma surgeries	23 (3.7)	3 (3.0)	0.731
Eye lid surgeries	129 (20.9)	0	**< 0.001**
Conjuctivas and tear ducts surgeries	26 (4.2)	0	**0.037**
Cornea surgeries	18 (2.9)	0	0.085
Intravitreal injections	48 (7.8)	0	**0.004**
Strabismus interventions	20 (3.2)	0	0.069
Other	7 (1.1)	12 (12.1)	**< 0.001**

*For the level of significance of 0.05

When it comes to surgeries, in the examined period in 2019, cataract surgeries were most often performed, [149 (24.1%)], followed by eyelid surgeries [129 (20.9%)] and eye tumor surgeries [87 (14.1%)]. In the same period in 2020, surgeries due to eye trauma were the most frequently performed [39 (39.4%)], vitreoretinal surgeries [19 (19.2%)] and eye tumor surgeries[14 (14.1%)].

During the examined period in 2020, there were no operations on the eyelids, conjunctiva and tear ducts, cornea, there were no intravitreal injections and strabismus operations. Significantly more vitreoretinal surgeries, eye trauma surgeries and operations due to eye infections were performed, while in 2020 there were significantly fewer cataract surgeries (Table 4).

DISCUSSION

The COVID-19 pandemic has shown a significant impact on the health system, affecting patients' ability to access emergency and elective care, reducing the number of surgeries, hospital admissions and clinic examinations [8]. Providing emergency surgical care in the early days of the COVID-19 pandemic was a unique challenge for surgeons. New factors, such as perioperative testing for SARS-CoV-2, the availability of adequate personal protective equipment, have overgrown traditional paradigms of surgical care [9]. Also, the COVID-19 pandemic has led to extensive quarantines around the world that have restricted the movement of billions of people [10]. The Eye Clinic of the University Clinical Centre Serbia, as a tertiary ophthalmological center with an emergency service that works 24 hours a day, has remained open for patients who need urgent ophthalmological care. The results of our research showed a significant difference in the care of ophthalmic patients during the state of emergency in the Republic of Serbia compared to the same period last year. With a large volume of elective procedures, ophthalmology was severely affected by the COVID-19 pandemic, losing almost 80% patients volume in March and April 2020 [8].

The average age of patients who were treated surgically during the state of emergency was significantly lower compared to the same period in 2019. This is most likely a consequence of the limited movement of citizens over the age of 65 who were most at risk from COVID-19, as well as citizens' fear of potential infection. Similar results were presented by dell'Omo et al. [4]. Pellegrini et al. [11] concluded that both surgeons and patients who were involved in surgical treatment were statistically significantly younger in April 2020 compared to April 2019.

Also, there was a statistically significant difference in sex distribution. Our study showed that there were more male patients than female operated on during the state of emergency in 2020, compared with the previous year, which is in line with research conducted by Pellegrini et al. [11] and Babu et al. [12]. This difference could be influenced by the fact that mostly women stayed at home to take care of children when schools were closed, so there was less chance of them getting sick or injured [8, 12].

Our study showed a difference in the distribution of surgically treated patients in different age groups. Children and adolescents were operated more often during the state of emergency, which was not the case according to the study of Pellegrini et al. [11] who noticed a decrease in representation of this age group. According to the study conducted by Babu et al. [12] there was an increase in the

prevalence of patients younger than 60 years during the state of emergency, which is in line with our research. In our study, a statistically significant decrease in the number of patients older than 65 was noticed during 2020, which is a consequence of the restrictions on the movement of this part of the population during the state of emergency in the Republic of Serbia.

The number of operations performed during the state of emergency was significantly lower compared to the same period in 2019, with a notable decline in elective interventions. This could be due to restrictions imposed during the state of emergency, such as recommendations to stay at home and limited movement at certain times of the day, which is in line with previously published studies [8, 11, 12, 13]. Although in 2020 the total number of operations decreased compared to the previous year, both elective and emergency, we noticed a large difference in the types of operations performed. During 2019, the largest number of operations performed were elective interventions, while in 2020 there were mostly emergency interventions that, if delayed, could lead to vision loss This data is in accordance with the measures envisaged during the state of emergency, as well as with the recommendations of the AAO [6]. Most elective interventions were postponed during the state of emergency, but a small number of these operations was performed which coincides with the results of other studies [4, 11, 12].

Cataract is one of the most common ophthalmic diseases in adults over the age of 50, and cataract surgery is the most common elective operation in developed countries [4]. Our study showed a statistically significant decrease in the number of these operations in the observed period, which was the most common operation in 2019, while in 2020 this number was significantly lower, as shown by studies by other authors [4, 12, 14]. Retinal detachment is an emergency that requires urgent intervention. In our study, we noticed a statistically significant decrease in these interventions, although in percentages they were more frequent in 2020 than in 2019. Similar results were presented by other authors [4, 11], explaining this as the reluctance of patients to risk exposure to the coronavirus in healthcare facilities. Eye tumor surgeries, which fall under emergency interventions, did not show a statistically significant difference in the number of operations performed in the observed period of both years. Eye trauma is an emergency in ophthalmology. Our study showed that the number of these cases increased in the observed period in 2020 compared to the previous year, and that this is far the most common surgical intervention performed during the state of emergency, probably because of the do-it-yourself tasks. On the other hand, dell'Omo et al. [4] concluded that the number of trauma cases decreased, which they had explained by limited outdoor activities, while Abdullatif et al. [13] in their study concluded that the number of trauma cases did not change compared to the pre-pandemic era.

Surgical treatment of many conditions was not performed during the state of emergency, and that includes operations on the eyelids, conjunctiva and tear ducts, cornea, strabismus, etc. These conditions are not classified as urgent and as such may be deferred, in accordance with AAO guidelines [6].

The limitation of this study is that it was conducted in one clinical center, so that these results cannot be generalized to others, although the Eye Clinic of the University Clinical Center of Serbia covers the entire territory of the Republic of Serbia. As a source of data, we used the operating protocol of the main operating room and compared the differences between the examined periods in 2019 and 2020. The other two protocols, from the day hospital for cataract surgery and the small operating room, were not used because the rooms were closed during the state of emergency, which may affect the data as a whole during the period under review in 2019. In future studies, we plan to include protocols from all three operating rooms to provide more detailed insight. In addition, we do not have data from private ophthalmology clinics in our study. Despite the limitations, we believe that our study provides valid information for further clinical research on the impact of the pandemic on the treatment of ophthalmic patients.

CONCLUSION

The coronavirus pandemic has led to numerous challenges in the daily care of ophthalmic patients. Many patients did not have access to adequate treatment, which certainly led to the impairment of many ophthalmic diseases. Many changes in practice during this pandemic will take place and become part of the new normal after the COVID-19 pandemic. With the help of new knowledge and studies, medical workers can prepare to deal with the consequences of the pandemic and they can plan safe, effective, and comprehensive protocols for the future.

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Утицај пандемије ковида 19 и ванредног стања на хируршко збрињавање офталмолошких пацијената у терцијарној офталмолошкој установи

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САЖЕТАК

Увод/Циљ Светска здравствена организација је у јануару 2020. прогласила пандемију болести коју изазива вирус корона под називом ковид 19. Ванредно стање у Републици Србији почело је 15. марта 2020. године, што је у великој мери утицало на лечење оних пацијената који нису били заражени ковидом 19.

Циљ овог рада је да се упореде најчешће офталмолошке операције током карантина са онима које су обављене 2019. године у истом периоду.

Методе Ово је ретроспективна студија. Прикупили смо податке из оперативног протокола главне офталмолошке операционе сале. Пратили смо промене у вези са хируршким захватима током ове две године.

Резултати Током ванредног стања урађено је значајно више операција на пацијентима мушког пола него женског у односу на исти период 2019. године ($p = 0,043$). У посматраном периоду, у 2019. години је хируршки лечено значајно више пацијената старијих од 65 година ($p < 0,001$). Током 2019. године било је 397 (64,3%) изборних и 220 (35,7%) хитних поступака, док је за исти период наредне године било 9 (9,1%) изборних и 90 (90,9%) хитних поступака. Током 2020. године обављено је значајно више хитних интервенција у односу на 2019. годину ($p < 0,001$).

Закључак Пандемија ковида 19 довела је до многих промена у лечењу офталмолошких пацијената. Многи пацијенти нису имали приступ адекватном лечењу, што је сигурно довело до погоршања многих офталмолошких болести.

Кључне речи: ковид 19; карантин; операције; офталмологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Importance, personal protective equipment, and our experience after first autopsies performed on COVID-positive deceased in Novi Sad, Serbia

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SUMMARY

Introduction/Objective Autopsy represents the gold standard for determining cause and mechanisms of death. With this paper, the authors wanted to acquaint colleagues with our experiences while performing autopsies of COVID-positive deceased patients.

Method The study included total of 12 autopsies related to COVID-19 infection, performed in our forensic pathology institution, from which one autopsy of suspected patient and 11 autopsies of confirmed COVID-positive patients. Confirmation of infection was obtained by antemortem polymerase chain reaction analysis of oropharyngeal and nasopharyngeal swabs and by postmortem swabs taken from upper airways and lungs.

Results In five cases, cause of death was directly attributed to COVID-19 infection. In two cases cause of death was due to heart attack, in two cases due to gastrointestinal hemorrhage, in one case due to multiple injuries, in one case due to trauma complications and in one case due to gunshot injury.

Conclusion Large number of autopsies in which cause of death has been established to be other than COVID, along with importance of these cases for litigation, strongly emphasizes the importance of forensic autopsy of COVID-positive deceased. If adequate personal protective equipment is used, there should be minimal exposure risk to virus remaining in body tissues.

Keywords: COVID-19; autopsy; PPE; cause of death

INTRODUCTION

At the end of the year 2019, cases of an unknown infectious disease, primarily targeting lungs and causing pneumonia of unknown etiology, were reported in Wuhan, China. The microorganism which was causing this infection was identified as novel corona virus and was named severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) [1]. The disease, named coronavirus disease (COVID-19), soon spread across Asia, then Europe, and then all over the world causing a pandemic. Recently, the result of a retrospective analysis of postmortem obtained sample of vitreous humor, showed that the first case of COVID-19 infection in Republic of Serbia was registered on February 5, 2020 [2]. Although there are multitude of literature data that provides insights of clinical manifestations of infection with Coronavirus, there were not so many articles related to pathomorphological characteristics, especially at the beginning of the pandemic. The reason for this deficiency of data was because the pandemic plunged the whole world into a healthcare crisis and also into an economic crisis, that caused initial shortage of protective medical equipment [3, 4]. The primary focus was on saving patients' lives and not adding pressure to already overcrowded health institutions and overburdened mortuary systems. In some countries, autopsy rooms were even used as morgues [5]. Accordingly, autopsies did not seem important at the time.

After an initial delay, it soon became obvious that the role of forensic pathologists and performing autopsy procedures on COVID-positive deceased, is vital for gaining a better understanding of COVID-19 pathological mechanism [6, 7]. Autopsy results (macroscopic, microscopic, microbiological), give us insight of how the virus is spreading through an organism, which organs are more affected, or even about possible later effects of the infection on an organism. Furthermore, autopsy represents the best tool for determining the exact cause of death and, in some cases, manner of death as well.

There were a few reported articles about pathological findings of COVID-positive patients, obtained by conducting biopsies and minimal invasive autopsies or partial autopsies which were systematically presented by Maiese at all. [8] The first full autopsy report was published in February 2020, in China [9] and after those articles, others were reported as well. Also, articles about necessary safety measures while performing autopsies were given by health organizations and governments of various states [10–13].

In Serbia, autopsies are performed in forensic institutions if they are ordered by the prosecutor's

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Table 1. Autopsy and pathohistological findings (Ph)

No	Brain	Heart	Lungs	Liver	Kidneys	Cause of death	Comorbidity	Heart (Ph)	Lungs (Ph)
1	Brain autopsy not performed	680 g, left ventricle 2.3 cm, right ventricle 0.8 cm, heart valves dysfunctional	Edema, 1010 g, 1140 g, pneumonia	Stasis	Stasis, initial nephrosclerosis	Heart failure	/	Hypertrophy, myofibrosis	Edema, emphysema, macrophages in the alveoli
2	Brain autopsy not performed	540 g, infarction scar, thinned left ventricle wall, atherosclerotic occlusion of RIP 99%	980 g, 940 g, edema, hepatization, initial ARDS	Stasis	Stasis	Reinfarction	Hypertension, obesity	Infarction scar, myofibrosis	Hyaline membranes, edema, septa with proliferated connective tissue and lymphocytes
3	Anemic	Atherosclerosis, aortic valve stenosis, aortic, mitral and tricuspid valve insufficiency	Bilateral pneumonia	Anemic	Anemic	Hemorrhagic shock, gastrointestinal bleeding	Hypertension	Infarction scar	/
4	Nonspecific	Nonspecific	Left pneumothorax, left rupture, bilateral pneumonia	Nonspecific	Nonspecific	ARDS, sepsis, polytrauma	/	Myofibrosis	Massive bronchopneumonia, focal intraalveolar hemorrhage, hyaline membranes
5	Brain autopsy not performed	Vegetation on mitral valve, chronic cardiomyopathy	Adhesions, ARDS	Anemic	Anemic	Hemorrhagic shock, gastric ulcer, gastrointestinal bleeding	Obesity	Myofibrosis, hypertrophy	Hyaline membranes, edema, septa with proliferated connective tissue and lymphocytes, massive intraalveolar hemorrhage
6	Nonspecific	Nonspecific	ARDS, bilateral pneumonia	Rupture, suture dehiscence, abscess	Shock	Sepsis, peritonitis, subphrenic abscess	Hypertension	/	Edema, septa with proliferated connective tissue and lymphocytes, atelectasis
7	Nonspecific	Atherosclerosis, STENT	Bilateral interstitial pneumonia, pleural effusion 150 ml	Nonspecific	Nonspecific	Interstitial pneumonia, COVID-19	Coronary atherosclerosis	/	/
8	Nonspecific	Atherosclerosis	Bilateral interstitial pneumonia	Cirrhosis	Nonspecific	Interstitial pneumonia, COVID-19	Alcoholic, malnourished	/	/
9	Brain autopsy not performed	Nonspecific	Edema, lobar pneumonia	Stasis	Stasis	Interstitial pneumonia, COVID-19	Obesity	Myofibrosis, lipomatosis	/
10	Nonspecific	Hypertrophy, 500 g, moderate atherosclerosis	1300 g, 900 g, ARDS, pneumonia	Stasis	Nephrosclerosis, cysts, shock	ARDS, pneumonia, COVID-19	/	/	Edema, hyperemia
11	Nonspecific	600 g, mild atherosclerosis	ARDS, bilateral pneumonia	Stasis	Nonspecific	Pneumonia, COVID-19	Hypertension	Hypertrophy, acute infarction	Hyaline membranes, edema, septa with proliferated connective tissue and lymphocytes, emphysema
12	Subarachnoid hemorrhage, contusions, edema	Nonspecific	ARDS, peripheral emphysema	Abscess	Nephrosclerosis	Gunshot wound	/	/	Emphysema, anthracosis, edema

ARDS – acute respiratory distress syndrome

office (in accordance with the Criminal procedure code) or if it is in accordance with certain paragraphs of the Healthcare law. Consent of the deceased closest family, in these cases, is not necessary and they cannot call off an autopsy procedure. It looks very similar to laws in Austria, as stated in the article by Skok et al. [3].

METHODS

From the beginning of the pandemic, until the end of 2020, 12 autopsies related to COVID-19 infection were performed at our forensic institution, from which one autopsy of suspected patient and eleven autopsies of confirmed COVID-positive patients. For most of these cases confirmation of infection was obtained by antemortem polymerase chain reaction (PCR) analysis of oropharyngeal and nasopharyngeal swabs and in two cases postmortem swabs from upper airways and lungs were taken during autopsy procedure, and then analyzed by PCR. Result of one suspected patient came out negative after the PCR analysis of postmortem sample. All bodies were placed in cold storage at least 36–48 hours before the beginning of the autopsy, in order to minimize the possibility of infection of forensic practitioners. It is possible that infectivity is unlikely if the cycle threshold values are greater than 30, but more postmortem studies can clarify the survival interval of the virus in a dead body and how dangerous it is for forensic practitioners to perform an autopsy [14]. It could also explain the possibility of virus transmission from infected corpse to forensic practitioner reported in Bangkok, Thailand [15].

This research was approved by the Ethics committee of our institution.

RESULTS

During an autopsy, all recommended safety measures were followed and personal protective equipment (PPE) was used. After gaining autopsy results, the cause of death was directly attributed to COVID-19 infection in only five cases, in which death occurred due to pulmonary manifestations (viral pneumonia). In these cases, lungs were firm, heavy and edematous, with whitish, foamy liquid leaking over the cut surface. Microscopically, pneumocyte hyperplasia, lymphocyte infiltration, multinuclear giant cells, increase in alveolar wall thickness and focal presence of hyaline membrane were seen, which are typical changes in viral pneumonia and acute respiratory distress syndrome (ARDS). Some of the autopsy and pathohistological findings are presented in Table 1, but surely more autopsies of COVID-positive patients are needed for a better understanding of these findings and also for understanding the pathogenesis of SARS-CoV-2 that the whole world is struggling with [16].

In two cases, the cause of death was due to heart attack, in two cases due to gastrointestinal hemorrhage, in one case due to multiple injuries (polytrauma), in one case due to trauma complications (sepsis), and in one case due to brain trauma associated to gunshot injury. In some death cases, non-related directly to COVID-19 infection, mild to moderate form of viral pneumonia was present, so it was not always easy to opt for the exact cause of death, which brings us to another problem, especially present among clinical colleagues – proper filling of death certificates. To help resolve this dilemma, we emphasize that if a patient has died from viral pneumonia and/or ARDS related to COVID-19 infection, then these diagnoses should be put in part one of death certificate (primary cause of death, direct and indirect cause of death) and if a patient dies from other cause, but has been COVID positive prior to death, with mild or moderate pneumonia (he died with COVID but not from COVID), then diagnoses of COVID infection should be put in part two (disease or trauma that contributed to the cause of death).

DISCUSSION

From the very beginning of the epidemic, there were several recommendations that autopsies of COVID-positive deceased should not be performed, with the exceptions of medicolegal cases. A protocol of actions in the case of such patients has been issued by experts from our country in March 2020 [17]. As far as performing autopsy is concerned, recommendations were that autopsies of COVID-positive patients should not be performed, except in cases of forensic significance, and that they must be performed using the adequate PPE.

Advisory Committee on Dangerous Pathogens has categorized pathogens (Table 2) according to their risk of human infection [18]. SARS and Middle East respiratory syndrome related coronaviruses (including Sars-Cov-2), hepatitis virus B, C, D and E, HIV, rabies, poliovirus, dengue, Creutzfeldt-Jakob disease agent, *Echinococcus*, *Mycobacterium tuberculosis* and many others are representatives of group 3 (Table 2).

Table 2. Advisory Committee on Dangerous Pathogens hazard group definitions

When classifying a biological agent it should be assigned to one of the following groups according to its level of risk of infection to humans	
Group 1	Unlikely to cause human disease
Group 2	Can cause human disease and may be a hazard to employees; it is unlikely to spread to the community and there is usually effective prophylaxis or treatment available
Group 3	Can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available
Group 4	Causes severe human disease and is a serious hazard to employees; it is likely to spread to the community and there is usually no effective prophylaxis or treatment available

Staff working in autopsy rooms is at high risk of exposure to Sars-Cov-2, because of specific procedures, during which a large number of viruses are released into the air in the form of aerosols. To minimize the risk of infection, use of appropriate PPE during autopsy is required. Minimum

Figure 1. Minimum of personal protective equipment

Figure 2. Oscillator saw with suction of the bone aerosol

of PPE as well as other recommendations are explained and summarized in several articles [3, 5, 7, 12, 13, 19, 20]. Recommended PPE implies the use of surgical scrub suit worn under impermeable gown or apron with full sleeve coverage, double surgical gloves interposed with a layer of cut-proof synthetic mesh gloves, disposable N95 respirator, goggles or face shield that covers the front and sides of the face, surgical caps, shoe covers (Figure 1). Also, there should be limited number of personnel present in the autopsy room, and a single practitioner should be operating within the body cavity at a time. Round-ended scissors and blades with blunted points should be used to minimize the risk of mechanical injuries, an oscillator saw with suction of the bone aerosol (Figure 2) should be used for sawing the skull. The fact that we did not have this device from the very beginning is the reason why in the first few autopsies we did not open the skull (in accordance with the recommendations on staff protection). It is essential to have all the necessary equipment at your disposal, to avoid the need to leave the area to find additional items.

Our autopsy room had air exchange with negative pressure and air recirculation to the other rooms was restricted. We did not have special autopsy room for infectious corpses with downward air suction directly at the autopsy table or Biosafety level 3 autopsy room or Airborne infection isolation room or 3M Versaflo powered air purifying respirators with M-series headgear as stated in some recent papers [7, 20]. The necessity of listed safety measures are still controversial, but more and more experts believe that these protective measures are excessive [21]. After performing larger number of autopsies on COVID-positive patients, a consideration should be given to the idea of establishing a national database, that will make it easier to obtain all the necessary data for better understanding of pathophysiology, diagnosis and treatment of this disease [22].

CONCLUSSION

With this paper, the authors wanted to acquaint colleagues with their experiences and possible problems while performing autopsies of COVID-positive deceased patients. Autopsy is still the gold standard for determining cause of death, mechanism of death and in some cases manner of death as well. Here, we represent the first autopsies of COVID-positive deceased performed in our country, and since the beginning of writing this paper, there were even more autopsies performed in our institution. If adequate PPE is used, there should be minimal exposure risk to virus remaining in body tissues. Also, we remind that plenty of autopsies infected with pathogens from the same hazard group were routinely done during past years or even decades. Such a large number of autopsies, in which the cause of death has been established to be other than COVID, along with importance of these cases for litigation, strongly emphasizes the importance of referring COVID-positive deceased to a forensic autopsy.

Conflict of interest: None declared.

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Значај, адекватна заштитна опрема и наша искуства после првих извршених обдукција преминулих особа позитивних на ковид у Новом Саду, Србији

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САЖЕТАК

Увод/Циљ Обдукција представља златни стандард за одређивање тачног узрока смрти и механизма умирања. Овим радом желели смо да упознамо колеге с нашим искуствима у вези с вршењем обдукција покојника позитивних на ковид.

Метод У нашој форензичкој установи извршено је укупно 12 обдукција повезаних са инфекцијом вирусом корона, од којих се за једну особу сумњало да је позитивна, а за 11 болесника је било потврђено да су позитивни на ковид. Потврда инфекције добијена је раније урађеном анализом полимеразне ланчане реакције орофарингеалног и назофарингеалног бриса, као и постмортално узетим брисом горњих дисајних путева и плућа.

Резултати У пет случајева узрок смрти директно се везује за инфекцију вирусом корона. У два случаја узрок смрти наступио је услед срчаног удара, у два случаја због гастроинтестиналног крварења, у једном случају због вишеструких повреда, у једном случају због компликација трауме, а у једном случају због повреде нанете из ватреног оружја.

Закључак Већи број обдукција код којих је откривено да узрок смрти није директна последица инфекције вирусом корона, заједно са значајем који ова открића имају за текуће судске процесе, наглашавају важност вршења судскомедицинских обдукција над преминулима који су били позитивни на ковид. Уз коришћење адекватне личне заштитне опреме и придржавања одређених мера приликом вршења обдукције, ризик од изложености вирусу у телесним ткивима покојника је минималан.

Кључне речи: ковид 19; обдукција; лична заштитна опрема; узрок смрти

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Different angiogenic response and bone regeneration following the use of various types of collagen membranes – *in vivo* histomorphometric study in rabbit calvarial critical-size defects

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SUMMARY
Introduction/Objective Success of guided bone regeneration depends on the size and morphology of defect, characteristics of barrier membranes and adequate angiogenesis.
The aim of the study was to reveal impact of three different collagen membranes on angiogenesis and bone production in critical-size defects.
Methods Defects were created in rabbit calvarias, filled with bovine bone graft and randomly covered with one of three investigated collagen membranes (Biogide – BG, Heart – PC, Mucograft – MG) or left without a membrane for the control group (C). After two and four weeks of healing, a total of 10 animals were sacrificed for histological and histomorphometric analysis of angiogenesis, bone regeneration, and inflammatory response.
Results In the early healing phase, the highest values of trabecular thickness and trabecular area were recorded with PC and BG membranes, respectively. After four weeks, significantly improved bone healing was noted in the MG group, as well as significantly pronounced inflammation. Initially, vessel density was significantly higher in the C group compared to all three membranes. After four weeks, significantly better results were observed in the MG compared to the other groups, BG compared to the rest of groups, and between PC and C groups.
Conclusion The use of collagen membranes significantly affects angiogenesis, reducing it in the early and enhancing it at the later healing phase. All three tested membranes in combination with bone graft significantly improved the amount of regenerated bone. Among the investigated groups, MG favored more pronounced angiogenic, osteogenic, and inflammatory response in the observation period of four weeks.
Keywords: collagen membrane; angiogenesis; guided bone regeneration; collagen matrix; pericardium

INTRODUCTION

Opposite to other connective tissues bone has a remarkable ability to completely restore its structure and function, recapitulating the embryonic processes of intramembranous and endochondral ossification. On the other hand, besides its substantial self-regenerative capacity, healing of intraoral bone defects largely depends on the size and morphology of the defect, number of bony walls, mechanical wound stability, healing environment and treatment protocols [1, 2].

Concept of guided bone regeneration (GBR) is based on the use of barrier membranes that selectively exclude migration of fast-growing soft tissue cells, thus allowing enough time for osteoprogenitors to populate and regenerate the entire defect. Among numerous available bioresorbable and non-resorbable barriers today, collagen is recognized as more frequently used due to its biocompatibility, hemostatic effect, osteoblast attraction, growth factor adsorption, and the active role in bone formation [3, 4]. In contrast, unpredictable resorption and poor mechanical stability are their main limiting factors [5]. These are partially compensated by combining a membrane with particulate bone graft and using membranes of improved structural characteristics and prolonged barrier longevity, such as cross-linked membranes, the two-membrane technique, membranes of more resistant source of collagen, or incorporation of antibacterial agents, growth factors, and ceramics within their structure [3, 6, 7].

For successful bone regeneration, angiogenesis is considered the prerequisite factor for bone formation, repair and remodeling [8]. New blood vessels, besides providing nutrition and gaseous exchange, bring important growth factors and stem cells into the healing zone [8, 9]. They temporally precede bone formation, starting exclusively from the surrounding bony walls and the periosteum [10]. While more

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blood vessels could mean more nutrition, growth factors, stem cells, and intensified regeneration, there is an assumption that one of the mechanisms of membrane efficiency might be the exclusion of blood vessels from the overlaying soft tissue that do not have the bone-forming potential [9].

Therefore, the aim of this study was to reveal how different collagen membranes effect angiogenesis in the critical-size defect model and its further reflection on bone healing. The inflammatory response was also evaluated with respect to its impact on bone regeneration.

METHODS

Study design and surgical procedures

Ten skeletally mature New Zealand White rabbits, weighing 3.5–4.5 kg, were included in this study. The study protocol was approved by the Institutional Ethics Committee, School of Dental Medicine, University of Belgrade (Approval number 36/10) and conducted in accordance with the European Union Directive 2010/63/EU for animal experiments.

The surgical procedure was performed under general anesthesia, achieved with intramuscular administration of 5 mg/kg of xylazine, 35 mg/kg of ketamine, and 0.75 mg/kg of acepromazine. In brief, after disinfection, incision, and flap elevation, four 8-mm circular bicortical defects were created in rabbit calvaria, two in the frontal and two in the parietal bones, using trephine drills. The defects were filed with bone substitute (Bio-Oss, Geistlich Söhne AG, Schlieren, Switzerland) and randomly assigned to one of following investigated groups: 1) BG (Biogide, Geistlich), 2) PC (heart pericardium membrane, Bioteck, Arcugnano, Italy), 3) MG (Mucograft, Geistlich), and 4) C (control group without membrane). All membranes were trimmed into the 10 × 10 mm quadrant shape, adapted over defects, and stabilized by suturing the periosteum with horizontal mattress sutures (Coated Vicryl 5-0, Ethicon Inc., Raritan, NJ, USA). The skin was closed with a continuous suture (Coated Vicryl 4-0, Ethicon Inc.). Postoperatively, the animals received antibiotics (15 mg/kg of oxytetracycline, intramuscularly) and analgesics (0.01 mg/kg of butorphanol, subcutaneously) for three days.

After two and four weeks of healing, five randomly assigned rabbits were sacrificed under general anesthesia, with an overdose of phenobarbital (100 mg/kg). Their cranial vaults were removed with a saw, rinsed with water, and immersed in 10% buffered formalin solution. Thereafter, formalin-fixed calvarial bones were cut with a low-speed diamond saw disc in the regions of previously created experimental bone defects.

Histologic processing and histomorphometric evaluation

Bone samples were further decalcified with 10% formic acid, dehydrated in ethanol, molded in paraffin blocks, and longitudinally sectioned through the center of the defects. Three central tissue sections of 5 μm thicknesses were cut from each block for hematoxylin-eosin staining. Histomorphometric analysis and histologic observation were done by an experienced pathologist blinded to the experimental groups. Slides were observed by optical microscopy (Olympus 5 microscope, Olympus Corporation, Tokyo, Japan) using Olympus Cell-B morphometric software.

Histomorphometric parameters were analyzed quantitatively, counted in the areas of the highest density (hot spots) at high power magnification (HPM) of 200 ×. The following parameters were measured: vessel density (VD, number of blood vessels within one microscopic field under HPM), blood vessel diameter (BVD, the largest vessel diameter, in μm), blood vessel area (BVA, in μm^2), trabecular thickness (Tb.Th, the widest dimension of bone trabeculae, in μm), trabecular area, (Tb.A, in μm^2), and multinucleated giant cells (MNGC, number of cells within one microscopic field under HPM).

Statistical analysis

Statistical analyses were made using IBM SPSS Statistics, Version 24.0 (IBM Corp., Armonk, NY, USA). All data were presented as mean and standard deviation (SD). Statistical methods for intergroup analyses included the Mann–Whitney U-test, or one-way ANOVA, due to the normality test. Intragroup comparisons within time were assess using the two-sample t-test or Mann–Whitney U-test. The level of significance was set at ≤ 0.05.

RESULTS

The healing was uneventful in all the animals. During the study there were no signs of infection, allergic reaction, wound dehiscence, or membrane exposure.

Histological findings

After two weeks, all membranes showed an angiogenic potential. BG and PC membranes had blood vessels in direct contact with membrane fibers that partially grew into the membrane (Figure 1A, B). In contrast, MG membrane was forming a blood vessels demarcation between the membrane and graft (Figure 1C). This relationship was maintained after four weeks (Figure 1D–F). Analyzing bone production, we noticed that all trabeculae were similar in cellularity, but there was a difference in their thickness. At the second week of healing, trabeculae in the MG group were thinner, more graceful and narrower (Figure 2C), than the other two membranes (Figure 2A, B). Contrary, in the fourth week newly formed bone was more voluminous and wider in MG group (Figure 2D-F). Considering inflammatory response, a limited infiltrate of nonspecific inflammatory cells was observed in all the groups at the second week of healing. On the other hand, after four weeks, in the MG group there were MNGC present, with diffuse membrane infiltration (Figure 1F), while

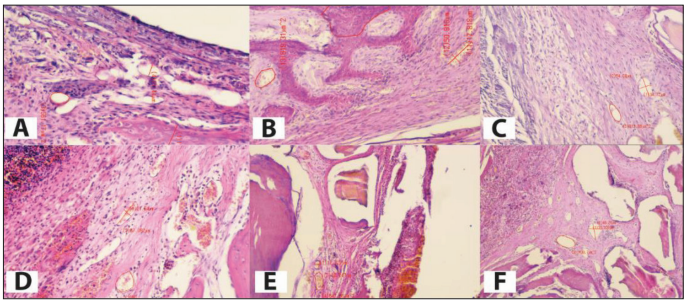

Figure 1. Histological images showing angiogenesis and inflammatory response concerning investigated membranes BG (A, D), PC (B, E), MG (C, F), and healing time: two weeks (A, B, C), four weeks (D, E, F); H&E, 200 ×

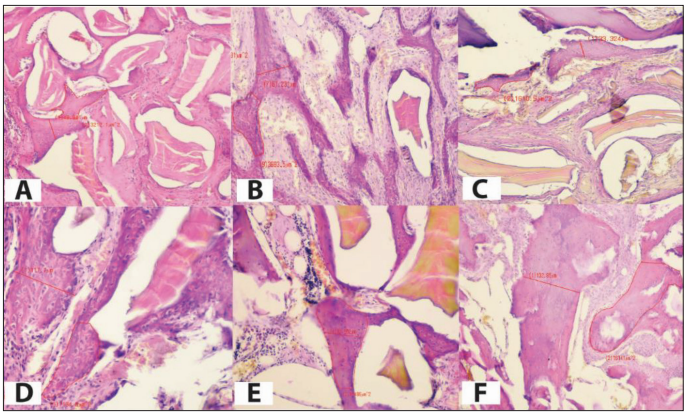

Figure 2. Histological images showing bone production according to different membranes; BG (A, D), PC (B, E), MG (C, F), and follow-up time: two weeks (A, B, C), four weeks (D, E, F); H&E, 200 ×

the other two membranes did not induce such an intense tissue response (Figure 1D, E).

Histomorphometric analysis

Angiogenesis (Table 1)

In two weeks, all the investigated parameters were significantly increased in group C compared to the other groups, except for BVD, which lacked significance between the C and MG groups. Inside the membranes, groups' statistical analyses showed significantly increased VD in group BG in comparison to PC and MG groups, which was also seen between PC group and MG group. On the other hand, BVD and BVA were significantly increased in MG compared to BG and PC. However, while BVD was significantly increased in BG compared to PC, BVA was significantly higher in PC than in BG. After four weeks of healing, MG group showed significantly increased values of all vascular parameters in comparison to the other groups. Considering VD, significantly higher results were also observed with BG compared to PC and C, as well as between PC and C. Contrary, BVD and BVA were significantly increased in C compared to BG and PC, although without significance for BVD between C and BG. Comparison between BG and PC regarding BVD showed significantly higher results for BG, while PC showed significantly increased values for BVA. Within the time frame, T4 *vs.* T2, all investigated parameters were significantly improved in the MG group, while all the other groups expressed decreased values.

Bone regeneration (Table 2)

Use of collagen membranes after two weeks of healing resulted in significantly more bone comparing to group C in both parameters. Analysis among investigated membranes after two weeks of healing demonstrated significantly increased Tb.Th in group PC, compared with BG and MG, as well as between PC and MG. In contrast, Tb.A was increased in BG compared to PC, and significantly higher in BG and PC compared to MG. Similarly, after four weeks of healing, collagen membranes significantly improved bone regeneration compared to the control. Among the investigated membranes, both parameters were significantly higher for MG in comparison to BG and PC. However, while Tb.Th was significantly improved in BG in comparison to PC, Tb.A was significantly higher for PC. Considering bone production with time, all groups showed significant increase of Tb.Th, which corresponds to significant increment of Tb.A.

Inflammatory response (Table 2)

Two weeks after the surgery, no MNGC was detected within any investigated group. After four weeks of healing, results of an inflammatory response were significantly higher for the MG group compared to all the other groups, as well as between the BG and the PC groups.

DISCUSSION

In recent years, a growing body of evidence has been suggesting that blood vessels in bone promote osteogenesis [10, 11]. However, there are scarce data regarding the influence of barrier membranes on angiogenesis, pointing out the need for its research [12].

Our study investigated the impact of three structurally different collagen membranes on angiogenesis and bone production in rabbit calvarial critical-size defects. Since previous research showed that membranes possess the greatest impact in the upper and central defect regions, we focused our analysis on that top half part of bone defect [4].

According to our results, it seems that in the early healing phase more pronounced angiogenesis can be expected at those sites where barrier membrane is not used. This result is in line with previous research of De Marco et al. [13], who pointed out that more intensive and extensive revascularization of autologous block bone graft were found in the group without occlusive membrane use, where new blood vessels proliferate not only from the bony walls of the recipient bed, but from the overlaying soft tissue as well.

Table 1. Histomorphometrical analysis of vascular parameters

Parameters	BG	PC	MG	C	Comparison between groups
T2					
VD (N/mm^2)	9.87 ± 1.73	8 ± 0.76	6 ± 0.66	14.40 ± 1.24	BG *vs.* PC p = 0.002; BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
BVD* (μm)	23.26 ± 2.63	19.7 ± 2.77	35.16 ± 3.45	37.07 ± 2.01	BG *vs.* PC p = 0.005; BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C p = 0.000; MG *vs.* C NS
BVA (μm^2)	552.86 ± 10.49	605.33 ± 18.77	808.35 ± 23.87	2960.40 ± 944.49	BG *vs.* PC, BG*vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4					
VD (N/mm^2)	8.2 ± 1.01	6.8 ± 0.94	11.33 ± 1.5	5.93 ± 0.80	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, MG *vs.* C p = 0.000; PC *vs.* C p = 0.019
BVD* (μm)	24 ± 2.15	12.85 ± 2.22	38.98 ± 1.9	25.88 ± 2.45	BG *vs.* C NS; BG *vs.* PC, BG *vs.* MG, PC *vs.* MG, PC vs C, MG *vs.* C p = 0.000
BVA (μm^2)	461.18 ± 23.02	527.93 ± 29.61	1479.11 ± 174.29	726.49 ± 21.07	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4 *vs.* T2§					
VD	0.003	0.001	0.000	0.000	
BVD	NS	0.000	0.001	0.000	
BVA	0.000	0.000	0.000	0.000	

Values are given as mean ± SD;
BG – BioGide; PC – pericardial membrane (heart); MG – Mucograft; C – control; T2 – two weeks; T4 – four weeks; VD – vessel density; BVD – blood vessel diameter; BVA – blood vessel area; NS – not significant;
Kruskal–Wallis test, post hoc Mann–Whitney test;
*one-way ANOVA, Bonferroni post hoc test (due to normality);
§two-sample t-test / Mann–Whitney test

Table 2. Histomorphometrical analysis of bone production and inflammatory response

Parameters	BG	PC	MG	C	Comparison between groups
T2					
Tb.Th* (μm)	49.05 ± 1.33	75.2 ± 5.37	32.77 ± 4.88	19.79 ± 4.49	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
Tb.A (μm^2)	3738.17 ± 332.45	3594.83 ± 192.62	1598.29 ± 68.11	1168.23 ± 117.12	BG *vs.*MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000; BG *vs.* PC NS (p = 0.059)
MNGC (N/mm^2)	-	-	-	-	
T4					
Tb.Th (μm)	118.06 ± 3.08	94.62 ± 2.92	130.38 ± 9.36	23.90 ± 2.33	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
Tb.A (μm^2)	7243.18 ± 425.70	15316.57 ± 1563.44	16490.59 ± 886.98	2239.16 ± 164.32	BG *vs.* MG, BG *vs.* PC, BG *vs.* C, PC *vs.* C, MG *vs.* C p = 0.000; PC *vs.* MG p = 0.011
MNGC (N/mm^2)	2.67 ± 0.97	0.93 ± 0.7	11.6 ± 1.96	0	BG *vs.* PC, BG *vs.* MG, BG *vs.* C, PC *vs.* MG, PC *vs.* C, MG *vs.* C p = 0.000
T4 *vs.* T2§					
Tb.Th	0.000	0.000	0.000	0.005	
Tb.A	0.000	0.000	0.000	0.000	

Values are given as mean ± SD;
BG – BioGide; PC – pericardial membrane (heart); MG – Mucograft; C – control; T2 – two weeks; T4 – four weeks;
Tb.Th. – trabecular thickness; Tb.A – trabecular area; MNGC – multinucleated giant cell; NS – not significant;
Kruskal–Wallis test, post hoc Mann–Whitney test;
*one-way ANOVA, Bonferroni post hoc test (due to normality);
§two-sample t-test / Mann–Whitney test

When we compare results among investigated membranes, the difference in their angiogenic response may be the result of their distinctive structure. The findings of this study revealed that in the early healing phase, the BG membrane produced the highest VD in the underlining bone defect, followed by the PC membrane, while the thick MG membrane expressed the lowest early angiogenesis. These results are in line with previous animal studies investigating angiogenesis inside the collagen membranes themselves, which showed early angiogenesis of the BG [14, 15], a somewhat slower angiogenesis of the bovine PC [14], and a delay in angiogenesis of the MG [16]. Even though angiogenesis and bone regeneration mainly arose from the surrounding bony walls, Schwarz et al. [17] found some localized areas of newly formed bone below the barrier membranes, which allow transmembranous angiogenesis, in contrast to the occlusive ones.

However, despite better result of angiogenesis with the use of BG in comparison to PC, we found significantly thicker bone trabeculae and improved bone Tb.A after two and four weeks, respectively, with the use of the PC membrane. Although angiogenesis plays a significant role in bone healing, modification of material surface properties, mechanical characteristics, thickness, porosity, and composition are recognized to be important issues in GBR [7]. In line with that, a recent study by You et al. [6]

revealed better bone regeneration using a porcine pericardial membrane compared to the BG membrane, where the smooth surface of the pericardial membrane promoted proliferation and differentiation of attracted human bone mesenchymal stem cells at a higher level, implying its potentially osteoconductive and osteinductive characteristics. Although we used the pericardial membrane from different animal species, similar multilayer composition of pericardium could probably have allowed more bone-forming cells to attach and proliferate on its lower surface, like in previous research. Other possible factors that may affect bone production are excellent mechanical properties of pericardium [18]. Regardless of its negligible impact on space maintenance ability due to bone graft use, mechanically stable environment and stiffer surfaces showed enhanced osteoblast differentiation [19]. Furthermore, heart pericardial sac consists of collagen and elastin, whose elastic fibers, in addition to improving tensile strength, might have a potential pro-osteogenic role [20].

Regarding MG, our results showed that in the early healing phase it provoked the lowest VD of the underlining defect, as well as significantly lower bone regeneration compared to the other two membranes. Similar results were demonstrated by Basudan et al. [21] in the same animal model after two, four, six, and eight weeks of healing, showing lower bone regeneration comparing MG and the cross-linked collagen membrane. They considered that a possible reason for a decreased bone regenerative potential may be slower vascularization of the dense, compact layer of MG [16]. Our results may also indicate that the compact layer of this thick matrix may be the reason for lower initial angiogenesis and bone regeneration outcome.

When we compared the result in the later healing phase, we found that angiogenesis was significantly higher with the use of collagen membranes compared to the control. That result is in line with data from Koerdt et al. [22], who found that in the augmentation model of sheep bone, the use of a bovine bone substitute and collagen barrier membrane improved vascularization of an autologous iliac bone graft in the later healing period. This finding could be explained by a lower metabolic demand of tissue in the control group, in which a barrier membrane was not used, so that the competing fibrous cells had access to the defect area. Moreover, we observed that the lowest bone formation after four weeks of healing was in the control group, which is in agreement with previous research of impaired bone production without membrane use [4].

Precisely with respect to results after four weeks of healing, the MG group showed significantly improved angiogenic and osteogenic response compared to other investigated groups. There are several potential reasons for this outcome. First of all, this collagen matrix has an open-pore structure on its lower surface, suitable for stem cell ingrowth [16]. Even though collagen matrices were primary constructed for soft tissue augmentation, according to the latest findings they are highly appropriate for bone forming cell adhesion, migration, proliferation and osteoblast differentiation [23]. Moreover, MG allowed for the highest percentage of cell penetration from the liquid platelet-rich fibrin, compared to BG and the bovine PC membrane [24]. Secondly, due to the highest thickness, it possesses the largest area of collagen strands available for absorption of various growth factors released from the deeper layers, after the initial burst release, over the next two weeks [25]. Although bone morphogenetic protein 2 is discharged at a low percentage in the early phase for the MG membrane, it has been shown that in the collagen–hyaluronic acid membrane more intensified discharge was noted between three and seven weeks, probably as a result of membrane degradation [25, 26].

Finally, after four weeks of healing, we found the presence of material-induced MNGC, which could be related to the material degradation process [27]. MNGC symbolize the syncytium of macrophages that could be pro-inflammatory (M1) or pro-regenerative (M2) [28]. Considering that a higher number of MNGC in the MG group is followed by the presence of intensified angiogenesis and bone production, it could be speculated that at least one part of these cells may have pro-regenerative potential. Moreover, a recent study showed a rather similar distribution of M1 and M2 macrophages after four weeks of soft tissue healing with MG, although without MNGC formation [29]. In addition, both types of macrophages can contribute to angiogenesis – M1 via vascular endothelial growth factor production in the initiation process, and M2 by releasing thrombocyte growth factor and matrix metalloproteinase responsible for vascular branching and maturation [30]. In our study, only the MG group showed significant angiogenesis enhancements with time, both in VD and size.

CONCLUSION

The use of collagen membranes significantly affects angiogenesis, reducing it in the early healing phase and enhancing it at a later one. All three tested membranes in combination with bone graft significantly improved the amount of regenerated bone. Among the investigated groups, MG favored more pronounced angiogenic, osteogenic, and inflammatory response in observation period of four weeks. Further studies with longer follow-up are needed to investigate whether this trend continues with time.

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Различит ангиогени одговор и коштана регенерација после примене различитих врста колагених мембрана – *in vivo* хистоморфометријска студија на критичним дефектима калварије кунића

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САЖЕТАК

Увод/Циљ Успех вођене коштане регенерације зависи од величине и морфологије дефекта, карактеристика баријерне мембране и адекватне ангиогенезе.

Циљ ове студије је био да се открије утицај три структурално различите колагене мембране на ангиогенезу и коштану продукцију у дефектима критичне величине.

Методе Дефекти су направљени на калваријама кунића, попуњени говеђим коштаним графтом и насумично покривени једном од три испитиване колагене мембране (*Biogide – BG, Heart – PC, Mukograft – MG*) или остављени без мембране за контролу (К). После две и четири недеље зарастања укупно 10 животиња је жртвовано ради хистолошке и хистоморфометријске анализе ангиогенезе, коштане регенерације и инфламаторног одговора.

Резултати У раној фази зарастања највеће вредности дебљине и површине трабекула су забележене редом код *PC* и *BG* мембрана. После четири недеље значајно боље коштано зарастање је уочено у групи *MG*, као и значајно израженија инфламација. Густина крвних судова је иницијално била значајно већа у групи К у поређењу са све три мембране. После четири недеље значајно бољи резулати су примећени у групи *MG* у поређењу са осталим групама, у групи *BG* у поређењу са преосталим групама и између група *PC* и К.

Закључак Примена колагених мембрана значајно утиче на ангиогенезу, смањујући је у раној а подстичући је у каснијој фази зарастања. Све три испитиване мембране у комбинацији са коштаним графтом су значајно повећале количину регенерисане кости. Међу испитиваним групама *MG* је фаворизовао израженији ангиогени, остеогени и инфламаторни одговор у периоду посматрања од четири недеље.

Кључне речи: колагене мембране; ангиогенеза; вођена коштана регенерација; колагени матрикс; перикард

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Use of antibiotics after lower third molar surgery – useful or harmful procedure? A randomized, double-blind, placebo-controlled trial

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SUMMARY

Introduction/Objective The aim of the present study was to investigate the effects of moxifloxacin and cefixime in preventing postoperative infection following mandibular third molar surgery.

Methods Double-blind study was completed by 157 patients undergoing surgical removal of mandibular third molars. The patients were randomly assigned to the following three groups: moxifloxacin (M), cefixime (C), and placebo (P). Patients in each group were classified into two subgroups: subgroup (a), without previous history of pericoronitis, and subgroup (b), with previous history of pericoronitis. All the patients were evaluated at the postoperative follow-ups on the first, second, and seventh postoperative day.

Results Postoperative infections were registered only in patients with a history of pericoronitis. Antibiotic prophylaxis with cefixime and moxifloxacin reduced the occurrence of postoperative infection. Overall incidence of postoperative infections was 6.4%. All postoperative infections were registered in the placebo-group, where the incidence of postoperative infection was 19.2%. Microbiological tests verified the clinically obtained results. Isolated microflora was resistant to penicillin-derived antibiotics in 50% of the cases.

Conclusion Prophylactic use of antibiotics after third molar surgery should be weighed against potential risks and benefits and could be considered in cases with previous history of pericoronitis, when complicated surgical extraction is performed.

Keywords: third molar surgery; antibiotic prophylaxis; postoperative complications; drug resistance; microbial susceptibility tests

INTRODUCTION

Amoxicillin (alone or in combination with clavulanic acid), as well as clindamycin and metronidazole, have a long history of success in the treatment of odontogenic infections. These are still the most commonly used antibiotics in oral surgery due to the fact that oral microorganisms are mostly susceptible to them. Therefore, it is not surprising that the majority of published studies related to the use of antibiotics in oral and maxillofacial surgery, both for prophylaxis and treatment of odontogenic infections, have been done with amoxicillin or antibiotics of similar antimicrobial spectrum [1].

However, due to the growing number of patients being allergic to penicillin derivatives as well as the increasing occurrence of oral microorganisms resistant to penicillin and several other antibiotics, there is a growing need for research directed towards antibiotics that could be an alternative to amoxicillin and other antibiotics with similar antibacterial spectrum in prevention and treatment of oral infections [2, 3]. Among novel antibiotics, it seems that fluoroquinolones (especially moxifloxacin) and the third generation of cephalosporins (especially cefixime) are promising in that regard. Both are effective against many microorganisms resistant to other antibiotics, including amoxicillin [4, 5]. Moreover, they have different pharmacokinetic properties from penicillin derivatives and may demonstrate (especially moxifloxacin) some anti-inflammatory and immunomodulatory effects, which is desirable in prophylaxis or treatment of odontogenic infections [6].

On the other hand, inadequate prescribing accelerates the development of bacterial resistance to a large number of antibiotics, which could have unforeseeable consequences for health care worldwide in the future. Antibiotic resistance is currently one of the biggest public

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health problems today, resulting in significant decreases in infection treatment efficiency, an increase in multidrug-resistant bacterial strains, and increased morbidity and mortality, with repercussions for the health system as a whole [7]. The World Health Organization report makes it clear that this is not a phenomenon in poor or developing countries – the problem of bacterial resistance is now being observed around the world [8].

We intended to test the efficacy of moxifloxacin and cefixime in preventing postoperative infection after mandibular third molar surgery [9, 10]. The goal of our study was to investigate whether prophylactic use of moxifloxacin and cefixime has significant impact on the rate of postoperative infections after third molar removal. Having an increased antibiotic resistance in mind, our goal was also to determine under what circumstances recommendations on their prophylactic use can be justified.

METHODS

This clinical research, approved by the Ethics Committee of the Dental Clinic of Vojvodina, with the decision number 01-33/8-2019 and registered with the NIH – ClinicalTrials.gov with ID NCT05027893, was carried out as a double-blind, prospective, placebo-controlled clinical study. All patients included in the study were ≥ 18 years old, indicated for surgical removal of the impacted mandibular third molars, with good systemic health (classified as ASA I and ASA II) [11]. Exclusion criteria were as follows: hypersensitivity to study drugs; history of systemic antibacterial therapy within six months prior to randomization; pregnancy or breastfeeding; fluoroquinolone-related tendon disorder; clinically relevant cardiac conditions or current use of QT interval prolonging drugs; severe hepatic insufficiency (Child–Pugh C); cases where, in addition to removing the impacted mandibular third molar, some other oral surgical procedure was performed; patients with currently present pericoronitis.

It was assumed that the percentage of infection in the prophylactic groups would be similar and no more than 1%, while in the placebo group it would be higher – approximately 15%. Sample size was calculated as a difference between the two proportions (1% and 15%). Using 80% power and alpha = 0.05, 56 patients per group was determined sufficient to achieve 80% to detect significant differences between the groups.

The patients were randomly and equally assigned to treatment groups. Randomization was performed using a complete randomization algorithm (R software for Windows, package "randomizeR"), allocation ratio 1:1:1, and a sample size of 165 (55 per group). The randomization list was created with three groups and consequent numbers from 1 to 165. The medication boxes were marked using a specific ID consisting of three digits and two letters, generated by R software. The IDs were randomly assigned to the randomization list numbers and the final randomization list consisted of ID and list number. Consecutive patients were assigned to a specific group by reading the list number and giving the medication box with specific ID for that number. Only third-party members, who were not involved in patient care, had all the lists in one place and could identify the medication (to be used for emergency unblinding purposes if needed). That person was available 24/7 via mobile phone number provided to the participants.

The manufacturers (Hemofarm DOO, Vršac, Serbia, and Alkaloid DOO, Skopje, North Macedonia) created the boxes for medications to be identical by instruction of the research team. The boxes with film-coated tablets were the same shape, size, color, and taste. Placebo tablets were created using the same instructions (PhytoNet DOO, Belgrade, Serbia).

After the screening, all participants that fulfilled the enrollment criteria (inclusion/exclusion) were blindly assigned to a specific group by the randomization list. Eight patients were classified as dropouts due to either non-compliance (n = 4) or failure to appear for the control visit (n = 4).

Mandibular third molar surgeries were performed by five oral surgeons (Figure 1). All surgeries were performed under local anaesthesia, using 4 ml of 2% lidocaine with adrenaline 1:80,000. Flaps were elevated, bone was removed with surgical burrs, and third molars sectioned as needed to facilitate removal. All wounds were sutured with non-resorbable 3-0 black silk sutures. The sutures were removed on the seventh postoperative day. The patients were advised to take an analgesic containing 200 mg of ibuprofen and 325 mg of paracetamol if needed.

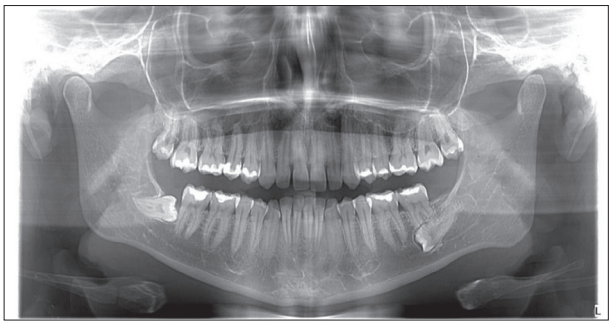

Figure 1. Panoramic radiograph of a patient included in this study (lower right third molar with previous history of pericoronitis was removed)

All the patients postoperatively received film-coated tablets with either 400 mg of moxifloxacin or 400 mg of cefixime *per os*. One-third of the patients received placebo tablets containing indifferent substances with no antimicrobial action (99% microcrystalline cellulose, 0.5% silicon dioxide, and 0.5% magnesium stearate). Surgeons who performed surgery were blinded to the type of tablet which patients received. All used film-coated tablets were administered for the first five days postoperatively, once a day, in a double-blind manner. All the patients were evaluated at the postoperative follow-ups on the first, second, and seventh postoperative day.

Postoperative infection was diagnosed based on the presence of local signs of inflammation and systemic signs

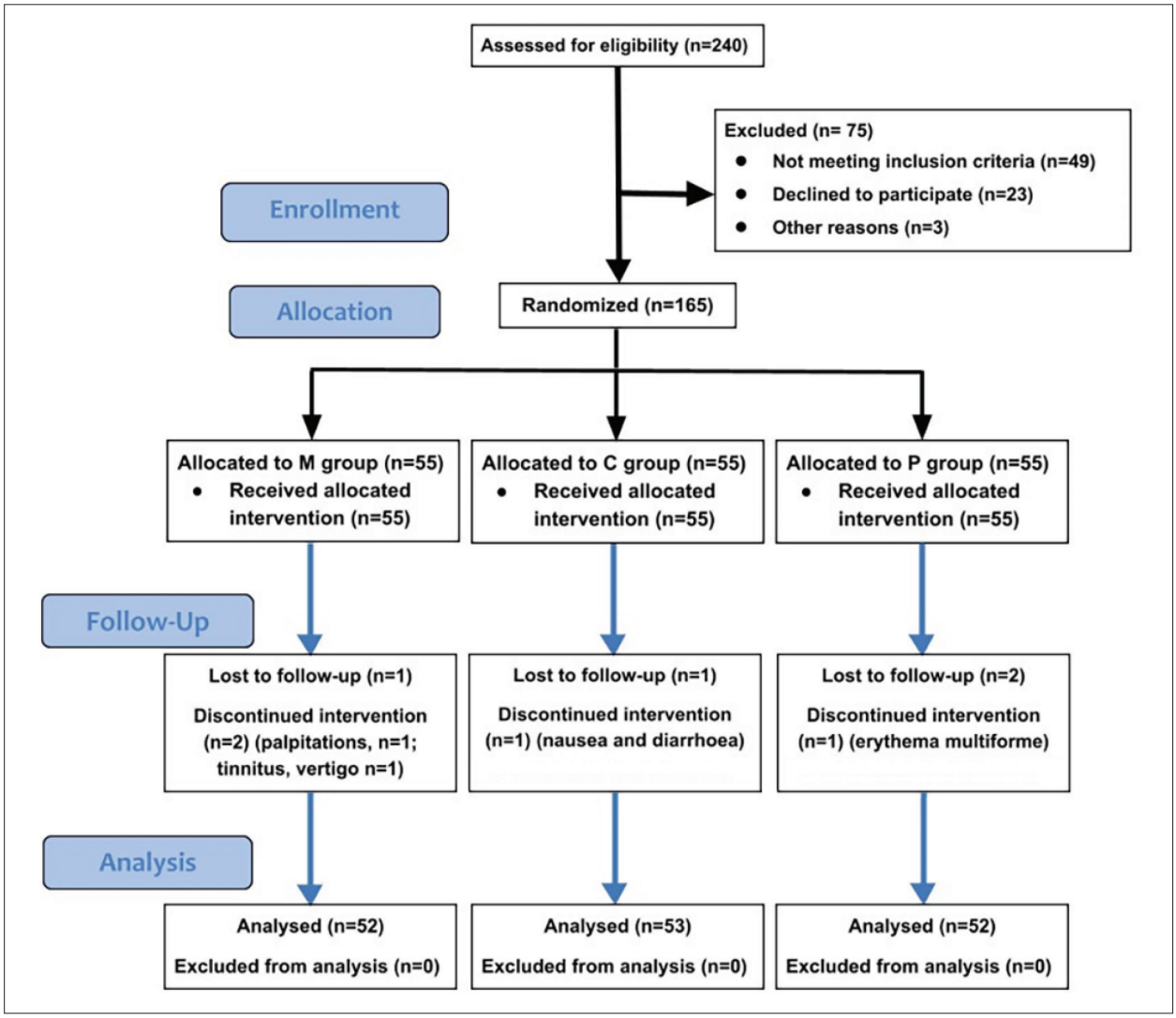

Figure 2. Flow diagram of the progress through the phases of a three-group parallel randomized trial [12]; [downloaded Sep 2, 2021]; available from: http://www.consort-statement.org/

of infection (elevated body temperature, accelerated erythrocyte sedimentation, leucocytosis).

Swab samples were obtained from surgical wounds of patients with signs of postoperative infection, and microbiologically analysed. The susceptibility of isolated microorganisms to moxifloxacin, cefixime, and other antibiotics commonly used in oral and maxillofacial surgery was tested by culture on antibiotic-containing media. Regarding the susceptibility to antimicrobial drugs, bacteria were classified according to the growth inhibition zone, into three categories: susceptible (S), intermediate (I), and resistant (R).

After the study was completed and the codebook was opened, the results were grouped by type of treatment into three groups: moxifloxacin (M), cefixime (C), and the placebo (P). Based on the previous history of pericoronitis, the patients in each group were classified into two subgroups: patients without previous history of pericoronitis (a) and patients with previous history of pericoronitis (b).

The manuscript was reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement [12]. Primary outcome was identified as occurrence of postoperative infection. Secondary outcomes were the subgroup analysis (previous history of pericoronitis or not) and identified as susceptibility of isolated microorganisms to antibiotics (in cases where swab samples were obtained).

Results are presented as count and percent (where appropriate). Group comparisons were performed using Fisher's exact test. P values less than 0.05 were considered significant. All data were analyzed using the IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) statistical software.

RESULTS

The clinical study included 165 patients who were indicated for surgical removal of an impacted mandibular third molar. Eight patients were excluded from the study due to non-compliance with the postoperative instructions or not showing up to the follow-up exams. The template for the CONSORT flow diagram is shown in Figure 2 [12]. The study, in accordance with the described method, was

completed by a total of 157 patients, so that the first study group, group (M), consisted of 52 patients [subgroup (a) of 39 patients, subgroup (b) of 13 patients], the second study group (C) of 53 patients [subgroup (a) of 39 patients, subgroup (b) with 14 patients], and the control group (P) of 52 patients [subgroup (a) of 39 patients, subgroup (b) of 13 patients]. Mean patient age was 26.7 years, with standard deviation of 8.85 years in group M; 24.2 years with standard deviation of 5.11 years in group C, and 25.5 years with standard deviation of 5.44 in group P. Gender distribution in groups was as follows: group M (59.6% female, 40.4% male); group C (73.6% female, 26.4% male), group P (75% female, 25% male).

In our study, the overall incidence of postoperative infections was 6.4%. Interestingly, we did not register any *de novo* infection – all cases of postoperative infection occurred in patients who had preoperative history of pericoronitis (subgroup b), and all these cases belonged to the placebo group (P) (Table 1).

Table 1. Incidence of postoperative infection in the research groups

Research groups			Postoperative infection		Total
			no	yes	
Group	M	n	52	0	52
		%	100	0	100
	C	n	53	0	53
		%	100	0	100
	P	n	42	10	52
		%	80.8	19.2	100
Total		n	147	10	157
		%	93.6	6.4	100

Group M – research group receiving moxifloxacin;
Group C – research group receiving cefixime;
Group P – control group receiving placebo

The difference between each of the study groups (groups M and C) and the control placebo group (group P) regarding the occurrence of postoperative infection was confirmed with Fisher's test of exact probability ($p < 0.001$).

There is a statistically significant difference in the predisposition to infection with previous history of pericoronitis (Table 2). Patients of the group P, subgroup (b), had a postoperative infection in 77% of the cases (10/13 patients). Patients without previous history of pericoronitis remained infection-free following surgery (0%, 0/39 patients).

Table 2. History of pericoronitis and postoperative infection

Parameter	Moxifloxacin (n = 52)	Cefixime (n = 53)	Placebo (n = 52)	p
History of pericoronitis	13 (25%)	14 (26.4%)	13 (25%)	0.982a
Postoperative infection	0	0	10 (19.2%)	< 0.001b

[a]Pearson χ^2;
[b]Fisher's exact test

Patients with diagnosed postoperative infection were under close supervision for three weeks after surgery. In cases where postoperative infection was diagnosed in the placebo group, standard therapy was administered – amoxicillin with clavulanic acid (or in case of allergy to amoxicillin, azithromycin was used). After the microbial identification of the causative agent(s), and in case of its resistance to amoxicillin with clavulanic acid or azithromycin, antibiotic therapy was modified in accordance with microbiological susceptibility.

Out of 23 analysed samples, five were taken preoperatively from the examinees from each of the study groups (M and C), who had a history of pericoronitis (subgroups b). A total of 13 samples were taken from the control placebo group (P); three samples were taken preoperatively around the tooth with a history of pericoronitis, and 10 samples were taken from the operative wound of the patients with signs of postoperative infection before giving antibiotic therapy. The samples were tested for susceptibility to the researched antibiotics, as well as to other antibiotics usually used in oral surgery (Table 3).

As shown in Table 3, all the swabs were susceptible to moxifloxacin. It is interesting to note that three swabs with strictly pathogenic *Staphylococcus aureus* were resistant to ampicillin, amoxicillin, amoxicillin with clavulanic acid, and had intermediate susceptibility to clindamycin. Resistance to moxifloxacin was not observed in any of the samples taken, and resistance to cefixime was noted in only one case.

DISCUSSION

Justification for the use of antibiotics relative to mandibular third molar surgery has been a controversial topic. Lang et al. [13] found as many as 42 different protocols for antibiotic administration following this procedure (in terms of type, dose, timing, and mode of delivery). Many authors conducted prospective, placebo-controlled clinical trials, with similar methods, and in most studies the antibiotic in the study group was amoxicillin (with or without clavulanic acid), administered in a single preoperative dose or three to five days after surgery. The conclusions are mainly reduced to the common consensus that there is sufficient evidence for the use of these antibiotics for third molar surgery, because the benefit does not outweigh the risk of side effects [14]. Recently, Cervino et al. [15] proposed a modified protocol based on the administration of amoxicillin or amoxicillin with clavulanic acid before and after intervention, but, again, many questions were left unanswered. The conclusion was that it was necessary to find an alternative to existing, especially penicillin-derived antibiotics [15].

Chugha et al. [9] observed heterogeneity in the design of the studies and the method of antibiotics administration. Better evidence and justification are needed in this area, as many strong recommendations are currently made on the basis of weak evidence [16].

Moxifloxacin and cefixime have been labelled by many authors as a realistic therapeutic alternative to existing antibiotics widely used in oral and maxillofacial surgery [5,

Table 3. Susceptibility of samples obtained from the infected postoperative wounds or from the space around the tooth to test antibiotic

Antibiotic	Research group												Total
	Moxifloxacin (M)			Cefixime (C)			Placebo (P)						
	History of pericoronitis			History of pericoronitis			History of pericoronitis			Postop. infection			
	S	I	R	S	I	R	S	I	R	S	I	R	
Moxifloxacin	5			5			3			10			23
Cefixime	5			5			2		1	9		1	23
Ampicillin	4		1	5			1		2	5		5	23
Amoxicillin	4		1	5			1		2	5		5	23
Amoxicillin + CA	4		1	5			2		1	6		4	23
Tetracycline	5			4		1			3	6		4	23
Clindamycin	5			4	1		3			8		2	23
Total samples	5			5			3			10			23

S – susceptible; I – intermediate; R – resistant; CA – clavulanic acid

17]. Cachovan et al. [18] demonstrated that moxifloxacin penetrates very well into oral tissues, reaches high concentrations in bone, and is well resorbed after oral administration. Moreover, moxifloxacin is very effective against oral pathogens, especially against the periopathogen *Aggregatibacter actinomycetemcomitans*, as well as against *Porphyromonas gingivalis*, *Prevotela intermedia* and *Tannerella forsythia* [19]. Efficacy of oral treatment with moxifloxacin and amoxicillin with clavulanic acid on oral function and quality of life after third molar surgery demonstrated that moxifloxacin shortened the period of postoperative recovery [20].

In our study, the frequency of postoperative infection in group P was extremely high (19.2%). Data on the frequency of infection after this surgery vary depending on the assessment method. Most studies indicate that the prevalence of postoperative infection is in 1–10% range, which is lower than in our study [21]. Patients with a previous history of pericoronitis had postoperative infection in 77% of cases, while none of the patients without previous history of pericoronitis developed postoperative infection (0%). All the cases of postoperative infection occurred in the group that did not receive antibiotics postoperatively. Some studies suggest that randomized controlled trials should be performed and a cause-and-effect relationship between a previous history of pericoronitis and more frequent postoperative infection after mandibular third molar surgery should be established, while others have shown that postoperative infection is statistically higher in patients with previous history of pericoronitis [22, 23].

We also observed three postoperative infections where *Staphylococcus aureus* was isolated from the sample; in all other cases we registered polymicrobial flora dominated by viridians streptococci. The results of microbiological analysis verified the results of clinical trials, because the microorganisms isolated from all swab samples were susceptible to moxifloxacin. All samples were susceptible to cefixime, except one case with coagulase-negative staphylococci. Also, in 50% of the samples taken, resistance to ampicillin, amoxicillin, and amoxicillin with clavulanic acid was registered, which is very concerning (Table 3). Microbiological analysis of samples obtained from odontogenic abscesses concluded that odontogenic infections are polymicrobial. Moxifloxacin had promising *in vitro* activity against odontogenic pathogens such as the viridans and hemolytic streptococci, *Strep. anginosus* and *Strep. mitis* group and *Neisseria spp.* [4]. Moxifloxacin was also effective against more than 90% of isolated strict anaerobes, predominantly *Prevotella spp.* [24]. Treatment of severe odontogenic infections comparing moxifloxacin, amoxicillin with clavulanic acid, and clindamycin, in the mixed aerobic-anaerobic bacterial flora, showed at least one of the pathogens was resistant to penicillin in 50% of the patients, and a rate of increase in resistance to clindamycin was also noticeable [4, 25]. Due to its high tissue penetration and concentration in bone tissue, moxifloxacin has shown promising results [18, 24]. Because of the broader activity and reduced dosing frequency (administration only once daily), the use of moxifloxacin instead of clindamycin in penicillin-allergic patients seems worth considering [4, 24].

Antibiotic prophylaxis with moxifloxacin and cefixime reduced the occurrence of postoperative inflammatory sequelae (pain, swelling, and trismus). It is interesting that both antibiotics, especially moxifloxacin, also contributed to reducing the incidence of postoperative dry socket, which is not provoked by inflammation [26].

Adequate antibiotic treatment comprises the administration of the appropriate antibiotic in optimal dose through the correct route of administration [27]. Nevertheless, antibiotics have been associated with considerable side effects, thus their use should be adequate and according to guidelines. Our finding that several patients in group P did not have postoperative infections confirms that antibiotics are not an absolute requirement in all surgical cases.

Side effects of fluoroquinolones include headaches, tendonitis, and transient neurological effects in elderly population [28]. In addition, some relatively benign side-effects are observed, such as nausea, vomiting, and diarrhea [29].

We did not include a group with amoxicillin (with or without clavulanic acid) due to a different dosing regimen, which makes double-blind design impossible, which is a relative limitation of the study. Another limitation of our study is a relatively small sample size, which should be considered when estimating the strength of data given here in. Finally, even though isolated microorganisms have demonstrated resistance to penicillin and its derivatives, we cannot claim with certainty that those species/strains have caused the infection, considering that all specimens contained polymicrobial flora.

Our study also indicates that antibiotics should not be prescribed generally in all cases. Overprescribing is fueling a global increase in bacterial resistance, which is becoming a major public health challenge around the world [7]. Considering the increase in antimicrobial resistance,

a growing awareness on the search for new antibacterial agents is essential, and the choice of moxifloxacin and cefixime to prevent postoperative infections after third molar surgery seems appropriate, not only because of the aforementioned benefits, but also because of the simplified dosing regimen, avoiding drug interactions in polymedicated patients (patients who use multiple medications), monotherapy options, and thus better compliance and adherence [30].

Ultimately, the decision to prescribe antibiotics, should be made on the case-by-case basis, considering the complexity of the surgical case as well as risks and benefits of antibiotic prophylaxis.

CONCLUSION

We found a cause-and-effect relationship between a previous history of pericoronitis and frequent postoperative infections after mandibular third molar surgery, while no infections were observed in patients without such history. This indicates that prior history of pericoronitis may be a decisive risk factor, and that prescribing prophylactic antibiotics may be unwarranted in cases where such a history is absent. Restraint and appropriate practices in antibiotics prescribing would be very helpful in limiting further spread of microbial resistance, and in maintaining efficacy of existing drugs. However, our study did not include the analysis of time elapsed between the last pericoronitis episode (in cases with history of pericoronitis) and date when surgery was performed, which may influence the relative risk of postoperative infection.

While strong recommendations require a more powerful study with a larger number of patients, our data provide evidence that in cases where prophylactic antibiotics are warranted, moxifloxacin and cefixime provide good protection against postoperative infection, and should be considered for use in that context.

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Примена антибиотика после хируршког вађења доњег трећег молара – корисна или штетна процедура? Рандомизована, двоструко слепа, плацебом контролисана студија

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САЖЕТАК

Увод/Циљ Циљ студије био је да се испита ефекат моксифлоксацина и цефиксима на сузбијање инфекције после хируршког вађења доњих трећих молара.

Метод Двоструко слепа студија спроведена је на 157 пацијената којима су хируршки уклоњени доњи трећи молари, а који су насумично распоређени у једну од три групе: моксифлоксацин (М), цефиксим (Ц) или плацебо (П). Пацијенти из сваке групе распоређени су у две подгрупе: подгрупу „а", без претходне историје перикоронитиса, и подгрупу „б", са претходном историјом перикоронитиса. Пацијенти су контролисани на постоперативним прегледима првог, другог и седмог постоперативног дана.

Резултати Постоперативна инфекција регистрована је искључиво код пацијената са претходном историјом перикоронитиса. Антибиотска профилакса цефиксимом или моксифлоксацином смањила је појаву постоперативне инфекције. Укупна инциденца постоперативних инфекција била је 6,4%. Сви случајеви постоперативне инфекције регистровани су у групи која није примала антибиотике већ плацебо, где је инциденца инфекција била 19,2%. Микробиолошке анализе потврдиле су клинички добијене резултате. Изолована микрофлора била је резистентна на деривате пеницилина у 50% случајева.

Закључак У склопу профилактичке примене антибиотика после хируршког вађења доњих трећих молара треба узети у обзир могуће ризике и добробите, а може се сматрати сврсисходном у случајевима са претходном историјом перикоронитиса, као и у случају компликованијих хируршких екстракција.

Кључне речи: хирургија трећих молара; антибиотска профилакса; постоперативне компликације; резистенција на лекове; тестови осетљивости микроорганизама

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Polymer characteristics and mechanical properties of bulk-fill, giomer, fiber-reinforced, and low-shrinkage composites

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SUMMARY

Introduction/Objective The objective was to determine the degree of conversion (DC), cross-link density, percentage of leachable monomers, flexural strength (FS), and hardness (HV) of nanohybrid, nanofilled bulk-fill, giomer, fiber-reinforced, and low-shrinkage composites.

Methods Standardized specimens (n = 5/group) of Tetric EvoCeram Bulk Fill, Filtek Bulk Fill, Beautifil, EverX posterior, Kalore, Filtek Z250 (microhybrid control), and Tetric EvoCeram (nanohybrid control) were subjected to micro-Raman spectroscopy, three-point bending, and HV. Cross-linking density and leachable monomers were ascertained based on the ratio of HV and DC before and after immersion in absolute ethanol.

Results DC was in the range 50.4–70.5%, the highest for Filtek Bulk and the lowest for Kalore. The highest %DC change was in Beautifil (10.3%) and the lowest in Filtek Bulk (1.4%) and Z250 (1.28%). FS ranged between 78.9 MPa (TEC) and 126.7 MPa (Filtek Bulk). HV ranged between 58.6 (Kalore) and 113.9 (Z250) and significantly decreased post-immersion (19–55%). HV48h inversely correlated to HV% loss (r = -0.761), whilst DC positively correlated with FS (r = 0.893).

Conclusion Filtek Bulk, EverX, and Z250 showed the highest DC. The lowest DC and mechanical properties were observed for Kalore. The greatest cross-link density was shown by Filtek Bulk. There were up to 10% of leachable monomers. DC and FS positively correlated.

Keywords: composite; conversion; cross-link density; flexural strength; hardness

INTRODUCTION

It has been widely adopted that dental composites are the materials of choice for most anterior and posterior restorations [1]. An intricate balance of primarily biomechanical, but also isolation, bonding, and aesthetic demands, has led to the development of different "subclasses" of composites recommended for direct posterior restorations.

Nanohybrid/nanofilled, microhybrid, and fiber-reinforced composites have all been indicated for posterior restorations for their improved mechanical properties, either based on increased filler load or the presence of short glass fibers [2, 3]. Giomer composites are nanohybrid, fluoride release and recharge composites, also in the sculptable bulk-fill group. Low-shrinkage composites, intended to overcome polymerization shrinkage as the major shortcoming of dental composites, employ high filler load or high-molecular weight cross-linking monomers as the main strategies [4]. Despite shrinkage that may be comparable to other composite types, lower shrinkage stress was reported for some low-shrinkage than for universal or flowable composites [5, 6, 7].

Bulk-fill composites combine some of the previous approaches with increased translucency or alternative photoinitiators for increased depth of cure and decreased the number of required increments in posterior cavities [8, 9, 10]. Bulk-fill composites are a variable group of materials with mechanical properties that are comparable, at best, to universal incremental composites [2]. Exception could be the fiber-reinforced bulk-fill composite everX posterior (GC International AG, Luzern, Switzerland; previously Xenius, StikTech) with consistently higher fracture toughness compared to universal, bulk-fill, and fiber-reinforced composites [3, 11, 12].

Degree of conversion (DC) is related to material composition, temperature, as well as curing conditions [13, 14, 15].

DC is measured directly as a ratio of C = C double bonds in cured and uncured materials using micro-Raman [16] or Fourier-transform infrared spectroscopy [13, 16, 17]. Hardness (HV) and flexural strength (FS) are arguably the most frequently tested mechanical properties of dental composites [18]. The percentage of hardness loss after chemical softening in ethanol has been used as an indication of cross-link density.

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The latter is important for hygroscopic and chemical stability and viscoelastic behavior of composites [19]. Higher degree of softening expressed as greater hardness loss has been related to lower cross-link density [20].

The aim of this study was to compare and correlate polymer characteristics – DC and cross-link density, and mechanical properties – HV and FS, of composites from different subclasses – nanohybrid and nanofilled bulk-fill, giomer, fiber-reinforced, low-shrinkage, and universal composites. The null hypotheses were as follows: (1) there are no statistically significant differences in the DC, cross-link density, FS, and HV between tested composites and (2) there is no correlation between polymer and mechanical characteristics of the tested composites.

METHODS

Table 1 presents details on materials used in the present study. For the DC, cross-link density, and HV, standardized plastic molds 5 mm in diameter and 2 mm or 4 mm deep were used to prepare composite specimens (n = 5/group). Uncured material was placed into each mold, covered with a glass slide, and pressed to extrude excess material. Light-curing was performed for 40 seconds using a monowave LED light-curing unit (LEdition, Ivoclar Vivadent AG, Schaan, Liechtenstein) at a standardized distance of 1 mm and an intensity of ~800 mW/cm^2 (Radiometer Model 100, Kerr Corp, Orange, CA, USA). The specimens were polished in wet conditions using SiC abrasive discs (600, 1000, and 2000 grit) and polishing cloth (TexMet, Buehler, Lake Bluff, IL, USA). For FS, square beam specimens were prepared in 2 × 2 × 25 mm molds as per the ISO standard 4049:2009. Light-curing was performed through a 1 mm thick glass slide in three exposures, 40 seconds each, whilst the final light exposure was performed by moving the light tip from one end of the specimen to the other over 40 seconds. Specimens were stored in dark, light-proof containers at 37°C for 24 hours prior to measurements.

The DC was determined using micro-Raman spectroscopy (XploRA, Raman spectrometer, Horiba Jobin Yvon) using the following parameters: laser 785 nm wavelength, spectrometer grating 1200 gr/mm, acquisition time 10 s, number of acquisitions 5, objective 50 ×. Uncured composite were used as reference materials to calculate the DC according to the following formula:

$$DC = \left(1 - \frac{Rcured}{Runcured}\right) \times 100$$

where Rcured and Runcured are the ratio of spectral peaks related to aliphatic (1640 cm^{-1}) and aromatic (1610 cm^{-1}) C = C bonds in cured and uncured material, respectively. Initial measurement was done after 24 hours of storage at three random points at the bottom, unexposed surface. The measurements were repeated using the same conditions after 48 hours of specimen storage in absolute ethanol.

FS was measured using a three-point bending test in a universal testing machine (Force Gauge PCE-FM200, PCE Instruments UK Ltd, Southampton, UK) at 1 mm/minute speed until fracture. FS was determined using the equation:

$$FS = \frac{3Fl}{2bh^2}$$

where F is the maximum load measured before fracture and l is the distance between supports (20 mm), b is sample width, and h is sample height.

HV measurements were done performed using Vickers indenter in a hardness tester (Buehler Indentament 1100 series, Buehler) at 100 g over 20 seconds. Hardness measurements were performed linearly in five or nine points in 2 mm or 4 mm thick specimens, respectively. Repeated measurements were done 24 hours and 48 hours of post-immersion in ethanol.

Table 1. Materials used in the present study

Materials	Manufacturer / Type / Increment thickness	Composition	Filler content
Tetric EvoCeram Bulk Fill (Code: TEC Bulk)	Ivoclar Vivadent/ nanohybrid bulk-fill / 4 mm	BisGMA, UDMA, BisEMA, Barium aluminium silicate glass fillers, prepolymer, ytterbium trifluoride and spherical mixed oxide	79.5wt% (62.5wt% filler and 19.7% prepolymer)
Filtek™ Bulk Fill Posterior Restorative (Filtek Bulk)	3M ESPE/ nanofilled bulk-fill / 4–5 mm	Aromatic UDMA, UDMA, DDDMA, 2,2-dimethyl-4-methylene- reaction products with glycidyl methacrylate, EDMAB, zirkonia / silica and YbF3 filler, titanium dioxide	76.5wt% 58.4vol%
Beautifil Bulk Restorative (Beautifil)	SHOFU INC./ giomer / 4 mm	Bis-GMA, UDMA, Bis-MPEPP, TEGDMA, S-PRG filler based on F-B-Al-silicate glass	83.3wt%
EverX Posterior (EverX)	GC EUROPE/ fiber-reinforced bulk-fill / 4 mm	Bis-GMA, TEGDMA, PMMA, SiO2, barium glass, glass fibers 1–2 mm length	76wt% 57vol%
Kalore (Kalore)	GC EUROPE/ Nanohybrid low-shrinkage / 2 mm	UDMA, BisEMA, BHT, dimethacrylate, DX-511 co-monomers, fluoroaluminosilicate glass, pre-polymerized filler, strontium glass, SiO2	82wt% 69vol%
Tetric EvoCeram (TEC)	Ivoclar Vivadent/ nanohybrid (control) / 2 mm	BisGMA, UDMA, BisEMA, barium glass filler, ytterbium trifluoride, mixed oxide, prepolymers	82.5wt% (48.5wt% filler and 34% prepolymer)
Filtek Z250 (Z250)	3M ESPE/ microhybrid (control) / 2 mm	BisGMA, TEGDMA, UDMA, BisEMA6, EDMAB, silane treated ceramic	78wt% 60vol%

Composition is based on manufacturers' technical data;
BisGMA – bisphenol-A-diglycidyl-dimethacrylate; UDMA – urethane dimethacrylate; BisEMA/BisMPEPP – ethoxylated bisphenol A dimethacrylate; DDDMA – 1,12-dodecane dimethycrylate; TEGDMA – triethyleneglycol dimethacrylate; Bis-MPEPP – bisphenol A polyethoxy methacrylat; PMMA – polymethylmethacrylate; BHT – butylated hydroxytoluene; EDMAB – ethyl 4-dimethyl aminobenzoate

Polymer cross-linking density was ascertained based on the ratio of hardness values before and after ethanol immersion. The percentage of leachable monomers was determined as the ratio of the DC before and after ethanol immersion.

Data for the DC was analyzed using general linear model for "material" and "time" factors with factor interaction included. In case of significant factor interaction, follow-up one-way analysis of variance (ANOVA) was performed for inter-material comparison at each time separately, whilst intra-material comparison of initial and 48-hour measurements was done using paired t-tests separately with the Bonferroni correction. HV and FS data were analyzed in Minitab 16 (Minitab Inc, State College, PA, USA) using one-way ANOVA with Tukey's post-hoc test. Pearson correlation and regression analyses were performed to assess the relationship between DC, cross-link density, HV and FS. The level of significance was set at 0.05.

The study was approved by the Ethics Committee of the University of Priština –Kosovska Mitrovica, Serbia.

RESULTS

Figure 1 presents DC values of the tested composites initially and after 48 h of ethanol immersion. General linear model for factors "composite" and "time" showed significant interaction ($p < 0.05$). The results of follow-up analyses are presented in Figure 1. Initially, the DC was in the range Filtek Bulk, EverX, Z250 > TEC Bulk, Beautifil, TEC > Kalore ($p < 0.05$). After immersion in ethanol, the DC was in the following order: Filtek Bulk ≥ EverX ≥ Beautifil, Z250, TEC Bulk, TEC > Kalore ($p < 0.05$). EverX, Beautifil, TEC Bulk, and TEC showed significantly higher DC after immersion than initially ($p < 0.05$), whilst no significant difference was found for Filtek Bulk, Kalore, and Z250 ($p > 0.05$). After 48 hours of ethanol storage, the %DC increase was significantly higher in Beautifil (10.3%) and TEC (8.5%), followed by TEC Bulk (4.7%) and EverX (3.5%) compared to initial DC ($p < 0.05$). The %DC change in Kalore (1.9%), Filtek Bulk (1.4%), and Z250 (1.28%) was not significant compared to initial values ($p > 0.05$), albeit Z250 showed slight decrease in DC.

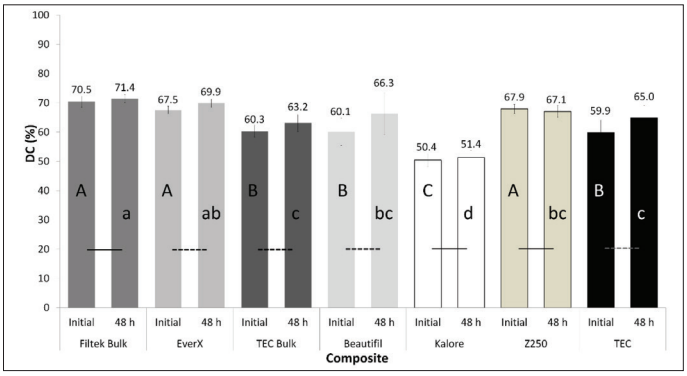

Figure 1. Degree of conversion of the tested composites initially and after 48 hours of ethanol immersion; columns represent mean and bars represent standard deviation values; different uppercase letters indicate statistically significant differences between composites initially; different lowercase letters indicate statistically significant differences between composites after immersion; dashed lines indicate statistically significant differences within each composite between different time intervals ($p < 0.05$); horizontal lines indicate no significant difference within each composite between different time intervals ($p > 0.05$)

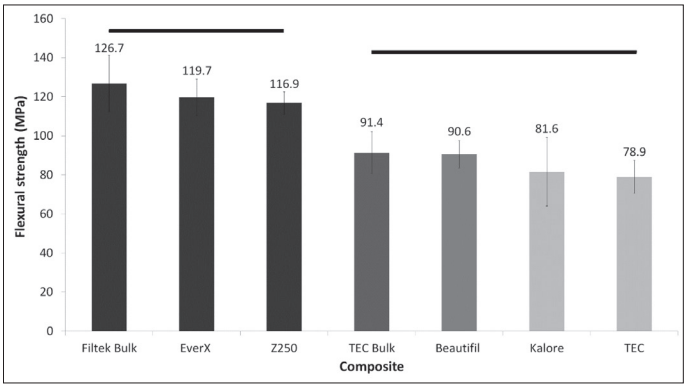

Figure 2. Flexural strength of the tested composites; columns represent mean and bars represent standard deviation values; groups connected with horizontal lines are not significantly different ($p > 0.05$)

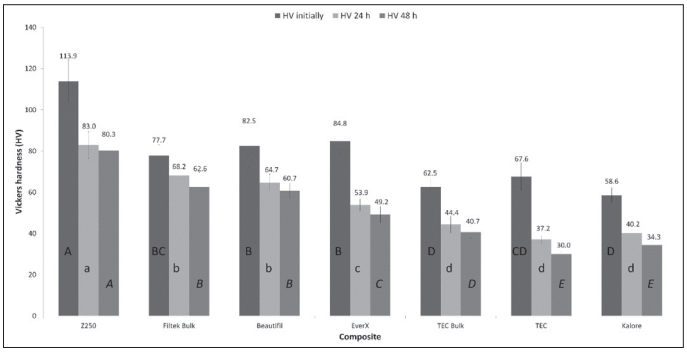

Figure 3. Vickers hardness values (mean and standard deviation) for the tested composites initially and after 24 hours and 48 hours post-immersion in ethanol; groups with different uppercase, lowercase, or italic letters are significantly different for each of the time periods ($p < 0.05$)

Filtek Bulk and EverX showed significantly higher FS than other composites, whilst the lowest values were measured for TEC ($p < 0.05$). Comparing bulk-fill composites, Filtek Bulk, and Everx had higher FS than TEC Bulk and Beautifil ($p < 0.05$) (Figure 2).

Figure 3 presents HV data for different time intervals. Z250 showed the highest HV at all time periods. Filtek Bulk and Beautifil showed consistently similar HV ($p > 0.05$), but lower than that of Z250. TEC Bulk, TEC, and Kalore exhibited lower HV than other composites ($p < 0.05$).

HV of all tested composites significantly decreased after ethanol immersion, ranging 55–19% HV loss (Figure 4). TEC showed significantly higher %HV loss (55.3%) than other materials ($p < 0.05$), followed by EverX (41.8%), Kalore (41.3%), and TEC Bulk (34.7%), whilst this decrease was the lowest in Z250 (29%), Beautifil (25.6%), and Filtek Bulk (18.8%) ($p > 0.05$).

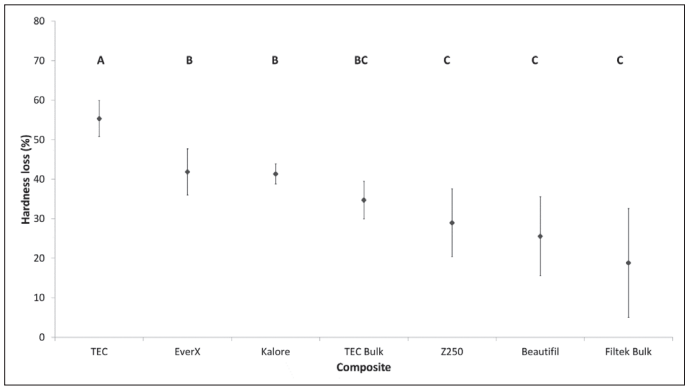

Figure 4. Percentage of hardness loss following ethanol immersion test; groups with different uppercase letters are significantly different ($p < 0.05$)

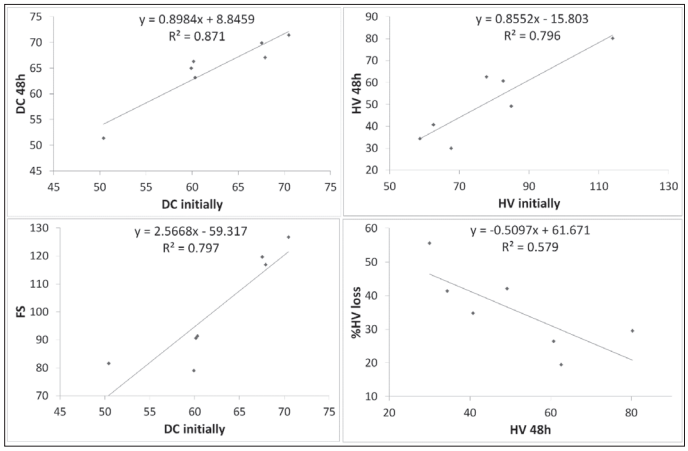

Figure 5. Regression analysis showing the relationship between tested properties with regression equations and R^2 values;
DC – degree of conversion; FS – flexural strength; HV – hardness

Figure 5 presents the results of regression analysis. Pearson's correlation showed significant positive correlation between DC initially and DC after storage as well as between HV initially and HV after storage, with Pearson's correlation coefficients r = 0.933 (p = 0.002) for DC and r = 0.892 (p = 0.007) for HV. HV after ethanol immersion was a better predictor of HV% loss than initial HV, as significant negative correlation was found between HV_{48h} and HV% loss (r = -0.761, p = 0.047) but not between $HV_{initial}$ and HV% loss (r = -0.389, p = 0.388). Significant positive correlation was found between DC and FS (r = 0.893, p = 0.007). No correlation was found between DC and HV (DC *vs.* HV initially: r = 0.669 and p = 0.100; DC *vs.* HV after storage: r = 0.545 and p = 0.206) or between FS and HV (r = 0.636, p = 0.124).

DISCUSSION

Both tested hypotheses were rejected. Tested composites showed significant differences in the DC, cross-link density, FS, and HV. Positive correlation was established between DC and FS as well as between DC and HV initially and after storage, whilst HV_{48h} and HV% loss were negatively correlated.

Sculptable nanofilled and nanohybrid bulk-fill composites – Filtek Bulk and TEC Bulk – showed similar DC to their universal counterparts – Z250 and TEC. Differences were notable between both bulk-fill as well as between universal composites. This is the case with TEC and TEC Bulk in that they have similar resin composition, prepolymer, and filler content. TEC Bulk contains Ivocerin, a benzoyl-germanium-based additional photoinitiator, a type I initiator that shows α-cleavage under formation of benzoyl and germyl radicals with no need for an amine co-initiator [8]. Ivocerin was shown to absorb light energy around 410–420 nm, unlike a similar alternative photoinitiator Lucirin TPO, making Ivocerin compatible with monowave light-curing units [8, 9]. Similar DC of TEC Bulk at full increment depth of 4 mm to TEC may be explained by increased translucency of TEC Bulk compared to TEC, allowing deeper light penetration [21]. Similarly, DC at full increment depth of TEC Bulk and TEC was seen in recent studies in different experimental setups, albeit with lower values measured [17, 21].

The present finding of higher DC of Filtek Bulk than TEC Bulk was reported previously for different curing conditions [21]. Resin composition of Filtek Bulk is considerably different from TEC Bulk and is based on a high-molecular-weight aromatic dimethacrylate monomer, addition-fragmentation monomer, UDMA, and 1,12-dodecanediol dimethacrylate, as per manufacturer's technical data. The main purpose of these monomers is the control of shrinkage and shrinkage stress of Filtek Bulk. High DC of Filtek Bulk could be related to an increased number of reactive sites and cleavage of an addition-fragmentation monomer to fragments during polymerization which may further react with other reactive sites of the developing polymer. In Z250, performing similarly to Filtek Bulk, low reactivity of BisGMA was likely compensated with TEGDMA, UDMA, and BisEMA, allowing a DC in the range of 65–70% of this universal composite similar to Filtek Bulk. A similar DC of Z250 was recently reported for a high-intensity polywave light-curing unit (~1100 mW/cm^2) and shorter curing time (20 seconds) as opposed to the presently used monowave unit (~800 mW/cm^2) and longer curing time (40 seconds), probably indicating that this is a maximum DC reachable for this material [21].

Kalore, a low-shrinkage composite based on a high-molecular-weight monomer DX-511, showed the lowest DC in the present study – around 50%. Previous studies show inconsistent results in terms of DC, with one study reporting a DC close to 45% [7]. Despite differences in experimental conditions, in terms of light-curing units, irradiance, and curing time, the material was cured according to the recommended curing times for a particular light-curing unit in all these instances. Inconsistent results indicate factors still unknown, affecting the polymerization behavior of this composite. An increase in the DC after 48 hours of storage in absolute ethanol reveals the percentage of uncured

monomers and potentially leachable small oligomeric species, trapped within the polymer network. This approach can potentially lead to elution of small oligomeric species, thus mimicking the exact percentage of uncured monomers.

Ethanol is a potent organic solvent that allows polymer softening, swelling and elution of uncured monomers. In this study, absolute ethanol was used to extract all leachable unreacted monomers to further assess the quality of polymer networks. Up to about 10% of uncured monomers were detected from the tested materials. The highest percentage was found for giomer Beautifil and the lowest for low-shrinkage Kalore and sculptable bulk-fill Filtek Bulk. Taking into consideration the DC and %DC change, the present results suggest that around 30–35% of unreacted C = C double bonds remain in the form of pendant groups within the polymer. This relatively high percentage of unreacted C = C bonds remain due to the reduced mobility of unreacted monomers and pendant groups during diffusion-controlled propagation until the reaction stops due to polymer vitrification [14].

High values for mechanical properties – FS and HV, were not related to a specific subclass of composites, as nanofilled bulk-fill, fiber-reinforced, and universal composite were the three materials with the highest FS and HV. Further, giomer Beautifil showed similar HV to Filtek Bulk and EverX. The discrepancy could be due to differences in the range of selected materials for testing, further confirming the heterogeneity of the bulk-fill subclass. A more consistent pattern was found between the present results for the low-shrinkage Kalore and universal composites Z250 and TEC and previously reported data [18], showing that Z250 performed better whilst Kalore and TEC showed inferior results in terms of mechanical properties in both experimental setups. Mixed performance by Kalore in terms of comparable or inferior mechanical characteristics and shrinkage and lower shrinkage stress than universal or flowable composites indicates that composites from other subclasses should be preferred by clinicians over Kalore [5, 6].

The present data regarding %HV loss after ethanol softening ranged largely between the tested composites, between around 19% and 55%. This points to considerable differences in cross-link density. Absolute ethanol was used in the present study as ethanol concentration was shown to affect the outcome of softening analysis with 75% ethanol/water solution not being able to expose the differences in cross-link density of composites [20].

Filtek Bulk has shown the least %HV loss suggesting the greatest cross-link density whilst TEC showed the opposite result. Increased cross-link density of Filtek Bulk may be associated primarily with addition-fragmentation monomer and its increased ability to react with reactive sites in the growing polymer. Also, the position of aromatic groups in the high molecular weight dimethacrylate monomers in Filtek Bulk could result in differences in cross-linkage compared to BisGMA and BisEMA, the main monomers with aromatic groups in TEC.

EverX has also shown considerable %HV loss indicating lower cross-link density compared to Filtek Bulk. This could be due to the presence of PMMA, which is known to be a linear polymer. Previously, higher %HV loss for EverX (~29%) than for Filtek Bulk (~19%) was reported after 75% ethanol/water immersion [12]. In the present study, virtually the same %HV loss was detected for Filtek Bulk after storage in absolute ethanol (~19%) whilst greater %HV loss was found for EverX (~42%) compared to the previous study [12]. This further highlights the difference in ethanol concentration and the need for maximum concentration for cross-link density analysis.

Correlation analysis between filler content and mechanical properties was not done due to inconsistent data between manufacturer's technical data and literature reports [2, 19]. The filler content was not measured in the present study due to the lack of equipment. Multiple correlations using various data from the literature and manufacturers data revealed no significant differences between filler content and HV/FS. This could be due to the fact that different types of fillers (glass, prepolymerized, S-PRG glass, silica, zirconia, glass fibers) are present in the tested materials.

Positive correlation was established between the DC and FS but not between the DC and HV as was the case in previous studies [9, 21]. For the given range of composites tested in the present study, the DC was a better indicator of FS than of HV. A recent study showed that filler volume rather than filler weight percentage is a better indicator of elastic modulus and solvent sorption illustrating the complex relationship between material composition and properties [2]. HV after ethanol immersion is a good indicator of %HV loss, albeit in an inverse relationship – the higher the HV post-immersion, the lower the HV% loss and, thus, greater composite cross-link density. A positive correlation between the DC initially and DC after ethanol immersion suggests consistent presence of uncured monomers within the polymer. HV initially and HV after ethanol immersion showed consistent behavior for the tested composites and were linearly correlated. This indicates that the variation in cross-link density does not significantly affect HV of dental composites.

CONCLUSION

Based on the results of this study, the following can be concluded.

The DC of bulk-fill composites was comparable or higher than universal or low-shrinkage composites. Up to 10% of unreacted, leachable monomers and oligomeric species containing unreacted methacrylate groups were detectable within the polymer network of the tested composites.

Nanohybrid, nanofilled, fiber-reinforced, and giomer bulk-fill composites showed comparable or higher HV than nanohybrid TEC, but lower than microhybrid Z250.

The greatest cross-link density, as indicated indirectly by changes in HV after alcohol softening, was shown by the nanofilled bulk-fill composite Filtek Bulk, whilst the low-shrinkage Kalore and fiber-reinforced composite EverX showed the lowest cross-link density.

Conflict of interest: None declared.

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Карактеристике полимера и механичке карактеристике *bulk-fill*, гиомера, влакном ојачаних и нискоконтракционих композита

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САЖЕТАК

Увод/Циљ Циљ овог рада је испитати степен конверзије, густину полимерне мреже, проценат излужених мономера, савојну чврстоћу и тврдоћу нанохибридних *bulk-fill*, гиомера, влакном ојачаних и нискоконтракционих композита.

Методе Стандардизовани узорци (*n* = 5/ група) *Tetric EvoCeram Bulk Fill*, *Filtek Bulk Fill*, *Beautifil*, *EverX posterior*, *Kalore*, *Filtek Z*250 (микрохибридна контрола) и *Tetric EvoCeram* (нанохибридна контрола) испитивани су применом микрораманске спектроскопије, тестом савијања у три тачке и анализом тврдоће по Викерсу. Густина полимерне мреже и излуженост мономера добијени су на основу односа тврдоће и степена конверзије пре и после потапања узорака у апсолутни етанол.

Резултати Вредности степена конверзије биле су у опсегу 50,4–70,5%, највеће за *Filtek Bulk* и најмање за *Kalore*. Највећа промена процента степена конверзије добијена је за *Beautifil* (10,3%), а најмања за *Filtek Bulk* (1,4%) и *Z*250 (1,28%). Вредности савојне чврстоће биле су између 78,9 *MPa* (*TEC*) и 126,7 *MPa* (*Filtek Bulk*). Вредности тврдоће кретале су се од 58,6 (*Kalore*) до 113,9 (*Z*250) са значајним смањењем после потапања (19–55%). *HV*48*h* је показао обрнуту корелацију са процентом смањења тврдоће (r = -0,761), док је степен конверзије имао позитивну корелацију са савојном чврстоћом (r = 0,893).

Закључак *Filtek Bulk*, *EverX* и *Z*250 показали су највеће вредности степена конверзије. Најмање вредности степена конверзије и механичких карактеристика уочене су за *Kalore*. Највећа промена густине полимерне мреже уочена је код композита *Filtek Bulk*. Излуживање мономера било је и до 10%. Вредности степена конверзије и савојне чврстоће показују позитивну корелацију.

Кључне речи: композит; конверзија; густина полимерне мреже; савојна чврстоћа; тврдоћа

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Rheumatoid arthritis and spondyloarthritis prevalence in four European countries – a comparative study

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SUMMARY

Introduction/Objective The objective was to compare rheumatoid arthritis (RA), spondyloarthritis (SpA) and subtypes of SpA prevalence in four European countries.

Methods A 33-items detection questionnaire, containing self-reported diagnosis, classification criteria for RA and SpA, personal and family history, was translated using cross-cultural adaptation and validated in France, Turkey, Lithuania and Serbia, where it was used on a population sample. Suspected cases were evaluated and confirmed by a rheumatologist. Prevalence estimates were age- and sex-standardized to European standard population.

Results In total, 33,454 people older than 18 years were screened and 31,454 interviewed: France 14,671, Lithuania 6,558, Serbia 6,213, Turkey 4,012. Standardized RA prevalence varied from 0.29% (95% CI: 0.17–0.40) in France to 0.57% (0.31–0.84) in Turkey; this inequality was mostly caused by differences in women prevalence (from 0.42% in France to 1.02% in Turkey) SpA prevalence was similar in France (0.30%), Serbia (0.35%) and Turkey (0.37%), but in Lithuania it was 0.89%, which could be caused by geographic and genetic differences, as SpA prevalence was higher in North and East Europe, as well as the human leukocyte antigen B27 presence. SpA prevalence was equally presented by gender for France and Serbia. Regarding SpA subtypes, ankylosing spondylitis prevalence varied from 0.07–0.30% (Serbia–Lithuania), PsA 0.10–0.26% (France–Lithuania), reactive arthritis was 0.09–0.18% (Serbia–Lithuania). Previously non-diagnosed SpA cases were found in 6.9% in France, 25.9% in Lithuania and 31.2% in Serbia.

Conclusion East–West decreasing tendency for the female RA prevalence was noted. SpA was higher in North-Eastern Europe than in its Western and Southern part. One quarter of the SpA patients in Lithuania and one third in Serbia were not previously diagnosed. The SpA population prevalence was higher than expected and similar to RA.

Keywords: prevalence; rheumatoid arthritis; spondyloarthritis; ankylosing spondylitis; psoriatic arthritis; reactive arthritis

INTRODUCTION

In different parts of Europe, rheumatoid arthritis (RA) and spondyloarthritis (SpA) prevalence was estimated variously [1, 2].

In time, prevalence estimates have shown tendency to decrease for RA and to increase for SpA which, in addition to new achievements in imaging, could be attributed to various approaches in studies. For example, the American College of Rheumatology (ACR) 1987 classification criteria for RA do not cover undefined, possible or probable RA cases, like it was before with Rome and American Rheumatism Association (ARA) criteria [1, 3, 4]; in addition, the last ACR 2010 criteria show better sensitivity, though it does not require radiography [5, 6]. The overtime tendency of the SpA prevalence to increase could be attributed to campaigns for better understanding of the SpA concept and for better identification of the SpA patients [7, 8].

Furthermore, the heterogeneity of the RA and SpA prevalence could be influenced by diverse methodological research methods, including different sampling, various criteria for case detection and case confirmation, different cut-offs for age, incoherent presentation of results (raw or standardized), etc. [1, 2, 7].

The aim of this manuscript was to compare RA, SpA and subtypes of the SpA prevalence in four European countries: France, Turkey, Lithuania, and Serbia. Results were derived by unique study method, comprising random

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sampling, identical cases detection method by using a 33-items detection questionnaire, confirmation of diagnoses by rheumatologist and standardization of results to a common-European population. Separate results by countries have already been presented, standardized to the particular country population.

METHODS

Prevalence estimates for RA, SpA and subtypes of SpA [ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis (ReA), enteropathic arthritis (EA), and undifferentiated SpA] were achieved by a two-phase study: in a first-detection phase, screening questionnaire was used by trained interviewers or self-help patient groups. In a second-confirmation phase, suspected cases were confirmed by rheumatologist. Study was announced by local press, radio and other means of public advertisement before launching, in order to assure response.

Study was approved by the European League Against Rheumatism (EULAR) Standing Committee of Epidemiology and Health Service Research (SCEHSR). It was also approved by the local Ethical Committee in Lithuania and Serbia, while there was no requirement for an Institutional Review Board authorization for this kind of observational study in Turkey and France at that time. Patients have provided informed consent for participation in a telephone or face-to-face interview, according to the country.

Questionnaire

Screening detection questionnaire was first developed and validated in France [9]. It included self-reported diagnosis, signs and symptoms attributable to RA and SpA according to ACR 1987 classification criteria for RA and the European Spondylarthropathy Study Group 1991 criteria for SpA, personal and family history.

Following guidelines for forward and back translation, the questionnaire was translated and tested separately for Serbia, Lithuania, and Turkey with four groups of patients: RA, SpA, degenerative musculoskeletal disorders and healthy persons, showing acceptable sensitivity and specificity [10, 11, 12].

The questionnaire comprised both past and current symptoms of the investigated disease. In addition to the self-reported diagnosis, if a respondent gave a positive answer to one of the two main questions: "Are you at present experiencing, or have you in the past experienced pain in your joints?" or "Have you or have you had pain in your neck, your back in your buttocks?", the whole questionnaire was used.

Sample

The questionnaire was applied either by telephone, or face-to-face.

Telephone numbers for dialing were recruited randomly from the local landline telephone list, by dividing a total number of phones in that area by designed number of participants: in Serbia every 100th telephone number [10], in Lithuania every 50th [11], in France random selection of telephone numbers was combined by the next birthday household member (selection of persons whose birthday is closest to the interview date). Phones owned by business organizations, public offices, social associations or institutions for elderly were excluded before dialing, as well as the second home numbers (for residents living in the place less than one year), to reduce redundancy. Due to low landline telephone coverage in Turkey, random selection of homes in each of the studied areas and provinces was done in order to apply the screening Questionnaire face-to-face [12].

For France, sample has covered seven areas with 20 counties (Bretagne-Ouest, Bretagne-Est, Nord-Picardie, Midi-Pyrénées, Provence-Côte d'Azur, Lorraine, Rhônes-Alpes), for Turkey seven geographical regions with 25 administrative provinces (regions: Western (Aegean), Northwestern (Marmaran), Southern (Mediterranean), Northern (Black Sea), Central, Eastern and Southeastern), for Lithuania two largest cities (Vilnius and Kaunas) and for Serbia two geographical regions with four towns (Belgrade in the north region and Čačak, Užice, and Kruševac in the south).

Sampling area was chosen either on the basis of a widespread coverage of the population distribution, or by selection of random sample within regions representative of the average population.

Case ascertainment: detection and confirmation

From 30 to 110 volunteer interviewers (patient representatives, trained practitioners, nurses and self-help group members) were engaged in each country in a detection phase. Interviewed persons had to be older than 18 years and resident at the place for at least one year. The survey took place from March to May 2001 in France [13], from September to October 2004 in Lithuania [11] and from April to October 2008 in Serbia [1]. In Turkey, face-to-face interview was done from August 2004 to June 2005 [12]. People who gave answers suggestive for RA or SpA or positive self-reported diagnosis were called again by rheumatologists who asked for additional information about diagnosis and medical history. If diagnosis could not be ruled out, clinical visit was scheduled and diagnoses were approved in a second-confirmation phase.

Statistical analysis

The sample size was calculated on the basis of the expected prevalence of 0.3–0.5%, by using the Poisson distribution assumption [13]. Accordingly, 4000 people contacted by phone would provide a 95% confidence interval (CI) of 0.14–0.54% around a 0.3% estimate, and 0.30–0.77% around a 0.5% estimate.

According to the expected prevalence of RA and SpA in the community of 0.5%, 1% and 2%, the negative predictive value of the Questionnaire: 0.99 for RA and 0.99 for SpA gave us a strong confidence that we would do accurate classification if we declared someone not a case [9].

Age and sex standardization was done by direct method to European standard population, defined as EU-27+European Free Trade Association average populations, based on 2010 estimates [14].

RESULTS

Detection and confirmation

In a detection phase 33,454 persons were screened. After exclusion of second homes, work places and public enterprise numbers, 27,442 people were interviewed by telephone: 14,671 in France, 6,558 in Lithuania and 6,213 in Serbia (response rate 64.7%, 64.7% and 63.3%, respectively) [1, 11, 13]. In Turkey, 4,012 people were interviewed face-to-face (response rate 96,6%) [12] (Figure 1).

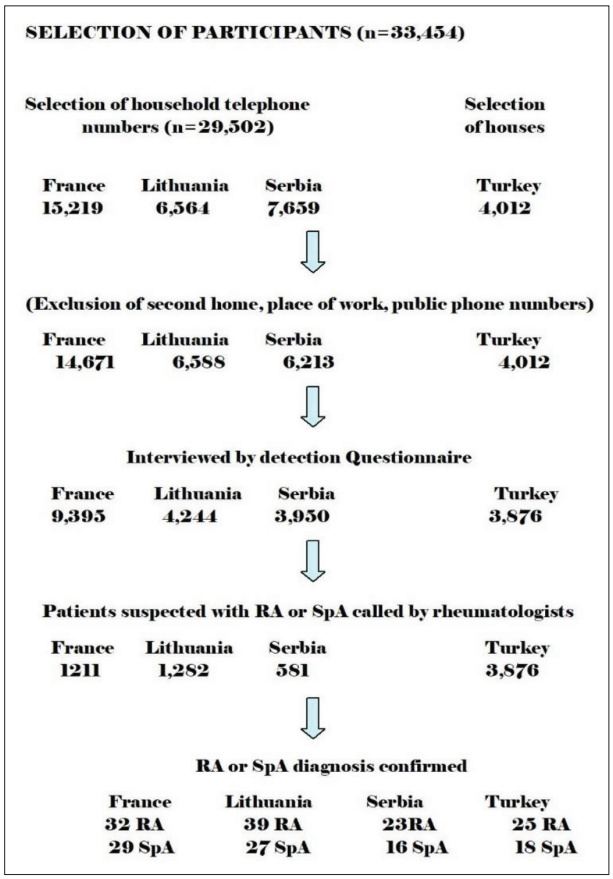

Figure 1. Sampling, case detection and case confirmation

RA was confirmed with 32 cases in France (two newly diagnosed), 39 in Lithuania (two newly diagnosed), 23 in Serbia (one newly diagnosed) and 25 in Turkey, comprising females in 84%, 100%, 82.6%, and 92% of cases, respectively [1, 11, 12, 13]. SpA was confirmed with 29 people in France (two newly diagnosed), 27 in Lithuania (seven newly diagnosed), 16 in Serbia (five newly diagnosed) and 18 in Turkey (representing males in 37.9%, 55.6%, 37.5%, and 16.7% of cases, respectively) [8, 11, 12, 15]. The age and sex distribution of samples and cases is given in Table 1.

Standardized RA prevalence estimates in four European countries are shown in Figure 2. RA prevalence for men was similar across countries (0.00–0.19%), but it has differed widely for women: from 1.02% (95% CI: 0.59–1.45) in Turkey and 1.01% (95% CI: 0.68–1.34) in Lithuania to 0.42% (0.26–0.58) in France and 0.52% (0.27–0.76) in Serbia. Considering SpA, higher prevalence was shown in North-Eastern Europe: Lithuania 0.89% (0.78–1) than in its Middle, Western and Southern part: France 0.30% (0.19–0.41), Serbia 0.35% (0.17–0.54) and Turkey 0.37% (0.18–0.56) (Figure 3).

AS prevalence was 0.10% (95% CI: 0.05–0.16) in France 0.12% (0.02–0.22) for males and 0.09% (0.02–0.16) for females; 0.30% (0.27–0.34) in Lithuania, that is 0.29% (0.04–0.60) for males and 0.32% (0.13–0.50) for females, and 0.07% (0.01–0.14) in Serbia, or 0.15% (0–0.36) for males.

PsA prevalence was 0.10% (0.04–0.16) in France, for men 0.09% (0–0.18) and for women 0.11% (0.03–0.19); in Lithuania it was 0.26% (0.2–0.32), for men 0.38% (0.04–0.71) and for women 0.14% (0.01–0.27); in Serbia it was 0.08% (0–0.15), e.g., 0.04% (0–0.11) for men and 0.11% (0.01–0.22) for women.

Prevalence estimates for ReA were 0.18 (0.07–0.29) in Lithuania, 0.15 (0.13–0.17) for males and 0.20 (0.18–0.23) for females and 0.09 (0–0.16) in Serbia, 0.12 (0–0.28) for males and 0.07 (0–0.17) for females.

EA prevalence was 0.02 (0–0.06) in Serbia, e.g., 0.04 (0–0.11) in women.

Undifferentiated SpA population prevalence was 0.04% (0–0.07) in France, e.g., 0.03% (0–0.08) for males and 0.04% (0.01–0.08) for females; it was 0.12% (0.09–0.16)

Table 1. Rheumatoid arthritis or spondyloarthritis, sample and cases for France, Lithuania, Serbia and Turkey

Age groups	FRANCE						LITHUANIA						SERBIA						TURKEY					
	Sample		RA cases		SpA cases		Sample		RA cases		SpA cases		Sample		RA cases		SpA cases		Sample		RA cases		SpA cases	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
18–24	330	447	0	0	1	1	144	236	0	1	0	0	76	95	0	0	0	0	238	347	0	1	0	2
25–34	684	960	0	1	2	4	152	356	0	1	1	2	188	281	0	0	2	1	355	546	0	5	1	6
35–44	749	1051	0	1	3	2	190	533	0	2	5	3	195	371	0	2	0	1	341	463	1	5	1	4
45–54	641	935	0	7	2	4	190	542	0	8	3	3	223	373	0	1	0	1	273	374	0	5	1	2
55–64	409	829	2	6	1	2	184	585	0	11	4	3	317	653	1	8	3	6	198	254	1	4	0	1
65–74	488	897	2	12	2	4	152	592	0	15	1	1	226	479	2	7	0	1	132	209	0	3	0	0
75–84	213	560	1	0	0	1	62	291	0	1	1	0	141	264	1	1	1	0	69	77	0	0	0	0
85 +	40	162	0	0	0	0	9	26	0	0	0	0	27	41	0	0	0	0	0	0	0	0	0	0

RA – rheumatoid arthritis; SpA – spondyloarthritis

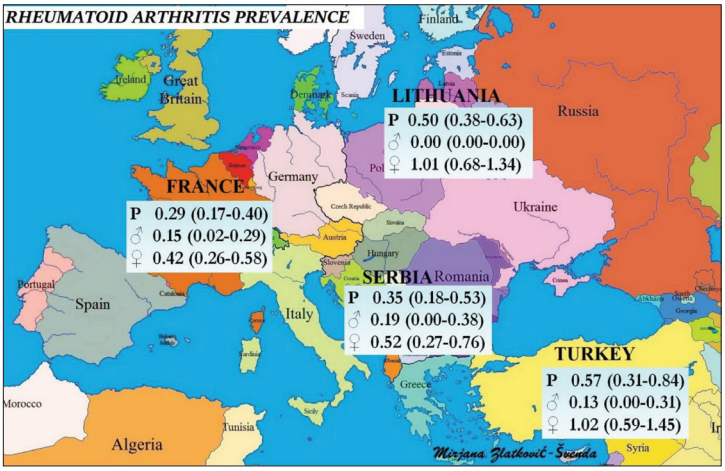

Figure 2. Rheumatoid arthritis prevalence, % (95% confidence interval) for France, Lithuania, Turkey, and Serbia for older than 18 years
P – population; ♂ – male; ♀ – female

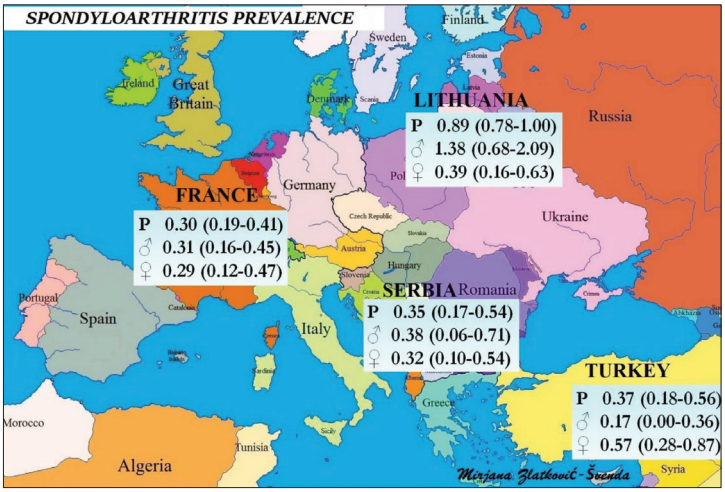

Figure 3. Spondyloarthritis prevalence estimates, % (95% confidence intervals) for France, Lithuania, Turkey, and Serbia for older than 18 years
P – population; ♂ – male; ♀ – female

in Lithuania, 0.22% (0–0.72) for males and 0.03% (0–0.08) for females and 0.02% (0–0.02) in Serbia, 0.13% (0–0.31) for males and 0.04% (0–0.09) for females.

DISCUSSION

The presented joint study effort, endorsed by EULAR, is a first investigation of the RA and SpA prevalence that was convoyed nationwide in countries located in different parts of Europe.

Sample was chosen within representative regions of population by random selection, and therefore the selection bias was considered minimized. The 33-items detection questionnaire comprised self-reported diagnosis, classification criteria for RA and SpA, personal and family history. Owing to main questions that covered both present and past symptoms, the questionnaire was able to include patients with active disease and those in remission, thus, allowing a full prevalence calculation. Diagnoses were confirmed by rheumatologists.

Up to date, RA and SpA prevalence were differently identified: by registry data, face-to-face or sent-by-post questionnaires or by elaborating database of blood donors. Diagnosis was confirmed either by using actual classification criteria or by doctor. Samples were derived from one or more geographic regions, representing the whole population or part of it, by using different age cut-offs, or by exploring one race or ethnicity group only [1, 2].

Since the age and sex mix of the populations is not uniform across countries, standardization of prevalence to a common population was needed; it also allows comparisons and monitoring of the disease progression over time.

Our study RA prevalence varied from 0.29% (95% CI: 0.17–0.40) in France to 0.57% (0.31–0.84) in Turkey (Figure 2). Up to date reported prevalence in Europe goes from 0.18% in Serbia [16] to 0.82% in Spain, 0.90% in Poland, and 0.8–1.1% in the United Kingdom [6, 4, 17].

RA prevalence in the present research decreases when going to the west and the lowest rate was found in far west of the France: Nord (Lille 0.13%) and Bretagne (Brest 0.14%), which is also the lowest rate of the European RA prevalence estimates ever reported [18]. First results of the East Europe RA prevalence were derived in Lithuania – 0.50%, which is in range with North Europe and Turkey [1].

RA mostly affects women and the female/male ratio of our study was 2.7:1 in Serbia, 2.8:1 in France and 7.8:1 in Turkey. High female RA prevalence in Turkey was already published [1, 12], but high Lithuanian RA prevalence in females was not noted before. Virtual absence of men with RA in Lithuania was like due to fluctuation in sample size, i.e., the men prevalence was so small that it could not be caught by the statistically determined sample size, based on the expected overall RA prevalence. This is not an uncommon case in epidemiology, as such lack of men in the RA prevalence was already reported by studies from Africa – Nigeria and Johannesburg, and in Australian Aboriginals, as well as in Thailand and Japan [1, 19].

According to our results, the East–West decreasing tendency of the European female RA prevalence was noted. The last meta-analysis estimation of the global RA prevalence, based on a systematic review, has shown that variations were mostly due to geographical locations and study limitations such as bias assessment and sample size [20]. As we have used identical methodology with the statistically determined sample size, in accordance to this meta-analysis, high women RA prevalence in the East Europe could be attributed to geographic longitude (e.g., less sun exposure due to low insolation in Lithuania, or due to national habits, like covering the face and the body in Turkey), and genetics. The present study SpA prevalence varies from 0.30% (95% CI: 0.19–0.41) in France to 0.89% (0.78–1) in

Lithuania. Published European SpA prevalence ranges from 0.21% in Scottish Highlands [21] to 1.6% in Portugal and 1.9% in Germany [22]. The last one was limited by methodology, as it has evaluated blood donors who were HLA-B27 positive in half of the cases. Stolwijk et al. [23] have given the pooled prevalence of the SpA in Europe of 0.54% (95% CI: 0.36–0.78). Generally speaking, SpA prevalence is lower in northern parts of Europe (0.21% in Scotland [19], 0.45% in Sweden [24] and 0.30% in France [25]) than the southern – 1.06% in Italy [26] and 1.05–1.35% in Turkey [27].

We have found no gender-specific SpA predominance, and our SpA male/female ratio was 1:1.6; 1:5; 1.25:1; and 1:1.7 for France, Turkey, Lithuania, and Serbia, respectively. AS was equally presented for males and females in France and Lithuania, PsA in France, ReA in Lithuania and Serbia, undifferentiated SpA in France.

Khan [28] has hypothesized about the prevalence of SpA, built upon the HLA-B27 prevalence. As HLA-B27 was found to be more prevalent in Ugro-Finnic, Slavic and Northern European populations (Norway, Sweden, Iceland) (7–16%) compared to the Western (6–9%) or Southern Europe (2–6%) and Turkey (2.8–11.1%), SpA would be higher in Northern and Eastern European countries than in its Western and Southern parts, which was confirmed here. The highest SpA prevalence rate in the present study was found in Lithuania (0.89%), followed by France, Serbia, and Turkey (0.29–0.39%). Turkish male SpA prevalence was surprisingly low: 0.17%. A previous regional SpA prevalence study in Izmir has shown female predominance as well (1.22% *vs.* 0.88% males) with SpA prevalence rate of 1.05% and 1.35% (0.5% for radiographic axial SpA and 0.8% for non-radiographic axial SpA) [27]. Maybe this prevalence difference could be attributed to heterogeneity of HLA-B27 frequency in different parts of Turkey.

Male SpA prevalence in Lithuania of 1.38% is the highest ever recorded, except for Portugal where it was reported to be 2.7%, but calculated only for men older than 50 years [21].

Different environment, living or eating habits, ethnicity or various genetic backgrounds could have the influence.

SpA prevalence derived from registers and hospital records could be underestimated, as we have revealed previously non-identified cases with SpA in 6.9% for France, 25.9% for Lithuania, and 31.2% for Serbia. SpA underestimation could be caused by the lack of familiarity of symptoms by patients or by doctors, wrongly considering patients as having degenerative disease, by inability of the health system to capture SpA patients, inadequate rheumatology coverage of certain areas or simply because of the complicated process of scheduling the examination.

Here, similar estimates of SpA and RA prevalence were found in France (0.30% *vs.* 0.29%), Serbia (0.35% *vs.* 0.35%) and Turkey 0.37% (95% CI: 0.18–0.56) *vs.* 0.57% (95% CI: 0.31–0.84). For Lithuania, SpA prevalence was even higher than the RA (0.89% *vs.* 0.50%).

The SpA prevalence estimates in the world are constantly growing, and in the current century have even exceeded that for RA in Japan, Germany, Turkey, China, Italy, Australian Aboriginals, and the United States [1, 7].

The strength of our study is the use of a unique survey method. For each of the observed countries study sample was population-based, therefore it is representative. The limitation is a wide study period across countries. However, data were collected in less than a decade (i.e., when there was little chance to observe a significant secular trend), and a systematic literature review has suggested little evidence for a substantial change in the RA frequency over the years [29]. Not exploring the HLA-B27 in SpA could be considered another study limitation, as confirmation of cases in our study was based on the European Spondylarthropathy Study Group criteria, which do not include HLA-B27. However, recent studies have indicated lower prevalence of non-radiographic axial SpA than previously reported [30].

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Преваленција реуматоидног артритиса и спондилоартритиса у четири европске земље – упоредна студија

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САЖЕТАК

Увод/Циљ Циљ је био упоредити преваленцију реуматоидног артритиса (РА), спондилоартритиса (СпА) и подтипова спондилоартритиса у четири европске земље.

Методе Упитник за откривање са укупно 33 питања, укључујући дијагнозу добијену од болесника, класификационе критеријуме за РА и СпА, личну и породичну анамнезу, преведен је уз транскултурну адаптацију и валидиран у Француској, Турској, Литванији и Србији, где је коришћен на случајно одабраном узорку популације. Сумњиви случајеви су евалуирани и потврђени од стране реуматолога. Резултати преваленције су стандардизовани према старости и полу у односу на популацију Европе.

Резултати Скрининговано је укупно 33.454 особа старијих од 18 година, а анкетирано 31.454: Француска 14.671, Литванија 6558, Србија 6213, Турска 4012. Стандардизована преваленција РА креће се од 0,29% (95% *CI*: 0,17–0,40) у Француској до 0,57% (0,31–0,84) у Турској; неједнакост је углавном узрокована разликама у преваленцији жена (од 0,42% у Француској до 1,02% у Турској). Преваленција СпА је слична у Француској (0,30%), Србији (0,35%) и Турској (0,37%), али је у Литванији 0,89%, што може бити узроковано географским и генетским разликама, пошто је преваленција СпА виша у северној и источној Европи, као и присуство хуманог леукоцитног антигена *B*27. Преваленција СпА има подједнак распоред по полу у Француској и Србији. У односу на подтипове СпА, преваленција анкилозирајућег спондилитиса варира од 0,07 до 0,30% (Србија–Литванија), ПсА 0,10–0,26% (Француска–Литванија), реактивни артритис је 0,09–0,18% (Србија–Литванија). Раније недијагностиковани оболели од СпА чине 6,9% у Француској, 25,9% у Литванији и 31,2% у Србији.

Закључак Уочена је тенденција опадања преваленције РА код жена источно–западно. Преваленција СпА је била виша у северноисточној Европи него у њеном западном и јужном делу. Четвртина болесника са СпА у Литванији и трећина у Србији није била претходно дијагностикована. Преваленција СпА у популацији виша је него што је очекивано и у најмању руку је слична као РА.

Кључне речи: преваленција; реуматоидни артритис; спондилоартритис; анкилозирајући спондилитис; псоријазни артритис; реактивни артритис

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Open surgical conversion and management of patients with ruptured abdominal aortic aneurysm after previous endovascular aneurysm repair

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SUMMARY

Introduction/Objective The objective was to present the results and technical considerations from high-volume center when performing late open surgical conversion (LOSC) after endovascular aneurysm repair (EVAR) in ruptured abdominal aortic aneurysm (RAAA) patients.

Methods This was a single center retrospective study. LOSC was performed whenever eventual endovascular reintervention failed, was not feasible due to hostile anatomy and unavailability of specific endograft materials, or when patient was hemodynamically unstable necessitating emergent surgery.

Results All previously implanted EVARs had bimodular configuration with suprarenal fixation. Total endograft explantation was performed in 40% of patients. Hospital mortality was 20%. Both patients who died had total endograft explantation with supraceliac clamp lasting more than 30 minutes. 30-day mortality was 30%, with one more patient who died from pulmonary embolism after hospital discharge and two hospital deaths were due to myocardial infarction.

Conclusion LOSC due to RAAA after previous EVAR carries greater mortality for the patient, suggesting multifactorial impacts on the outcome. The appropriate choice of surgical method and technical success are of ultimate importance, with total graft explantation having negative impact on patient's survival.

Keywords: ruptured abdominal aortic aneurysm (RAAA); endovascular aneurysm repair (EVAR); late open surgical conversion (LOSC)

INTRODUCTION

Most of the stent-graft failures are managed with secondary endovascular techniques (aortic or iliac endograft extensions, embolization, and endograft relining). However, late open surgical conversion (LOSC) may sometimes be the only available option to repair a failing endovascular aneurysm repair (EVAR) [1, 2].

The incidence of ruptured abdominal aortic aneurysm (RAAA) after EVAR is low, and estimated to be 0.9% [3]. Nowadays, the incidence may be even higher than previously reported, because of the follow-up delays during COVID-19 pandemic.

In the previous multicentric study we already reported that morbidity and mortality rates for LOSC after EVAR are generally higher than in standard open elective or semi-elective circumstances [4, 5]. Currently, insufficient data are available in the latest European Society for Vascular and Endovascular Surgery guidelines on the Management of Abdominal Aorto-iliac Aneurysms to recommend a particular strategy when performing LOSC in RAAA setting, and surgeon's preference still plays a major role [6].

That is why the objective of this paper is to present the results and technical considerations from high-volume center when performing LOSC after EVAR in RAAA patients, that might help vascular surgeons when dealing with this challenging condition.

METHODS

This was a single center retrospective study. A total of 236 elective EVARs were performed between January 2010 and January 2020. Ten patients were operated due to ruptured aneurysm following EVAR, however in five patients previous EVAR was performed in other hospitals. LOSC was performed whenever eventual endovascular reintervention failed, was not feasible due to hostile anatomy and unavailability of specific endograft materials, or when a patient was hemodynamically unstable needing urgent surgery.

Computerized tomography angiography was performed in all patients to determine the extent and anatomy of the RAAA. All procedures were performed in a fully equipped operating room including intraoperative cell saving system, under the general anesthesia. All patients were treated by experienced vascular surgeons proficient at both open and endovascular surgery.

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Following data were collected and analyzed: demographics (age and sex), baseline clinical characteristics (presence of hypertension, diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease, chronic renal insufficiency, hostile abdomen, time from EVAR to LOSC), endovascular reinterventions before LOSC (type of stent-graft, previous endovascular attempt to correct the culprit lesion, indication for LOSC), operative data (surgical approach, site of aortic cross-clamping (ACC), type of reconstruction, total blood loss, number of allogenous blood transfusion), as well as postoperative data such as complications, in-hospital and 30-day mortality.

To assess for normal distributions, we used Shapiro–Wilk test. All results were expressed as arithmetic mean (X) ± standard deviation for normally distributed variables and as median and lower and upper interquartile range for non-normally distributed variables. Categorical variables were presented as absolute and relative frequencies.

Informed consent for the procedure was obtained from all conscious patients. In those with distracted consciousness or intubated prior to admission due to aortic rupture, consent for surgery was obtained from family members. The study was approved by the institutional committee on ethics and was conducted according to the principals of the Helsinki Declaration.

RESULTS

The mean patient's age was 76 ± 6.86 years, the majority were males (87.5%), had coronary artery disease (62.5%), 50% of them had chronic renal failure and 25% had chronic obstructive pulmonary disease. All previously implanted EVARs had bimodular configuration with suprarenal fixation (five Endurant® and three Zenith®). The most common culprit for the development of RAAA was type Ia endoleak (50%) and the mean interval from initial EVAR until rupture was 48 ± 24.43 months. Previous endovascular attempt to correct the underlying endoleak was attempted in 40% of patients (Table 1).

Median laparotomy was performed in all patients. For proximal bleeding control supraceliac aortic clamp was applied in all patients. Total endograft explantation was performed in four (40%) patients. An aortobiiliac bypass was performed in the majority of patients (80%), with mean proximal clamp duration of 29.1 ± 7.9 min, mean total operative time of 179 ± 63 minutes and mean blood loss of 3417 ± 992 milliliters.

Hospital mortality was 20%. Both patients who died had total endograft explantation with supraceliac clamp lasting more than 30 minutes. Two patients developed transmural colon ischemia needing colectomy, while one had additional surgical bleeding requiring reintervention. 30-day mortality was 30%, with one patient who died from pulmonary embolism after hospital discharge, while two in-hospital deaths were due to myocardial infarction. From four patients who underwent total endograft explantation, two died (50%).

When comparing patients with total and partial graft explantation (Table 2), there was no significant major cardiovascular risk profile difference. The proximal clamping time as well as total operation duration seemed to be longer in patients who had total graft explantation implying the overall increased complexity when whole stent graft is explanted. Both myocardial infarctions occurred in total explantation group which led to the fatal outcome.

Table 1. Baseline data, indications, type of repair and postoperative outcomes

Baseline characteristics		Intraoperative variable	
Age	76.2 ± 6.05	Median laparotomy	10 (100%)
Male	9 (90%)	Total endograft explantation	4 (40%)
Hypertension	10 (100%)	Type of reconstruction	
Diabetes mellitus	5 (50%)	Graft interposition	2 (20%)
Coronary artery disease*	6 (60%)	All bypass	8 (80%)
COPD	3 (30%)	Proximal supraceliac clamp	100 (100%)
Renal failure	6 (60%)	Proximal clamp duration (min)	29.1 ± 7.9
Hostile abdomen**	2 (20%)	Total operative time	179 ± 63
Interval from EVAR to LOSC (months)	48 ± 24.43	Blood loss (ml)	3417 ± 992
EVAR*-related data		Postoperative outcome	
Type of endograft		Hospital mortality	2 (20%)
Endurant®	7 (70%)	30-day mortality	3 (30%)
Zenith®	3 (30%)	Surgical bleeding	1 (10%)
Suprarenal fixation	10 (100%)	Wound infection	1 (10%)
Previous endovascular reintervention	4 (40%)	Dialysis	1 (10%)
Indication for LOSC		Colon ischaemia	2 (20%)
Type Ia endoleak	6 (60%)	Acute limb ischaemia	1 (10%)
Type Ib endoleak	1 (10%)	Acute coronary syndrome	2 (20%)
Type III endoleak	2 (20%)	Stroke	0 (0%)
Stent-graft migration	1 (10%)	Prolonged ventilation (more than 48h)	3 (30%)

COPD – chronic obstructive pulmonary disease; EVAR – endovascular abdominal aortic aneurysm repair; LOSC – late open surgical conversion; AII – aortobiiliac bypass; *coronary artery disease was defined as presence of angina pectoris or previous myocardial infarction, percutaneous coronary intervention or coronary artery bypass; **hostile abdomen was defined as previous major abdominal surgery or radiation

Table 2. Baseline data, indications, type of repair and postoperative outcomes for patients who had total and partial stent-graft explantation

Parameters	Patients with total stent-graft explantation				Patients with partial stent-graft explantation					
Baseline characteristics	Patient No I	Patient No II	Patient No III	Patient No IV	Patient No V	Patient No VI	Patient No VII	Patient No VIII	Patient No IX	Patient No X
Age	66	81	67	80	73	82	77	79	83	74
Coronary artery disease*	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No
COPD	No	Yes	No	No	No	No	No	No	Yes	Yes
Renal failure	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes
Hostile abdomen**	No	Yes	No	No	No	No	Yes	No	No	No
Preoperative data										
Interval from EVAR to LOSC (months)	13	96	38	23	29	48	72	60	54	47
Type of endograft	Endurant®	Endurant®	Zenith®	Zenith®	Endurant®	Endurant®	Endurant®	Endurant®	Endurant®	Endurant®
Indication for LOSC	Stent-graft migration	Type Ia endoleak	Type III endoleak	Type Ia endoleak	Type Ia endoleak	Type Ib endoleak	Type III endoleak	Type Ia endoleak	Type Ia endoleak	Type Ia endoleak
Operative data										
Proximal clamp duration (min)	46	35	31	32	30	21	19	23	26	23
Type of reconstruction	All bypass	All bypass	Graft interposition	All bypass	All bypass	All bypass	All bypass	All bypass	Graft interposition	All bypass
Total operative time	300	180	210	240	210	170	90	150	110	135
Blood loss (ml)	5200	3200	2700	3300	4300	3000	2400	3200	2800	1600
Postoperative outcomes										
Colon ischaemia	Yes	No	No	No	No	No	Yes	No	No	No
Acute coronary syndrome	Yes	Yes	No	No	No	No	No	No	No	No
Prolonged ventilation (more than 48h)	Yes	No	No	Yes	No	No	No	No	Yes	No
Hospital mortality	Yes	Yes	No	No	No	No	No	No	No	No

COPD – chronic obstructive pulmonary disease; EVAR – endovascular abdominal aortic aneurysm repair; LOSC – late open surgical conversion; All – aortobiiliac bypass; *coronary artery disease was defined as presence of angina pectoris or previous myocardial infarction, percutaneous coronary intervention or coronary artery bypass; **Hostile abdomen was defined as previous major abdominal surgery or radiation

DISCUSSION

Although reported late, RAAA rate after EVAR is low, with the progressive expansion of EVAR, especially to more complex anatomies, frequently outside manufacturer's instructions for use, the need for LOSC is likely to increase, especially during COVID-19 pandemic [1, 7]. In only four of our patients previous endovascular reintervention was attempted, suggesting that better compliance with surveillance protocols would have resulted in elective endovascular or open surgical correction of EVARs.

In our institution, EVAR is mainly performed in patients of advanced age with significant comorbidities making them unfit for open repair. [8] Performing open reintervention that often exceeds the extent of hypothetic primary open repair is an extreme challenge. Although some reports suggest that these patients are less hemodynamically unstable than primary ruptures, our experience is different [1]. All our patients were in severe state of hemorrhagic shock on admission.

In a meta-analytical population of 791 patients treated with LOSC (617 elective and 174 urgent procedures), those treated in an urgent setting had a 10 times higher risk for mortality, suggesting that among various indications for urgent conversion, type I/II endoleak and infection, rupture possibly contributes to a larger extent to this event [4]. This underscore not only the necessity of close surveillance but also the importance of timely LOSC to approach the ideal scenario of avoiding non-elective LOSC because EVAR does not provide significant survival advantage when the aneurysm ruptures [9]. In a single-center cohort evaluating LOSC in the urgent setting, with high proportion of ruptured cases (57%), Perini et al. [10, 11] presented similar results, i.e., the mortality rate was 33%.

Three important technical elements of LOSC are: surgical approach, the level of proximal ACC and the procedure with stent-graft [5]. We advocate transperitoneal approach through midline laparotomy, since this provides adequate proximal and distal bleeding control, and enables fast cardiopulmonary reanimation if necessary. However, this approach could be challenging in patients with hostile abdomen that often-dictates the indication for initial endovascular procedure (two of our patients). In all our RAAA patients regardless of rupture etiology, we use liberal approach to supraceliac ACC. Especially in cases with previous EVAR and suprarenal stent fixation, we think that supraceliac ACC is mandatory. In this manner, the lesion of the metallic skeleton (including perforation) and the dissection through hematoma is avoided. This lowers the chance of retroperitoneal organ lesion, and in case of

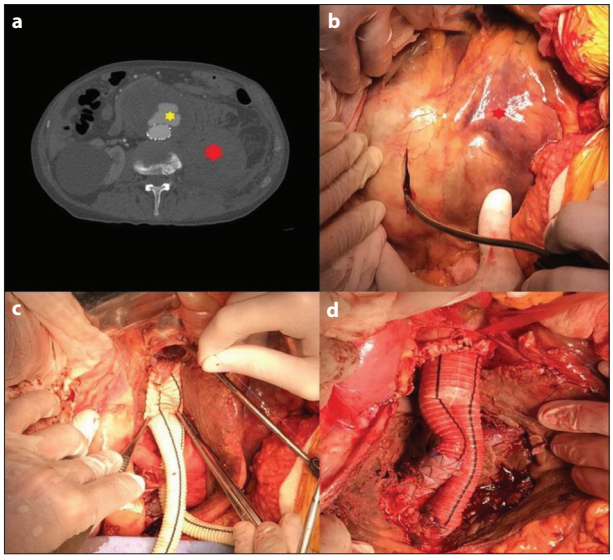

Figure 1. A patient undergoing partial stent-graft explantation: (a) preoperative computed tomography scan where yellow asterix shows type Ia endoleak and the red one retroperitoneal hematoma; (b) intraoperative photo at the moment of aneurysm sac opening after the supracoeliac clamp has been positioned, with red asterix the retroperitoneal hematoma is marked; (c) proximal anastomosis creation using "Bonvini" neo-neck technique; (d) bifurcated bypass presentation at the end of the procedure

hemodynamic instability it provides quick and efficient rise of blood pressure.

The final step is procedure with stent-graft, which includes its complete or partial removal. No clear recommendations exist regarding stent-graft management during open conversion (i.e., complete stent-graft removal *vs.* partial preservation), which is a controversial subject. Our opinion is that total endograft explantation should be avoided in order to perform the simplest reconstruction, especially when there is suprarenal fixation and absence of infection. Total explantation was associated with longer proximal ACC time, more blood loss and more extensive reconstruction (both patients died). Therefore, attempts should be made to partially remove the stent-graft whenever possible, and to perform a proximal suture, as the "neo-neck" technique (Figure 1) [12]. The proximal segment of new anastomosis between preserved stent graft and new graft should include three layers: stent graft, teflon pledgets and aneurysmal wall.

CONCLUSION

Emergent LOSC due to RAAA after previous EVAR carries greater mortality for the patient, suggesting multifactorial impacts on the outcome. This underlines the importance of surveillance after EVAR in order to avoid non-elective LOSC. The appropriate choice of surgical method and technical success are of ultimate importance, with total graft explantation having negative impact on patient's survival.

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Отворена хируршка конверзија и лечење болесника са руптуром анеуризме абдоминалне аорте и претходним ендоваскуларним третманом

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САЖЕТАК

Увод/Циљ Циљ рада је да се прикажу резултати и технички аспекти третмана са отвореном конверзијом после ендоваскуларног третмана код болесника са руптуром анеуризме абдоминалне аорте.

Методе Ово је била уницентрична ретроспективна студија. Отворена конверзија је рађена код претходно неуспелог ендоваскуларног третмана, када он није био могућ због хостилне анатомије и недоступности ендографтова, или када је болесник био толико хемодинамски нестабилан да је захтевао ургентну хирургију.

Резултати Сви претходни ендоваскуларни третмани анеуризме абдоминалне аорте су имали бимодуларну конфигурацију са супрареналном фиксацијом. Тотална експлантација ендографта рађена је код 40% болесника. Хоспитални морталитет је био 30%. Оба болесника која су преминула имала су тоталну експлантацију ендографта са супрацелијачним клемовањем које је трајало дуже од 30 минута. Тридесетодневни морталитет је био 30%, од чега је један болесник преминуо од плућне емболије после отпуста из болнице, а два смртна случаја у болници била су због акутног инфаркта миокарда.

Закључак Отворена конверзија због руптуре анеуризме абдоминалне аорте после претходног ендоваскуларног третмана носи велики морталитет за болеснике. Избор адекватне хируршке методе и технички успех су од највеће важности, при чему потпуна експлантација негативно утиче на преживљавање болесника.

Кључне речи: руптурирана анеуризма абдоминалне аорте; ендоваскуларни третман анеурзиме абдоминалне аорте; отворена конверзија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Influence of comorbidity on postoperative course and mortality in patients with hip fracture

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SUMMARY

Introduction/Objective Epidemiological research shows that we have a dramatic increase in the number of people with hip fractures, especially those over 65 years of age.
The objectives of this study are to assess the association between preoperative comorbidity and the risk of postoperative complications and mortality and postoperative worsening of comorbid conditions and their relationship to mortality within one year of hip fracture surgery.

Methods In this retrospective study, from January 2018 until January 2020, 64 patients with hip fractures were operated on at the Department of Orthopedic Surgery in Kosovska Mitrovica. We monitored the number of comorbidities and their significance on the preoperative risk and the course of concomitant diseases in the postoperative period and one-year mortality after surgery, in patients with hip fractures.

Results We collected data on patients from the moment of admission to discharge from the hospital accompanied by medical histories, and after discharge after follow-up examinations, six months and one year from discharge. Of the total number of subjects, 23 (35.9%) had one or two comorbidities, most often of cardiac and neurological nature, in 25 patients (39.1%) we had three concomitant diseases, and in 11 (17.2%) four and more comorbidities. The mean age of the patients was 72.51 years (69–92 years).

Conclusion Approximately 45–60% of men and women who suffer a hip fracture have three or more comorbid states. In older people with hip fractures, the presence of three or more comorbidities is the strongest preoperative risk factor.

Keywords: hip fracture; comorbidity; elderly; mortality

INTRODUCTION

Hip fractures present one of the biggest medical, social, and financial problems in the world, especially in the developed countries of the West. About 20% of all hospitalized orthopedic-traumatological patients are patients with hip fractures [1, 2, 3]. The number of hip fractures increases exponentially with age [2, 4]. It is a well-known fact that hip fractures, as a rule, lead to the worsening of existing chronic diseases from which the injured suffer, and which require the full engagement of doctors of other specialties. As one of the complications of osteoporosis, both due to its high incidence and due to the associated morbidity and mortality, hip fractures represent a significant problem in health, social, economic, and family terms [1, 5]. The basic precondition for the elderly to achieve "optimal physiological condition" to be able to perform the planned operation is the cooperative teamwork of orthopedists, anesthesiologists, and internists [3, 6]. All hip fractures are divided into: intracapsular (femoral neck fractures) and extracapsular (intertrochanteric and subtrochanteric fractures). The method of choice for patients with hip fractures is surgical treatment. In intra-articular fractures, hemiarthroplasty or total arthroplasty is used, and in extracapsular fractures, open repositioning and internal fixation of fractures are used [3, 4, 7]. Non-operative treatment is applied only in patients in whom the general condition is so bad that the risk of surgery is greater than the advantage of early fixation. Hip fractures have been considered one of the leading causes of death in the elderly population [1, 4]. McBride et al. [8] have shown that the state of mobility of the elderly and a small number of concomitant chronic diseases are very reliable prognostic indicators of the outcome of treatment of patients with hip fractures, while the type of fracture, type of implant, and age are not. In their study, Sterling et al. [6] show that survival in patients with chronic diseases is very low. He proved that the length of postoperative survival of patients after hip surgery is related to their activities in everyday life before fracture [5, 6]. Psychiatric diseases/delirium, depression and hip fractures are very common in elderly patients, so the outcome has a very poor prognosis [5, 9]. This study aimed to show the relationship between the health condition that the patient had before the hip fracture and the risk of postoperative complications and possibly mortality within one year of surgery. The impact of comorbidity and

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poor physical status on the development of postoperative complications and on mortality leads to the idea that the health condition reported by the patient at admission may be critical to predict the postoperative course. We were particularly interested in assessing the general status of patients with hip fractures immediately after injury, and monitoring comorbidities one year after surgery.

METHODS

The retrospective study included operated patients with hip fractures. The inclusion criteria were age over 65 years, and fracture of the proximal end of the femur. Patients with simultaneous bilateral fractures, periprosthetic and pathological fractures, patients younger than 65 years, and those who were not treated with operative methods were not included in the study. We started the preoperative preparation at the admission department, when, in addition to routine diagnostic procedures, we placed great emphasis on a well-taken anamnesis, whether the patient was physically active before the fracture and whether they had other concomitant diseases and which. A plan of consultative examinations was made, primarily for internist-cardiologists and anesthesiologists. We used a questionnaire that contained general data about the patient: sex, age, previous illnesses, and operations. The second part of the questionnaire referred to the level of physical activity before the fracture (IV levels), the mechanism of injury, the type of fracture, the existence of associated injuries, data on comorbidities. Appropriate therapy was administered at the ward. We performed complete medical, sanitary and psychological preparation for each patient. Each patient was informed of the treatment plan. In our paper, we used the Carlson comorbidity index (CCI). Depending on the type of variables and the normality of the distribution, the data description is shown as n (%), arithmetic mean ± standard deviation, or median (range, min–max). Among the methods for testing statistical hypotheses, the following were used: t-test, Mann–Whitney test, χ^2 test, and Fisher's test of exact probability. Logistic regression was used to analyze the relationship between binary outcomes and potential predictors. Statistical hypotheses were tested at the level of statistical significance (alpha level) of 0.05. All data were processed in the IBM SPSS Statistics 22 (IBM Corp., SPSS Statistics for Windows, Armonk, NY, USA) software package.

Committee for Ethical Topics of Health Center in Kosovska Mitrovica has approved the research before the beginning of this study.

RESULTS

The examined sample represents elderly patients who suffered a hip fracture. The study included 64 patients: 26 males (40.6%) and 38 females (59.4%). The youngest patient was 69 years old and the oldest is 92. There were 29 patients (45%) over the age of 80. The average age was 72.5 years. Many world studies classify sex as a very important factor influencing mortality after hip fractures.

In our study, long-term mortality was more common in females (70.8%) compared to males 29.2% (Table 1). Older age is one of the main risk factors for mortality in patients with hip fractures. People over the age of 85 have a high absolute mortality rate, especially when it comes to short-term mortality. The reasons are biological in nature, such as age, and certainly a higher number of comorbid conditions in the elderly. It has been proven that extracapsular fractures occur more often in the elderly than femoral neck fractures, but also that the median survival time was higher by about 10 months in patients with intertrochanteric fractures than for patients with intracapsular hip fractures.

Table 1. Distribution of hip fractures in relation to sex

Comorbidity			Yes	No	Total
Sex	male	Count	19	7	26
		% Comorbidity	47.5	29.2	40.6
	female	Count	21	17	38
		% Comorbidity	52.5	70.8	59.4
Total		Count	40	24	64
		% Comorbidity	100	100	100

In our paper, age, the type of fracture, as well as the mechanism of injury, were not statistically significant (Table 2). The strongest preoperative risk factor for the development of postoperative complications and mortality is the presence of comorbidities in persons with hip fractures. Less than 25% of elderly patients do not have a chronic disease (high blood pressure, anemia, ischemic heart disease, chronic obstructive pulmonary disease, metabolic diseases – diabetes and thyroid disease, dementia). Most patients have several concomitant diseases.

In our study, cardiovascular diseases were the most common comorbidities in 39 patients (60.9%), followed by anemia in 36 patients (56.3%), and respiratory diseases in 32.8% of subjects. However, we did not obtain statistical significance for any single disease (Table 3). Out of the total number, 42 patients had one or two so-called moderate concomitant diseases, while 22 patients had more severe comorbidities. In 11 subjects we had four or more

Table 2. Age in patients with hip fracture

/	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	0.071	0.791	-2.065	62	0.043	-2.600	1.259	-5.117	-0.083
Equal variances not assumed	/	/	-2.040	46.765	0.047	-2.600	1.274	-5.164	-0.036

F – test statistics for ANOVA; Sig. – p-value in output SPSS Statistics; t – test statistic for t-test; df – number of free degrees

Table 3. Prevalence of individual comorbidities in patients with hip fracture

Comorbidity n (%)	Total	Mortality	Living	p
Anemia	36 (56.3)	19 (79.2)	17 (42.5)	0.004
Cardiovascular disease	39 (60.9)	17 (70.8)	22 (55)	0.209
Respiratory disease	21 (32.8)	11 (45.8)	10 (25)	0.086
Neurological	20 (31.3)	7 (29.2)	13 (32.5)	0.781
Psychiatric	16 (25)	8 (33.3)	8 (20)	0.233
Endocrine system	18 (28.1)	8 (33.3)	10 (25)	0.473
Gastrointestinal tract	4 (6.3)	0 (0)	4 (10)	0.288
Urinary tract	9 (14.1)	3 (12.5)	6 (15)	1.000

comorbidities. Four patients with severe comorbidities who also developed surgical complications (two deep infections and one cut-out complication) died at the hospital. During the first postoperative year, another 16 patients with severe comorbidities and four patients with moderate comorbidities died. Comorbidity was monitored based on the CCI. We opted for CCI, primarily because CCI in a large number of studies has produced a predicted probability of mortality with a small degree of variation (Table 4).

Table 4. Presence of multiple comorbidities in subjects with hip fracture

Comorbidity			Mortality.ALL		Total
			No	Yes	
Number of comorbidities	0	Count	5	0	5
		% Within Mortality.ALL	12.5	0	7.8
	1	Count	2	0	2
		% Within Mortality.ALL	5	0	3.1
	2	Count	16	5	21
		% Within Mortality.ALL	40	20.8	32.8
	3	Count	12	13	25
		% Within Mortality.ALL	30	54.2	39.1
	4	Count	5	6	11
		% Within Mortality.ALL	12.5	25	17.2
Total		Count	40	24	64
		% Within Mortality.ALL	100%	100%	100%

Table 5. Overview of the significance of worsening of concomitant diseases on one-year mortality after surgical treatment of hip fractures

Independent variable	B	p	OR	95% confidence interval	
				Lower limit	Upper limit
Age	0.013	0.870	1.01	0.86	1.19
Existence of surgical complications during the operation	1.840	0.096	6.30	0.72	55.09
Worsening of comorbidity after surgery	4.272	**< 0.001**	**71.67**	7.82	657.06

B – gradient coefficient in the regression mode; p – value; OR – odds ratio

About 15–30% of bedridden patients with a hip fracture had serious complications during the acute phase of the fracture. The main medical complications of these fractures are pain, anemia, respiratory and cardiovascular complications (pneumonia, respiratory infections, myocardial infarction, stroke), urinary tract infections, delirium, decubitus ulcers, and therefore – from the moment of hospitalization of these patients should begin thromboembolic prophylaxis with complete diagnosis by treating different comorbidities. In the multivariant logistic regression model, a statistically significant predictor of death within one year of surgery was worsening of comorbidity after surgery (B = 4.272; p < 0.001), whose odds ratio was OR = 71.67. This shows that subjects with worsening comorbidities after surgery have over 70 times a higher chance of death, with control of all other factors in the model (Table 5).

DISCUSSION

A large number of papers have been published in the world literature which try to find the factors that influence the prognosis of hip fractures, both in the physical recovery of the patient and in their survival [1, 3, 7, 9]. Knowing the predictors of mortality is very useful because of the treatment plan for each patient individually, and in that way, the risk of death would be reduced [4, 6, 8]. Factors affecting mortality after hip fracture are: age, sex, poor mobility of the patient before fracture, poor mental status, dementia or cognitive impairment, diabetes, heart disease, cancer, comorbidities, higher ASA score, type of fracture, operative delay [5, 10]. The mortality rate during a hospital stay (intrahospital mortality) for patients with hip fracture in persons older than 70 years is 2–20% [7, 10, 11]. It usually ranges 5–7%, although in some studies it is significantly higher and is most often associated with the effects of acute trauma on the patient, length of hospital stay in the postoperative period, worsening of existing comorbidities, and possible surgical postoperative complications [4, 5, 11]. In our study, intrahospital-short-term mortality occurred in four patients and amounted to 6.25%. Mortality after discharge from the hospital – post-hospital mortality, can be determined at three, six, 12 months after discharge and later. The highest mortality rate is in the first three months after the fracture because patients in this period have to overcome physical and mental trauma caused by the fracture, which imposes functional and mental limitations associated with accompanying diseases that are characteristic of this life period [5, 9, 12]. Mortality in the first year after fracture is long-term mortality and it ranges around 25–30% [9, 13, 14]. However, most patients do not regain their previous functional results even after the first year of surgery and are not independent to perform basic life tasks, so they need the help of another person or require accommodation in special rehabilitation centers. It is estimated that only one-fifth of patients who walked independently before the fracture do so six months after surgery [5, 6, 15]. Age in patients with hip fractures is one of the main predictors of mortality. People with hip fractures older than 85 have an absolutely high mortality rate [4, 7, 11]. The mortality rate increases exponentially with age, while in persons under 75 it is about 7% per year, in persons over 85 it is about 33% in the first postoperative year [7, 16]. Life expectancy is estimated to be reduced by

up to seven years in people over 80 after a hip fracture. Many studies indicate that the mortality rate after hip fractures is higher in males and that hip fractures are more common in women. Men have a higher mortality rate especially when it comes to long-term mortality after five years [4, 6, 14]. This difference in sex has no specific explanation. It is considered that the causes of the sex difference are that in men we have more bad habits (alcohol consumption, smoking), and then a higher number of comorbidities compared to females of the same age [14, 17]. In our study, mortality was more common in females at 70.8% compared to males at 29.2%. Patient mobility before hip fracture (walking distance, ability to go shopping, use of walking aids) plays a very important role in predicting mortality in the first year after surgery. Research has shown that mobility in itself is a more important factor than where the fracture itself occurred (indoors or outdoors) [7, 18]. However, the problem is that to date there is no generally accepted method of assessing mobility. Vestergaard et al. [9] described a significant association between poorer mobility (inability to walk / walk only indoors) and increased risk of early mortality. The presence of preoperative comorbidities and the risk of developing postoperative complications and mortality is the strongest postoperative risk factor [4, 7, 19]. Almost three-quarters of patients with hip fractures have a disease noted on admission (heart failure, anemia, dementia, diabetes mellitus, thyroid dysfunction, etc.). In most patients, we have the presence of three or more accompanying chronic diseases, which greatly complicate the complete recovery of patients with hip fractures. The mental status of a person who has suffered a hip fracture plays a significant role in the choice of treatment method and the final result of [17, 20]. Some studies show a persistently increased mortality of [5, 21], while others suggest either no long-term increased mortality [7, 17] or only moderately increased long-term mortality compared to that expected in the elderly [3, 6, 22]. In many studies, mortality appears to be attributed to the hip fracture itself, ignoring the fact that these are patients with an already increased risk of death from other causes [18, 21, 23]. In a meta-analysis, Brauer et al. [5] show that mortality is 5–8 times higher during the first three months after a hip fracture than in patients of the same age who did not have fractures. However, in the same study, they also compared with a control group of patients who did not have hip fractures but matched by age, sex, and who were of similar functional status and with similar comorbid conditions, long-term mortality (after two years) did not show differences between groups [5]. Mortality in the first year of surgery, the so-called long-term mortality, in our study was 31.25%. Meunier et al. [11] showed that as many as 78% of hip fracture patients who underwent surgery had a higher one-year survival rate. The cause of death among conservatively treated patients is mainly attributed to worsening of existing comorbidities, which is not the case with surgically treated patients [8, 20, 24]. In the first 30 days after the fracture, the most common causes of death are related to the worsening of the existing disease, and not to the appearance of postoperative complications. There are also opinions that the rate of 30-day postoperative mortality is the basic indicator of the quality of hospital operative treatment of hip fractures [1, 3, 14]. Within three weeks after the fracture, 7.6% of these patients die, reaching 8.3% at the end of the month. During the next three months after the fracture, the highest mortality rates occur [10, 19, 25]. Precisely because in this period, patients have to overcome psychological and physical traumas caused by a fracture that imposes functional and mental limitations associated with pathologies characteristic of this life period [3, 4, 5, 22]. Six months following the fracture, we encounter medium-term mortality of patients whose general condition has worsened and, in most cases, failed to reach the functional status from before the fracture [11, 26]. There is evidence that about 20–30% of elderly patients with hip fractures die in the first year after fracture, that about 30% of these patients require placement in special rehabilitation centers, while only 30–40% of patients regain their previous functional independence [5, 7, 16]. Most patients have a residual disability that leads to loss of ability to live independently after a fracture. It is estimated that only one-fifth of patients who walked independently before the fracture do so six months after the fracture [19, 27]. About 78.4% of patients with proximal femoral fracture treated with a surgical technique had a higher one-year survival rate (72%) than those treated conservatively (50%) [19, 28]. Numerous studies show that delaying surgery after 72 hours of fracture approximately doubles the risk of death before the end of the first year after surgery and is a very important risk factor for mortality [12–16, 27]. Saul et al. [22] have described in a meta-analysis that delay in surgery was associated with a significant increase in the risk of death and recommended that most patients with hip fractures should be operated on within 48 hours of fracture. In addition, early fracture fixation and mobilization of these patients reduce the economic burden because it can reduce the total length of stay and thus the total cost [13, 18, 29].

CONCLUSION

Most studies point out that preoperative health is the most effective criterion for predicting postoperative mortality in hip fractures. It can be said that older men, with more chronic diseases (heart failure, chronic obstructive pulmonary disease, diabetes) and with a higher degree of dependence in daily activities are at the highest risk of dying during the first year of the fracture. Analysis of comorbidities and causes of death is extremely important for identifying risk factors, predicting the course of the disease, and timely prevention of complications. Usually, patients in orthopedic wards and clinics around the world receive "faster" help: preparation, anesthesia, surgery, and short-term rehabilitation. However, there are opinions that with a little additional engagement, organizing additional multidisciplinary specialist programs to support surgically treated patients with hip fractures, where the introduction of orthogeriatric specialists is possible, provides

the potential to improve functional outcomes and reduce mortality. Patients with hip fractures have a significantly higher mortality rate than the rest of the population of the same age. We are witnesses that almost one-quarter of patients with hip fractures require lifelong home care and the help of another person, and only half regain all the functions they had before the fall. In older people with a hip fracture, the presence of three or more comorbidities is the strongest factor influencing long-term comorbidity. Complications on the organs of the respiratory system and heart failure are the most common postoperative complications that lead to increased mortality. During the research, we proved that the worsening of comorbidities (primarily cardiovascular and respiratory diseases) after hip surgery increases the possibility of death in the first postoperative year by as much as 70 times. Although it is well known that mortality is increased after hip fractures, there is still controversy about the extent to which mortality can be reduced by hip fracture prevention, as those with the highest risk of hip fractures are weak and older and already have an increased risk of mortality. Lifestyle changes, calcium and vitamin D supplementation, smoking cessation, regular exercise, and reduced alcohol intake can all contribute to reducing the incidence of hip fractures. In short, although it is possible to prevent early deaths, reducing long-term mortality is likely to be very difficult. Some research shows that prevention of falls with the use of certain safety measures (e.g., protective clothing) may have limited benefits in prolonging overall life expectancy due to the multiple risks faced by weak older people.

Conflict of interest: None declared.

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Утицај коморбидитета на постоперативни ток и морталитет код пацијената са преломом кука

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САЖЕТАК

Увод/Циљ Епидемиолошка истраживања показују да имамо драматичан пораст броја особа са преломом кука, посебно оних старијих од 65 година.

Главни циљеви ове студије су да се процени повезаност преоперативног коморбидитета и ризика од постоперативних компликација и морталитета и постоперативног погоршања коморбидних стања и њиховог односа са морталитетом у току једне године од операције прелома кука.

Методе У овој ретроспективној студији, у периоду од јануара 2018. до јануара 2020. године на Одељењу ортопедске хирургије у Косовској Митровици, оперисали смо 64 пацијента са преломом кука. Код пацијената са преломом кука пратили смо број коморбидитета, њихов значај на преоперативни ризик и ток пратећих болести у постоперативном периоду и једногодишњи морталитет од операције.

Резултати Прикупили смо податке о пацијентима од момента пријема до отпуста из болнице и после контролних прегледа, шест месеци и годину дана након отпуста. Од укупног броја испитаника, 23 (35,9%) испитаника су имала један или два коморбидитета, најчешће кардиолошке и неуролошке природе, код 25 пацијената (39,1%) имали смо три пратећа обољења, а код 11 (17,2%) четири и више коморбидитета. Просечна старост пацијената била је 72,51 година (69–92 године).

Закључак Око 45–60% мушкараца и жена са преломом кука имају три или више коморбидних стања. Код старијих особа са преломом кука присуство три или више коморбидитета је најјачи преоперативни фактор ризика.

Кључне речи: прелом кука; коморбидитет; старије особе; морталитет

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Silicon breast implants' texture affecting bacterial biofilm formation

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SUMMARY

Introduction/Objective The most important etiologic factors for both, capsular contracture (CC) and breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is subclinical infection, defined as a response of an organism on presence of biofilm on the implant surface.
The aim of this research was to examine the possibility of biofilm formation of four different bacteria (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Ralstonia picketti*) on three differently textured silicone breast implants (Siltex, Mentor, pore size 70–150 μm; MESMO®sensitive, Polytech, pore size 50–900 μm; and SilkSurface, Motiva pores 13 μm) *in vitro.*

Methods Samples of silicone breast implant capsules (sized 1 × 1 cm) were divided into three groups according to texture. After sterilization, 30 samples in every group were contaminated with 100 μl of examined bacterial broth, followed by incubation which led to biofilm formation. For testing the capability of biofilm formation, modified technique with microtitar plates described by Stepanović was used.

Results All four examined bacteria *(Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Ralstonia picketti)* form more biofilm on implants with pore sizes 50–900 μm compared to implants with pore size 70–150 μm and those with 13 μm. Statistical significance was found in biofilm formation on implants with pores 70–150 μm compared to implants with pores 13 μm. The only exception was *P. aeuruginosa* which did not show significant difference in biofilm formation on implants 70–150 μm and 13 μm.

Conclusion Silicone breast implants with micro and nanotexture should be chosen in order to prevent biofilm formation and possible consequent complications.

Keywords: biofilm; bacterial adhesion; prosthesis-related infections; breast implants; silicon elastomers

INTRODUCTION

Breast implant surgery is followed with high level of satisfaction; however, occasional complications prolong treatment, increase costs in general, and reflect on quality of life of a patient [1]. The most common complication after breast implant surgery is capsular contracture (CC) which is also the most common cause for the reoperation [2]. The incidence of CC is up to 50% [2, 3]. It is usually left untreated, when CC is first or second degree of Regnault classification, while third and fourth stage cause breast disfigurement sometimes followed with mastodynia. This kind of CC requires reoperation including capsulotomy, capsulectomy and implant removal or exchange [3].

The most severe complication after breast implant surgery is breast implant-associated anaplastic large cell lymphoma (BIA-ALCL). Firstly, it was published by Keech and Creech in 1997, but it was not until 2011 when it was distinguished as a separate disease by defining specific immunophenotype CD30, found only in patients who developed ALCL and had silicone breast implants [4]. There is a growing number of reported patients with BIA-ALCL every day [5]. BIA-ALCL can have two forms: localized, presented as a solid mass on the capsule or late seroma or both; and systemic form. Localized disease is surgically treated by removing the implant and complete capsulectomy, while systemic disease needs multimodal therapy [4, 5].

Precise cause for both CC and BIA-ALCL remains unknown. However, many etiologic factors have been associated with its pathogenesis [6, 7, 8]. Common and most important risk factors for both CC and BIA-ALCL are presence of bacterial biofilm and silicone implant surface texture, where silicone implant surface is a distinguishing factor by itself for bacterial adherence [9]. According to Hu et al. [8], presence of bacterial biofilm promotes immunological response leading to BIA-ALCL. The most common bacteria isolated from biofilms found on silicone breast implants in patients with CC are *Staphylococcus epidermidis*, *Propionibacterium specieae*, *Staphylococcus aureus*, while *Ralstonia pickettii* is most common bacteria found in patients with BIA-ALCL [9–12].

Retrospective analysis showed increased incidence in formation of CC in patients with

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smooth surface implants in contrast to textured implants, while BIA-ALCL is found almost exclusively in patients with textured silicone implants [5, 7]. For these reasons, nowadays there are plenty of different breast implant textures on the market. According to pore sizes and implant surface roughness, many classifications have been suggested, such as: smooth, micro and macro textured [13]. In the literature there is a few papers published comparing possibility of biofilm formation on different textures [3, 7, 11, 14].

The aim of the study was to determine possibilities of biofilm formation *in vitro* of four bacteria (*Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Ralstonia picketii*) on three different silicone breast implant textures.

METHODS

This research was conducted at the Clinic for Plastic and Reconstructive Surgery, Clinical Center of Vojvodina and at the Laboratory for Microbiology at the Institute for Public Health of Vojvodina in Novi Sad, Serbia. For the experiment, three differently textured breast implants, divided into three groups, have been used:

- Group 1 – texture with pore size 70–150 μm (SILTEX, MemoryGel®, Mentor, CA, USA);
- Group 2 – texture with pore size 50–900 μm (MESMO®sensitive, Polytech Health & Aesthetics GmbH, Dieburg, Germany);
- Group 3 – texture with pore size 13 μm (SilkSurface, TrueMonobloc®, Motiva, Establishment Labs S.A., Coyol de Alajuela, Costa Rica).

Capsules of implants were cut into pieces 1 × 1 cm, which were sterilized with hydrogen peroxide plasma (STERRAD 100S, Johnson & Johnson, New Brunswick, NJ, USA). In total, 30 sterile samples, from each different texture, were contaminated with four different bacteria: *Staphylococcus epidermidis* (n = 30), *Staphylococcus aureus* (n = 30), *Pseudomonas aeruginosa* (n = 30), and *Ralstonia pickettii* (n = 30), which consisted of 360 samples in general. For detecting capability of biofilm formation, modified technique with microtiter plates according to Stepanovic et al. [15] was used. This method considers growth of bacteria in liquid trypticase soy broth in polyvinyl microtiter plates in previously determined conditions, which enable growth of biofilm (previously refreshed 24-hour bacterial culture, incubated on 37 degrees C in aerobic conditions and resuspended in trypticase soy broth).

According to absorbance, all bacteria were divided in four categories, where cut-off absorbance (OD_c) was defined as three standard deviations of mean absorbance negative control, as shown in Table 1.

Table 1. Intensity of bacterial strain adherence

$OD \leq OD_c$	non adherent bacterial strain
$OD_c < OD \leq 2 \times OD_c$	weakly adherent bacterial strain
$2 \times OD_c < OD \leq 4 \times OD_c$	moderately adherent bacterial strain
$4 \times OD_c < OD$	very adherent bacterial strain

OD – absorbance; ODc – cut-off absorbance

Statistical analysis was done in program SPSS v.20 (IBM Corp., SPSS Statistics for Windows, Armonk, NY, USA).

RESULTS

χ^2 test of independence detected statistically significant influence of breast implant texture on *Staphylococcus epidermidis* biofilm production (χ^2 (4) = 44.628, p = 0.000). The results are shown in Figure 1. Results of χ^2 test were confirmed with Kruskal Wallis test, which detected statistically significant difference in biofilm production of *S. epidermidis* on all three types of breast implants (χ^2 (2) = 42.365, p = 0.000). According to Cohen criteria, breast implant texture has an intermediate influence on biofilm formation of *S. epidermidis* (0.2479). In order to detect differences among different textures, Mann–Whitney U test was used with Bonferroni correction alpha (0.05/3 = 0.017). It was confirmed that bacteria *S. epidermidis* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 297, p = 0.005) and in Group 2 compared to Group 3 (U = 57.5, p = 0.000). Finally, biofilm of *S. epidermidis* is produced more on implants in Group 2 compared to Group 1 (U = 185, p = 0.000).

χ^2 test of independence detected statistically significant influence of breast implant texture on *Staphylococcus aureus* biofilm production (χ^2 (4) = 71.036, p = 0.000). The results are shown in Figure 2. Results of χ^2 test, confirmed with Kruskal–Wallis test, detected statistically significant difference in biofilm production of *S. aureus* on all three types of breast implants (χ^2 (2) = 55.504, p = 0.000). According to Cohen criteria, breast implant texture has a high influence on biofilm formation of *S. aureus* (0.3946). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *S. aureus* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 195, p = 0.000) and in Group 2 compared to Group 3 (U = 30, p = 0.000). Finally, biofilm of *S. aureus* is produced more on implants in Group 2 compared to Group 1.

χ^2 test of independence detected statistically significant influence of breast implant texture on *Pseudomonas aeruginosa* biofilm production (χ^2 (4) = 17.872, p = 0.001). The results are shown on Figure 3. Results of χ^2 test were confirmed with Kruskal–Wallis test, which detected statistical significant difference in biofilm production of *Pseudomonas aeruginosa* on all three types of breast implants (χ^2 (2) = 16.856, p = 0.000). According to Cohen criteria, breast implant texture has a low influence on biofilm formation of *Pseudomonas aeruginosa* (0.099). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *S. aureus* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 426, p = 0.694) and in Group 2 compared to Group 3 (U = 255, p = 0.000). Finally, biofilm of *P. aeruginosa* is produced more on implants in Group 2 compared to Group 1 (U = 258, p = 0.000).

χ^2 test of independence detected statistically significant influence of breast implant texture on *Ralstonia picketii*

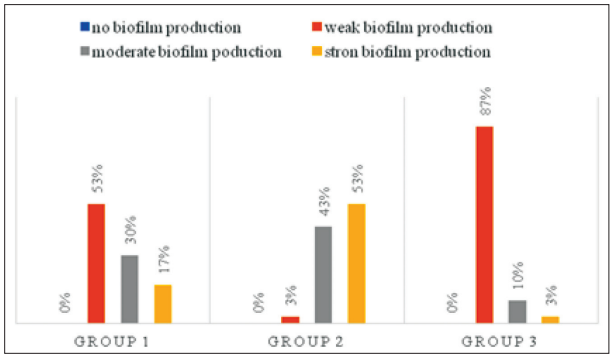

Figure 1. Frequency of *Staphylococcus epidermidis* biofilm production in all three differently textured silicone breast implants

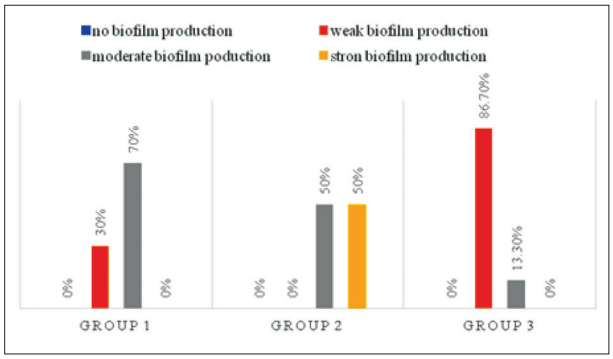

Figure 2. Frequency of *Staphylococcus aureus* biofilm production in all three differently textured silicone breast implants

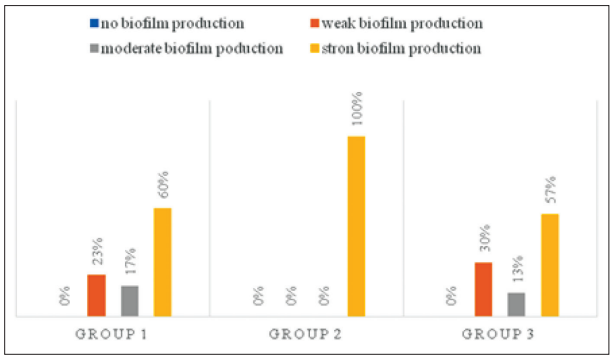

Figure 3. Frequency of *Pseudomonas aeruginosa* biofilm production on all three differently textured silicone breast implants

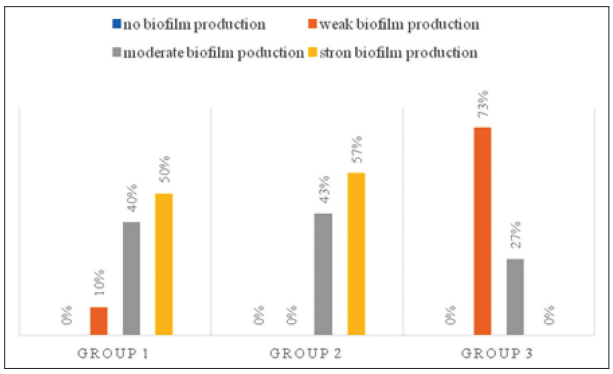

Figure 4. Frequency of *Ralstonia picketii* biofilm production in all three differently textured silicone breast implants

biofilm production (χ^2 (4) = 18.872, p = 0.001). The results are shown on Figure 4. Results of χ^2 test were confirmed with Kruskal–Wallis test, which detected statistical significant difference in biofilm production of *Ralstonia picketii* on all three types of breast implants (χ^2 (2) = 46.366, p = 0.000). According to Cohen criteria, breast implant texture has an intermediate influence on biofilm formation of *Ralstonia picketii* (0.2867). Mann–Whitney U test with Bonferroni correction alpha confirmed that bacteria *Ralstonia picketii* produce statistically more biofilm on silicone breast implants in Group 1 compared to Group 3 (U = 105, p = 0.694) and in Group 2 compared to Group 3 (U = 52, p = 0.000). Finally, biofilm of *Ralstonia picketii* is produced more on implants in Group 2 compared to Group 1 (U = 270, p = 0.000).

DISCUSSION

Breast implant surgery is one of the most common procedures in plastic and reconstructive surgery. Even though it has a high satisfaction rate, infrequent complications do happen. The most common complication is CC with the incidence of 8–50%, while the sincerest one is BIA-ALCL [4, 8].

The etiology of CC is still not known; however, many papers have been discussing it. [16–19]. Del Pozo et al. [17] found more bacteria on implants that were taken out due to the CC compared to those that were extracted for some other reason. Tamboto et al. [18] proved, in his *in vivo* experiment, that pocket inoculation with *Staphylococcus epidermidis* before positioning the implant increases the risk of CC four times [18]. Pocket inoculation with *Staphylococcus epidermidis* provokes CC in 80% of implants, while contracted capsules have 25% more bacteria compared to noncontracted, as published by Jacombs et al. [7]. Bergmann et al. [19] published an article detecting thicker capsule around those implants that were previously contaminated with *Staphylococcus epidermidis*.

Hu et al. [8] suggest that BIA-ALCL can arise as a consequence of chronic infection, such as chronic infection can be a cause for gastric lymphoma development in patients with *Helicobacter pylori* [20]. On experimental model *in vivo,* they detected more lymphocytes' infiltrate on textured implant surfaces compared to smooth surfaces. Furthermore, in that infiltrate T lymphocytes predominated in contrast to B lymphocytes, while polyurethane implants had significantly more bacteria than other implants with textured surfaces. Hu et al. [8] also detected bacteria *Ralstonia spp*. in capsules in 80% of patients diagnosed with BIA-ALCL.

In this study, possibility of biofilm formation of four different bacteria (*S. epidermidis, S. aureus, Pseudomonas aeruginosa* and *Ralstonia pickettii*) on three different breast implant surface textures was tested. *S. epidermidis* was tested as it the most common identified bacteria found, not only on the capsule but also on the implant itself, in patients with CC [10]. *S. aureus* is a common saprophyte on human mucosa and can be a virulent cause of sometimes

sincere infections. Gramm negative bacteria are rare cause of breast implant infections, but nevertheless *P. aeruginosa* was found to be the second most common cause of these infections and therefore was tested in this study [12]. Finally, *Ralstonia pickettii* was chosen as it was found in 80% of capsules in patients diagnosed with BIA-ALCL [8].

Cheesa et al. [10] tested the virulence and biofilm formation possibility of *S. epidermidis* and *S. aureus* taken from breast implants during the routine implant exchange or due to the complications. They found out that those bacteria were significantly stronger producers of biofilm compared to its controls. Prolonged incubation and biofilm formation allow them longer survival during time. *S. epidermidis* has an ability to produce slime which enhances attachment to different surfaces, and which act as a cement for other bacteria. Also, *S. epidermidis* is responsible for coagulase negative nosocomial infections, specifically infections on different implanted devices [3]. Presence of bacteria without signs of infection cause subtle inflammatory response and is called subclinical infection [21].

Since bacteria in biofilm are immersed in matrix, common swabs from infected surfaces are not sufficient for microbial detection. Those swabs are often negative. For biofilm detection on different surfaces, there are a few available methods [22]. Still, the most spread method in use today is sonification process. Even precise, this method besides providing information of biofilm presence and its intensity does not give any other information like number of bacteria [22]. Besides sonification, for biofilm detection electron microscopy, polymerase chain reaction and fluorescent *in situ* hybridization are being used. However, most of those methods are not easily reachable since they are found in specialized laboratories for biofilm research.

In his experiment, Rieger et al. [23] did sonification of whole protheses. Pajkos et al. [11] used only pieces of protheses (2 × 2 cm) as it was done in this study, but they also expected the samples with electron microscopy. According to their research, sonification process detected only one sample which was negative for biofilm, while electron microscope found biofilm on that sample [11]. In experiment conducted by Jacombs et al. [7], on four from six mini silicone breast implants installed into pigs, no biofilm was seen with electron microscopy, while sonification method showed its presence. Tamboto et al. [18] counted only biofilm identified with electron microscopy. In this study, for biofilm detection traditional method was used, concerning sonification process, bacterial growth and its identification with standardized microbiological procedures. Furthermore, the experiment was done on samples sized 1 × 1 cm, derived from capsules of three differently textured breast implants with pore sizes 70–150 μm, 50–900 μm and 13 μm. Using particles from breast implant capsules is not new. Pajkos et al. [11] used samples sized 2 × 2 cm, some authors used samples 1 × 1 cm and others even smaller 5 × 5 mm [3, 14].

Up till now, many studies have been published which examine the possibility of biofilm formation on different surfaces, but not so many on silicone breast implants [9]. Most studies compare biofilm formation on textured and smooth implants. In 1989, Sanger found with electron microscopy not only bacteria in pores of polyurethane implants but also in irregularities of smooth implants explanted for different reasons [24]. In study by Jacombs et al. [7], 20 times more bacteria were found in vivo and 72 times more in vitro, attached on textured breast implants compared to smooth. However, they did not specify which textured implant they used [8]. Del Pozo et al [17] examined bacterial cultures from contracted capsules and from implants itself explanted for different reasons. Majority were textured implants. In more than half of CC positive cultures were diagnosed on implants with sonification process.

Today, there are plenty of producers and production technologies of breast implants, so it is not enough to say only "textured" implants rather to precisely define texture. In 2016, Atlan et al. [25] compared characteristics of three differently textured implants with electron microscopy, X-ray microtomography and mechanical microscopy and found huge differences in surface textures which can reflect on clinical course. Abramo et al. [26] divided textured implant: on microtextured with open pores (Biocell®, McGhan) (600–800 μm) and depth (150–200 μm), and microtextured (Siltex®, Mentor) which have uniformly distributed wavy texture with open pores (70–150 μm) and depth (40–100 μm). A few years ago, implant with pore diameter 13 μm were produced, which according to Barr et al. [27] would be called mesotexture, but according to Maxwell intermediate texture [28].

Histologic studies show differently oriented collagen fibers in capsules around textured and smooth implants [24]. These fibers are intersected around textured implants, therefore lowering the incidence of CC as is shown in study by Stevens et al. [2] conducted among 5000 patients. The importance of textured surface is lowering the possibility of CC and anatomic implant rotation. However, the disadvantage is that they allow for more bacterial adhesion and biofilm formation [8, 29]. This is due to the fact that textured implants have more total surface area than smooth implants. Danino et al. [30] found quantitative increase in biofilm formation when bacterial count exceeds 2000 organisms per mm^2 and since macrotexture keeps more bacteria, it is no wonder that there is more biofilm formation. However, it is not only the texture that it is responsible for this phenomenon but the composition of capsule itself. Bacteria adhere easily to hydrophobic surfaces as it is case with elastomer. Hydrophilic surfaces lower bacterial count on the implant surface and contribute to apoptosis of inflammatory cells. Roughness of implant texture increase the contact angle with bacteria and lower the degree of wetness of hydrophobic surfaces, therefore allowing easier bacterial adhesion [25].

On the other hand, *in vivo* studies show different effects of bacterial contamination of macrotextured implants and effects of texture as a whole. Even though Jacombs et al. [7] found in their study more bacteria on textured implants compared to smooth ones, higher incidence of CC was not found around textured implants. She postulated hypothesis that there must be critical level of biofilm colonies, which, when exceeded, lead to CC. Furthermore,

this concentration is not dependent on surface texture. Bergamann et al. [19] made an experiment on the rats, which were implanted with textured implants and those covered with polyurethane foam. Half of the implants were contaminated with *S. epidermidis*. They expected the incidence of CC, histologic composition and possible infection. Even though they found significantly more inflammatory cells in capsules around polyurethane compared to smooth implants, this higher number did not correlate with bacterial contamination. Their results show thicker capsule around those implants (either textured or polyurethane) that were previously contaminated. Furthermore, they found significantly more T lymphocytes in both contaminated and non-contaminated polyurethane implants in contrast to textured one [19]. This would mean that texture itself would promote host inflammatory response, which, according to Hu et al. [8] can be one of the reasons for development of BIA-ALCL. In their multicentric study, Rieger et al. [23] observed capsules and implants extracted for whatever reason. They found out that bacterial contamination, confirmed with sonification method, contribute to intensity of CC but does not correlate with implant texture. Texture has higher influence on histologic capsule composition [19]. It is speculated that macrotextured implants, which usually have pores larger than cell size, influence the intensity of foreign body reaction therefore helping the tissue ingrowth on its surface and contribute to double capsule formation and late seroma accumulation. This is not the case with micro textured and meso textured implants [27].

In this research, three differently textured implants (70–150 μm (SILTEX, Mentor); 50–900 μm (MESMO®sensitive, Polytech Health & Aesthetics GmbH,); and 13 μm (SilkSurface, TrueMonobloc®, Motiva, Establishment Labs S.A.) were used. These implants are found at the moment on the market in our country and are used most often. Possibility of biofilm formation on these implants *in vitro* was examined. Results show that all examined bacteria (*S. epidermidis, S. aureus, P. aeruginosa* and *Ralstonia pickettii)* form statistically more biofilm on implants with pore sized 50–900 μm compared to pores 70–150 μm and compared to pores 13 μm, as well as on implant with pores 70–150 μm compared to 13 μm ($p = 0.000$). The only exception is *Pseudomonas aeruginosa* where no statistical difference was found in biofilm production on implants with pores 70–150 μm compared to those with pores 13 μm ($p = 0.694$).

CONCLUSION

In this experiment, it is shown that different implant surface texture influences different potential for biofilm formation of examined bacteria, which are most commonly found in contracted capsules and in capsules in patients who developed BIA-ALCL. Since bacterial contamination occurs most probably during the implant placement, further studies would be needed to identify which irrigation protocol would be recommended for each bacterium and texture.

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Утицај текстуре силиконских имплантата за дојку на формирање бактеријског биофилма

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САЖЕТАК

Увод/Циљ Најважнији фактор ризика за настанак капсуларне контрактуре и анапластичног лимфома великих ћелија удруженог са силиконским имплантатима за дојку је суклиничка инфекција, која се дефинише као одговор организма на присуство биофилма на површини имплантата.
Циљ рада је био да се испита могућност формирања биофилма четири различите бактерије (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* и *Ralstonia picketti*) на три различито текстурисана силиконска имплантата за дојку (*Siltex*, *Mentor*, величина пора 70–150 *μm*; *MESMO®sensitive*, *Polytech*, величина пора 50–900 *μm* и *SilkSurface*, *Motiva*, величина пора 13 *μm*) у *in vitro* условима.
Методе Узорци (величине 1 × 1 *cm*) капсула силиконских имплантата за дојку су подељени у три групе према текстури. После стерилизације, 30 узорака из сваке групе контаминирано је са 100 *μl* испитиваног бактеријског бујона, после чега је уследила инкубација која је довела до формирања биофилма. За тестирање могућности формирања биофилма коришћена је модификована техника са микротитарским плочама по Степановићу.
Резултати Све четири испитиване бактерије (*S. epidermidis*, *S. aureus*, *P. aeruginosa* и *Ralstonia pickettii)* више су формирале биофилм на имплантатима са порама 50–900 *μm* у односу на имплантате са величином пора 70–150 *μm* и 13 *μm*. Статистичка значајност је утврђена у формирању биофилма на имплантатима са величином пора 70–150 *μm* у односу на оне са порама 13 *μm*. Једини изузетак је био *P. Aeuruginosa*, који није показао значајну разлику у формирању биофилма на имплантатима са порама величине 70–150 *μm* и 13 *μm*.
Закључак У циљу превенције формирања биофилма и следствених компликација требало би користити микротекстурисане и нанотекстурисане силиконске имплантате за дојку.
Кључне речи: биофилм; адхезија бактерија; инфекције узроковане уградњом протеза; имплантати за дојку; силиконски еластомер

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Tomographic changes after corneal collagen cross-linking for progressive keratoconus – one-year follow-up study

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SUMMARY

Introduction/Objective The aim of this study was to evaluate the outcome of corneal collagen cross-linking (CXL) in patients with progressive keratoconus.

Methods This retrospective single-centered interventional study included 52 eyes of 41 patients who underwent epithelium-off CXL procedure at the age > 18 years. Corneal tomography data, uncorrected, and best spectacle-corrected distant visual acuity (UDVA and CDVA, respectively) were analyzed at baseline and postoperatively over the initial 12-month period. In addition, the natural course of corneal tomographic changes was demonstrated at one, three, six, nine, and 12 months after the CXL procedure.

Results At one year, mean UDVA improved significantly from 0.15 ± 0.22 (0.3 min – logMAR – 0 max logMAR) at baseline to 0.06 ± 0.09 logarithm of minimum angle resolution (logMAR) (0.1 min logMAR – 0 max logMAR) (p = 0.024). Mean CDVA was 0.45 ± 0.39 0.45 ± 0.39 logMAR (0.8 min logMAR – 0 max logMAR) at baseline and 0.06 ± 0.13 logMAR (1 min logMAR – 0 max logMAR) at one year (p = 0.039). Maximum keratometry showed a significant flattening of 1.36 D ± 1.53 D (p = 0.0032) at one year after CXL. Minimum keratometry significantly decreased with a mean change of 1.15 ± 1.20 (p = 0.011). Mean anterior and posterior best fit sphere (ABFS and PBFS, respectively) remained stable during the entire follow-up period. Mean reduction of corneal thickness after CXL was 47 ± 61 μm (p = 0.003). At one year, 29 (56%) eyes showed K max regression, 22 (53%) showed stabilization, and one (2%) showed progression. Spearman correlation coefficients were calculated to assess the correlation between difference in preoperative corneal thickness (CT), in posterior elevation corneal thickness (PECT), and minimum corneal thickness, ΔCT (PECT – minCT) and radius difference Δr (r1-r2). Spearman rs > 0.3 proved statistical significance and correlation.

Conclusion In our study, CXL effectively prevented progression of keratoconus in 98% of eyes at one year, while improving visual acuity. The effect of CXL can be evaluated at the earliest after six months; at that time, the stability of the corneal shape was provided by following the CXL procedure. The main limitation of this study is the small number of patients included.

Keywords: corneal collagen cross-linking; keratoconus; CXL

INTRODUCTION

Keratoconus (KC) is an asymmetric, bilateral, progressive ectatic disorder, which leads to corneal thinning and protrusion. Reduced visual acuity occurs because of induced progressive myopia, irregular astigmatism and scarring. KC typically appears in the teenage years and progress until the fourth decade of life [1, 2]. The origin of this corneal ectatic disorder was believed to be multifactorial with an autosomal recessive or dominant pattern of inheritance [3, 4]. It can arise as an isolated condition or as a result of genetic predisposition triggered by environmental factors. KC is commonly associated with atopy, Down syndrome, eye rubbing habit, and the use of contact lenses [3, 5, 6]. The disease is widespread, with an annual incidence of approximately 1 in 2000, and a prevalence of 54.5 cases per 100,000 population [7].

Conservative treatment modalities, such as spectacles and gas-permeable rigid contact lenses become insufficient for visual rehabilitation in advanced stages of KC. Thus, it is important to diagnose KC as early as possible. The introduction of corneal collagen cross-linking (CXL) significantly reduced the need for corneal transplantation, which is usually required in advanced stages of KC [5].

Corneal crosslinking was introduced by Wollensak et al. [7], to slow down or stop the progression of KC. This process also occurs physiologically with aging. Spoerl and Seiler developed photochemical CXL with riboflavin (vitamin B_2) and ultraviolet A (UVA) (370 nm) [7]. Upon excitation by UVA, photosensitizer riboflavin generates reactive oxygen species. These free radicals induce formation of additional covalent bonds between collagen molecules resulting in corneal stiffening. Riboflavin also protects deeper ocular structures including endothelium, lens, and retina from UVA damage [8].

The main aim of CXL is to increase corneal biomechanical stability in keratoconic eyes. Wolensak et al [9] reported an increase in corneal rigidity of the human cornea by up

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to 300% after CXL. Stiffening of the cornea flattens the corneal apex and may improve uncorrected visual acuity, whereas changes in corneal symmetry may lead to the improvement of corrected visual acuity.

The aim of this study was to report visual and tomographic outcomes of standard CXL procedure for progressive KC in Serbian patients treated at a single corneal surgical center. In addition, natural course of tomographic changes across the initial 12 months after CXL was demonstrated.

METHODS

Patients and methods

This retrospective single-center study included 52 eyes of 41 patients at the age > 18 years, who underwent standard corneal CXL treatment for progressive KC between November 2014 and November 2017. All the patients were followed up for at least one year. The study was performed in adherence to the tenets of the Declaration of Helsinki, and the institutional and informed consent regulation of the Clinic for Eye Diseases, University Clinical Center of Serbia.

All the patients underwent complete eye examination at all follow-ups including visual acuity measurement (Snellen chart), slit-lamp biomicroscopy, and corneal tomography. The following data were analyzed from medical records: uncorrected and corrected distant visual acuity and corneal tomography. These data were obtained at baseline (before CXL), one month, three, six, nine, and 12 months after the CXL procedure. Visual acuity was converted to logMAR values for statistical analysis.

Corneal tomography was performed using the Orbscan Topography System (Orbscan II, Bausch and Lomb, Rochester, NY, USA). The following parameters were analyzed: maximum keratometry (K max), minimum keratometry (K min), simulated keratometry (Sim K), posterior elevation height (PEH), anterior and posterior best fit sphere values (ABSF and PBFS, respectively), radius of ABFS (ABFSr) and PBFS (PBFSr). Corneal thickness was estimated in two spots including corneal thickness in the spot of the highest posterior elevation (PECT) and the spot of minimum corneal thickness (MCT). The position of these two spots was determined as the radius from the corneal apex (PECT-r1 and MCT-r2, respectively).

Changes in K max were defined as K max regression (> 1 D decrease in K max), K max stabilization (< 1 D change in K max), and K max progression (> 1 D increase in K max) in comparison with baseline values (before the CXL procedure) as described by Koller et al. [10]. In our study, this was also applied for changes in K min values. The main limitation of this study is small number of patients included.

Surgical procedure

Standard epithelium-off (Dresden) CXL procedure was performed as previously described by Wollensak et al. [7]. Central 9-mm epithelium was removed by mechanical debridement. Isotonic 0.1% riboflavin-20% dextran solution (10 mg riboflavin-5-phosphate in 10 ml dextran solution) was administered topically at intervals of two minutes for 30 minutes. Slit lamp examination was used to confirm riboflavin absorption throughout the corneal stroma and anterior chamber. Then, central cornea was exposed to UVA irradiation (365 nm) for 30 minutes using a UV light lamp (Intacs XL corneal crosslinking system, Addition Technology, Des Plaines, IL, USA) at irradiance of 3 mW/cm^2 and 5.4 J/cm^2 total energy dosage. During UVA exposure, isotonic riboflavin was administered every two minutes. Postoperatively, ofloxacin eye drops four times a day (quid) for one week, fluorometholone eye drops quid with taper for one month, and artificial tears quid for six months were administered. Therapeutic soft contact lens was applied until complete reepithelization of the cornea.

Statistical analysis

The values are expressed as mean ± standard deviation in the tables and graphs. To explore normal distribution, a Shapiro–Wilk analysis was performed. Data obtained from preoperative and postoperative follow-ups were compared using the analysis of variance (ANOVA). Spearman correlation coefficients were calculated to assess the correlation between difference in preoperative corneal thickness, in PECT and MCT, ΔCT (PECT – min CT) and radius difference Δr (r1-r2). Spearman rs > 0.3 proved statistical significance and correlation. The level for statistical significance was set as less than 0.05 ($p < 0.05$) and high statistical significance as less than 0.01 ($p < 0.01$). Statistical analysis was performed using PASW Statistics, Version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

In this retrospective study, 52 eyes of 41 patients with progressive KC were included. All the patients underwent standard epi-off CXL procedure. Thirty (73.17%) patients were male and 11 (26.83%) were female. Mean age at the time of surgery was 25.28 ± 5.71 years. All the patients have been followed up for at least one year.

Uncorrected and corrected distant visual acuity

Mean UDVA improved significantly from 0.15 ± 0.22 logMAR at baseline to 0.06 ± 0.09 logMAR at one year ($p = 0.024$). Mean spectacle CDVA was 0.45 ± 0.39 logMAR preoperatively and 0.06 ± 0.13 log MAR at one-year follow up ($p = 0.039$).

Corneal tomography

Maximum keratometry value significantly decreased 12 months after CXL with a mean change of 1.36 ± 1.53 D in comparison with preoperative value ($p = 0.0032$). Significant flattening of K max value was observed at six

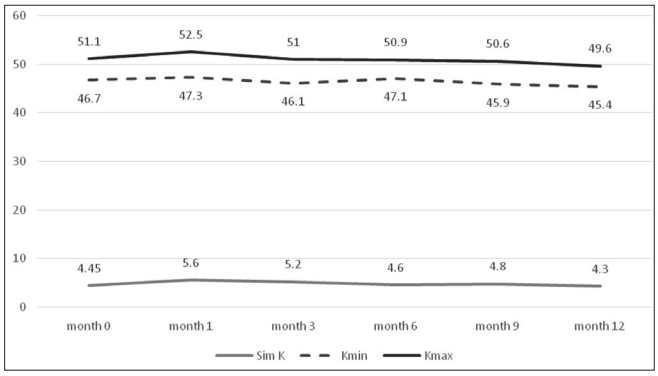

Figure 1. Changes in K max, K min, and Sim K over one-year follow-up period;
Sim K – simulated keratometry; K min – minimum keratometry; K max – maximum keratometry

Table 1. Preoperative and postoperative one-year tomographic values and their statistical significance

Parameter	Preoperative	Postoperative (1 year)	p
Sim K	4.45 ± 2.5	4.3 ± 2.4	> 0.05
K max	51.1 ± 5.6	49.6 ± 5.3	0.003
K min	46.7 ± 4.6	45.4 ± 4.7	0.011
ABFS	43.92 ± 1.98	43.6 ± 2.09	> 0.05
PBFS	55.2 ± 3.2	55.6 ± 2.9	> 0.05
ABFSr	7.7 ± 0.3	7.8 ± 0.4	> 0.05
PBFSr	6.1 ± 0.4	6.1 ± 0.3	> 0.05
PEH	0.1 ± 0.05	0.14 ± 0.1	> 0.05
PECT	432.1 ± 58.5	392.9 ± 71.7	0.003
MCT	423.5 ± 60.62	379.7 ± 74.3	0.001
r1 (PECT)	1 ± 0.4	1.6 ± 0.5	> 0.05
r2 (min CT)	0.9 ± 0.4	0.9 ± 0.5	> 0.05

K max – maximum keratometry; K min – minimum keratometry; Sim K – simulated keratometry; PEH – posterior elevation height; ABSF – anterior best fit sphere; PBFS – posterior best fit sphere; ABFSr – radius of ABFS; PBFSr – radius of PBFS; PECT – corneal thickness in the spot of highest posterior elevation; MCT – minimum corneal thickness; r1 – radius in PECT; r2 – radius in MCT; min CT – corneal thickness in the spot of the thinnest cornea

Table 2. Changes in Kmax and Kmin and their correlation at one year follow-up period

	ΔK max > 1D↓	ΔK max < 1D	ΔK max > 1D↑	
ΔK min > 1D↓	26 (50%)	3 (5.76%)	0	29 (55.76%)
ΔK min < 1D	3 (5.76%)	18 (34.61%)	0	21 (40.38%)
ΔK min > 1D↑	0	1 (1.92%)	1 (1.92%)	2 (3.84%)
	29 (55.76%)	22 (42.31)	1 (1.92%)	

ΔK max – change of maximal keratometry; ΔK min – change of minimal keratometry

months follow up (p = 0,0108) and remained stable afterwards (Figure 1, Table 1).

Furthermore, K min value significantly decreased with a mean change of 1.15 ± 1.20 D at one year after CXL procedure (p = 0.011). A significant improvement of K min was observed at nine months (p = 0.036) and remained statistically significant until the end of the follow-up period (Table 1, Figure 1).

The mean Sim K values showed statistically significant steepening at one month after CXL (p = 0.016), becoming not statistically significant after the third month until the end of the follow-up period (Table 1, Figure 1).

K max regression at one-year follow-up, with corneal flattening ≥ 1 D in comparison with preoperative value, was found in 29 eyes (55.76 %). Stabilization of K max (ΔK max < 1 D) was observed in 22 eyes (42.31%). Interestingly, K min regression, as demonstrated in Table 2, was documented in 29 eyes (55.76%), whereas stabilization of the flatter meridian at one year was observed in 21 eyes (40.38%). Progression of KC, with an increase in both meridian, K max and K min, was found in one eye (1.92 %) (Table 2).

The baseline and follow-up measurements showed that both ABFS and PBFS values remained stable after CXL treatment (p > 0.05) (Table 1, Figure 3). As presented in Figure 4, compared with the baseline, the mean radius of both anterior and posterior BFS increased at six-months follow-up; however, this change was not statistically significant (p > 0.05). There was also no statistically significant difference between mean preoperative and postoperative posterior elevation height values until the end of the follow-up period (Table 1).

Pachymetry

Mean values of corneal thickness in the highest posterior elevation spot (PECT) and in the spot of the thinnest cornea (MCT) at baseline at all follow-ups are shown in Table 1 and Figure 3. These measurements of corneal thickness were highly statistically decreased after CXL procedure during the entire follow up period (p < 0.01) (Figure 5 and Table 1). Furthermore, as shown in Figure 3, there was no significant difference in mean corneal thickness between these two spots following CXL procedure (p > 0.05). Mean radius values in the spot of the lowest corneal thickness (r1) and in the spot of the maximum posterior elevation height (r2) were not significantly different at any of the follow-ups as well (p > 0.05) (Table 1). However, statistically significant difference in corneal thickness between these two spots was observed at baseline in cones with mismatched position of minimum CT and maximum PEH, (p < 0.001). Preoperative difference in corneal thickness in these spots was in high correlation with the difference in their radius from corneal apex (Spearman rs = 0.3).

DISCUSSION

The effectiveness and safety profile of standard epithelium-off CXL procedure in halting the progression of KC has been already demonstrated [11]. Cornel CXL using riboflavin as photosensitizer and UVA light increases biomechanical stiffening of the keratoconic corneas as well as its resistance to collagenase activity. The effect of CXL is limited to the anterior 300 μm [7, 9, 12].

The procedure stimulates corneal remodeling while effectively prevents KC progression. This is usually accompanied by an improvement in visual acuity and topographic indexes. The efficacy of CXL procedure in the majority of

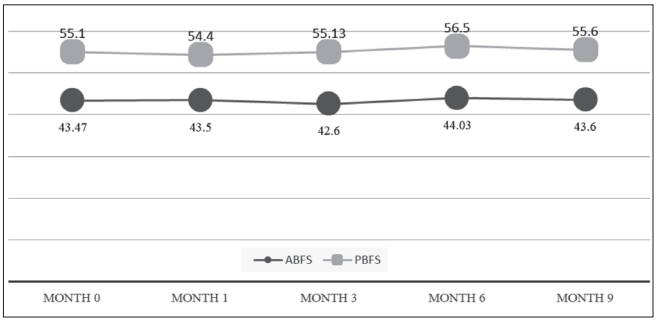

Figure 2. Changes in ABFS and PBFS over one-year follow-up period;
ABFS – anterior best-fit sphere; PBFS – posterior best-fit sphere

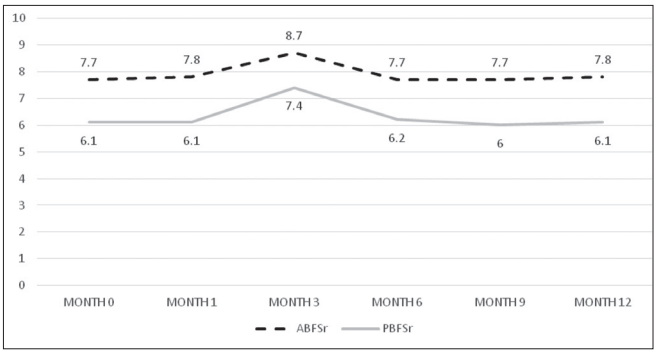

Figure 3. Changes in radius of anterior and posterior best fit sphere over one-year follow-up period;
ABFSr – anterior best fit sphere radius; PBFSr – posterior best fit sphere radius

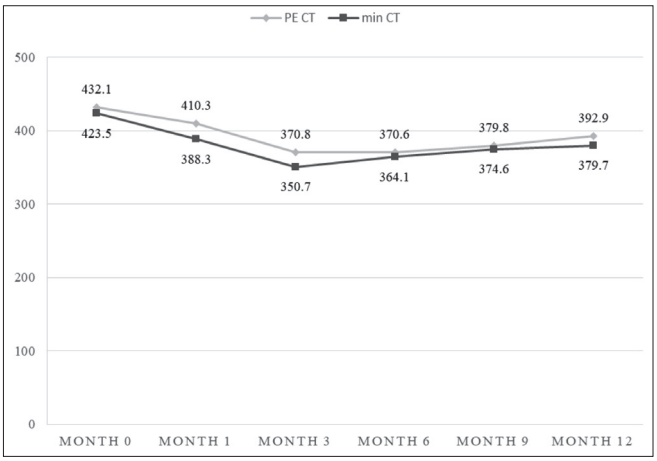

Figure 4. Changes in pachymetry values over one-year follow-up;
PECT – corneal thickness in posterior elevation spot; min CT – corneal thickness in the spot of the thinnest cornea

studies is determined by comparing pre- and postoperative K max values. Thus, maximum keratometry parameter is currently the standard in evaluating the efficacy of CXL for progressive KC [11, 12, 13].

In our study, standard CXL procedure halted KC progression in 98% of eyes at one-year follow-up with an average of 1.36 D decrease in K max. Nonetheless, it seems that the evaluation of both K max and K min gives better insight into postoperative regularization of the anterior corneal surface following CXL. Here, we demonstrated that 85% of cross-linked patients developed flattening of both orthogonal meridians as well. This was associated with a significant improvement in UCDVA and CDVA. Some other studies have also reported a decrease in K max while improving visual and refractive outcomes at one year after CXL procedure [8, 11, 13]. Natural course of topographic changes after CXL is important for proper visual rehabilitation. Better spectacle correction and contact lens tolerance following CXL was observed in our and similar studies [13, 14, 15]. In addition, it is of particular interest to evaluate treatment response after CXL at different time points.

Significant worsening of topographic values at one month followed by flattening until six months was observed in our patient cohort. Interestingly, there was a plateau between six and 12 months. This was also shown in the study by Chang and Hersh [16]. However, continual improvement in K max with long-term flattening was also reported in some studies [17].

The trend observed in the natural course of postoperative keratometry allows us to evaluate cross-linking efficacy at six to 12 months. In our study, KC progressed in one eye (2%) after receiving CXL. Progression of KC, defined as an increase in K max of 1 D over the preoperative value, has been reported after CXL procedure ranging 8.1–33.3% of cases [18].

According to the Global Consensus KC, KC progression is defined by "a consistent change in at least two of the following parameters where the magnitude of the change is above the normal noise of the testing system: steepening of the anterior or posterior corneal surface and thinning and/or an increase in the rate of corneal thickness change from the periphery to the thinnest point" [19]. However, due to the natural changes in tomographic indices after CXL, there is still no clear consensus for defining KC progression after CXL. Corneal thinning has been observed in cross-linked corneas in our and similar studies [15]. We also observed a significant decrease of corneal thickness in both spots, maximum PEH and MCT, during one-year follow-up. This is in line with other studies [20]. Mean corneal thickness in both points, PEH and MCT, was the thinnest between three and six months with subsequent improvement until the end of the follow up; however, corneal pachymetry remained significantly decreased at one-year follow-up [8].

Steepening of the anterior and posterior corneal surface has been generally considered a sign of KC progression in untreated patients. Interestingly, Sedaghat et al. [20] showed that ABFS had not been changed significantly after CXL, but posterior BFS increased six months postoperatively, and remained stable afterwards. In contrast, Grewal et al. [21] reported neither of BFS changes following CXL. Likewise, in our study, there was no significant change in ABFS and PBFS mean values during the initial 12 months after CXL. Furthermore, mean values of posterior elevation height remained stable during the follow up period. These data indirectly indicate biomechanical corneal stability in keratoconic eyes after receiving CXL procedure. We also demonstrated regularization of corneal shape following CXL; baseline difference in mean preoperative radius between the spot of maximum PEH and the spot of minimum CT was lost following CXL.

CONCLUSION

In summary, here we reported visual and tomographic outcomes of CXL for progressive KC in Serbian patients treated at a single corneal surgical center. Standard CXL procedure effectively halted KC progression in 98% of eyes at one-year follow-up. We also confirmed efficacy of CXL in regularisation of anterior corneal geometry while improving visual acuity. The effect of CXL can be evaluated at the earliest after six months, at which time the stability of corneal shape is the result of the natural course of topographic changes following CXL procedure.

Conflict of interest: None declared.

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Томографске промене након корнеалног колагенског *крос-линкинга* код прогресивног кератоконуса – студија једногодишњег праћења

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САЖЕТАК

Увод/Циљ Циљ ове студије је да се анализира исход корнеалног *крос-линкинга* (ККЛ) код болесника са прогресивним кератоконусом.

Метод Ова ретроспективна једноцентрична студија интервенције обухватила је 52 ока код 41 болесника, старијих од 18 година, који су подвргнути ККЛ-у са уклањањем епитела. Вредности корнеалне томографије, некоригована и коригована видна оштрина анализирани су преоперативно и током 12 месеци постоперативно. Такође, природни ток корнеалних томографских промена приказан је један месец, три, шест, девет и 12 месеци после процедуре ККЛ.

Резултати Средња коригована видна оштрина значајно се побољшала од 0,45 ± 0,39 до 0,06 ± 0,13 (0,8 мин. логMAP – 0 макс. логMAP) логаритма минималног угла резолуције (логMAP) након годину дана (p = 0,011). Такође, значајно се побољшала и средња некоригована видна оштрина од 0,15 ± 0,22 до 0,06 ± 0,09 логMAP (0,3 мин. логMAP – 0 макс. логMAP). Максимална кератометрија показала је значајно заравњење од 1,36 Д ± 1,53 Д (p = 0,0032) годину дана после процедуре. Минимална кератометрија значајно се смањила, са променом средње вредности од 1,15 Д ± 1,20 Д (p = 0,011). Средња вредност дебљине рожњаче после процедуре смањила се за 47 ± 61 микрометара (p = 0,003). После годину дана 29 (56%) очију показало је регресију максималне кератометријске вредности, 22 (42,31%) стабилизацију, а једно (2%) прогресију кератоконуса. Спирманов коефицијент корелације је коришћен да би се израчунала корелација између разлике у преоперативној дебљини рожњаче (ДР), у дебљини рожњаче на задњој елевацији (ДРЗЕ) и минималној дебљини рожњаче, ΔДР (ДРЗЕ – мин. ДР) и разлике полупречника Δр (р1-р2), и доказао је статистичку значајност и корелацију (p > 0,3).

Закључак У нашој студији прогресија кератоконуса ефикасно је спречена код 98% очију уз побољшање видне оштрине. Ефекат ККЛ-а може се анализирати најраније после шест месеци с обзиром на то да се тада постиже стабилност облика рожњаче након процедуре ККЛ. Највећи ограничавајући фактор ове студије је мали број укључених болесника.

Кључне речи: корнеални колагенски *крос-линкинг*; кератоконус; ККЛ

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Dry eye examination – benefits of Ocular Surface Disease Index (OSDI) questionnaire with clinical testing

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SUMMARY

Introduction/Objective Dry eye is a multifactorial disease with incidence up to 50% in the general population. It is characterized by a loss of homeostasis of the tear film and accompanied by ocular symptoms. Ocular Surface Disease Index (OSDI) questionnaire is designed to provide a rapid assessment of the symptoms. The aim of this study was to evaluate the diagnostic capacity of OSDI.

Methods A prospective, randomized and observational study was conducted at the Clinic for Eye Disease, University Clinical Center of Serbia, between December 2018 and February 2019. The OSDI questionnaire was used to rate the severity of dry eye disease. Schirmer I test, tear break-up time test (TBUT), Rose Bengal test and lid-parallel conjunctival folds (LIPCOF) test were performed as a clinical proof of the symptoms.

Results A total of 27 patients, 15 male (55.4%) and 12 female (44.6%), with mean age of 60 ± 15 years were included in the study. The average value of OSDI score was 26.37 ± 23.98 (0–80). Schirmer I test and Rose Bengal test for the right and the left eye, as well as the TBUT test for the left eye were positively correlated with OSDI score (Spearman correlation coefficient).

Conclusion OSDI questionnaire is a fast, reliable, and inexpensive test. In our study we have found a correlation between the OSDI score and other clinical tests, except with LIPCOF test. At this moment, the questionnaire that could be the gold standard for dry eye disease diagnosis does not exist, therefore further studies concerning this topic are needed.

Keywords: dry eye; OSDI questionnaire; LIPCOF; Schirmer test; TBUT test; Rose Bengal test

INTRODUCTION

Dry eye is multifactorial eye surface disease, characterized by the loss of tear film homeostasis and eye symptoms [1]. It is one of the most frequent reasons for visiting an ophthalmologist, so it represents a significant outlay for the health care system [2]. Most of the patients have mild symptoms, but sometimes very complex interventions are necessary to avoid further progression to corneal ulcer and conjunctival scaring [3]. Contact lens wear and refractive surgery can cause a dry eye [4]. In the etiology of dry eye main factors are tear film instability, hyperosmolarity, inflammation, eye surface damage, and neurosensory abnormalities [1]. Sjögren syndrome, transplantation (graft versus host reaction), and aging can also cause dry eye [5].

Following present knowledge, ocular and lacrimal inflammation take the main role because they are making defects of corneal and conjunctival cells and causing symptoms [1]. What occurs has been proven on the molecular and biochemical level, where it has also been shown that lower levels of androgen and higher levels of pro-inflammatory cytokines followed by the loss of immunologic homeostasis of the lacrimal gland and the eye surface lead to pathological changes [6]. Neurogenic mechanisms – loss of innervation and lower sensitivity – can also cause dry eye [4]. Dry eye disease is one of the most prevalent ophthalmic disorders in the general population, which can go up to 50% in different studies [2].

In the classification of dry eye disease, we have two large categories: aqueous tear-deficient dry eye (Sjögren syndrome dry eye) and evaporative dry eye (non-Sjögren syndrome dry eye) [7]. Aqueous tear-deficient dry eye implies that dry eye is due to a failure of lacrimal tear secretion, and it represents about 10% of all cases. Evaporative dry eye is due to deficiency in lipid part of tear film, which is manifested with higher evaporation. The main cause is Meibomian gland disfunction, and it represents 85% of all cases [8]. Blepharitis, eyelid margin inflammation is a cause, as well as a consequence, of Meibomian gland disfunction, but in differential diagnosis we also need to think of rosacea, atopy, seborrheic dermatitis and staphylococcal infection [1].

Etiologically important factors in dry eye disease are female sex and ageing (low levels of androgen play the main role in Meibomian gland disfunction) [8]. Except those, important factors are also lagophthalmos, decreased blinking, systemic autoimmune diseases, atopy,

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vitamin A deficiency, and external conditions with low air humidity [9]. A number of questionnaires have been developed, and they are in use in combination to help make a dry eye diagnosis, but none of them, separately, has required sensitivity and specificity to be a gold standard [10]. The clinical presentation of dry eye disease varies a great deal, which makes a diagnosis even more difficult. Patients frequently have unspecific symptoms, such as visual disturbance, ocular discomfort, photophobia, itching, and irritation. Patients can sometimes experience excessive tearing due to discomfort. Symptoms do not have a strong correlation with clinical findings, especially if there is low pain tolerance [11]. For the purpose of reaching a diagnosis, checking severity of the disease, starting treatment and follow-up, many questionnaires have been made, and among them are the Dry Eye Questionnaire (DEQ-5) and the Ocular Surface Disease Index (OSDI) [12, 13].

The OSDI questionnaire has been made by the Outcomes Research Group at Allergan Inc. in order to provide fast evaluation of ocular irritation symptoms in connection with dry eye disease and their impact on vision [13]. Therefore, the aim of this study was to evaluate diagnostic capacity the OSDI questionnaire in assessment of dry eye disease and the severity of the disease relative to clinical diagnostic procedures.

METHODS

This was a prospective, randomized, and observational study, conducted at the at the Department of Cornea and External Eye Disease, Clinic for Eye Disease, University Clinical Center of Serbia, between December 2018 and February 2019. Patients were randomized upon the arrival for a clinical examination such as cataract or blepharitis, and had no previous history of dry eye treatment. Anamnestic characteristics were collected at the beginning of the study. Participation was voluntary and informed consent was obtained from each participant. Ethical approval was obtained from the Institutional Review Board.

The 12-item OSDI questionnaire is a self-administered questionnaire used to rate the severity of dry eye disease. Responses to each item were scored on a five-point Likert scale, where 0 indicates "none of the time"; 1 indicates "some of the time"; 2 indicates "half of the time"; 3 indicates "most of the time," and 4 indicates "all of the time." The OSDI score calculates on the basis of the given formula: OSDI = ((sum of scores for all questions answered) × 100) / ((total number of questions answered) × 4)). The OSDI is assessed on a scale of 0 to 100, with higher scores representing greater disability [14].

For the purpose of this study, additional clinical measures were performed.

Schirmer I test

The Schirmer I test was used to determine the flow of tears produced by the tear glands and measures the basal and reflex secretions of the main and accessory glands. It is performed using calibrated, bent strips of non-toxic filter paper. On the lateral and middle-third of the lower eyelid, a shorter folded end is attached, in order to avoid irritation of the cornea. This test is performed without previously applied anesthesia, and on both simultaneously. The test length is 5 minutes, and after removing the strips from the lower eyelids, we measured the amount of wetting of the paper strips. The limit values of Schirmer I test for dry eye disease are ≤ 10 mm / 5 minutes [15].

Tear break-up time test

Tear break-up time (TBUT) is a clinical test used to assess the stability of the tear film. It is performed by instilling a small amount of fluorescein on the ocular surface of the lower eyelid, after which the respondent was asked to blink in order to spread the fluorescein evenly across the surface of the eye. Then, patient is instructed to keep their eyes opened, without blinking. Using cobalt blue illumination, the TBUT is recorded as the number of seconds elapsed between the patients last blink of an eye and presence of the first defect in the tear film. The normal values of the TBUT test are over 10 seconds, and the results below this value indicate that there is a disruption in the quality of the tear film [15].

Rose Bengal test

The Rose Bengal test is used to indirectly measure the presence of reduced tear volume, detecting damaged and devitalized epithelial cells that have lost the role of creating tears. The results of this test can be read immediately. On the surface of the eye, we observe three zones: the cornea, the nasal, and the temporal part of conjunctival staining. Points from 0 to 3 are assigned to each of these zones, depending on whether there is coloring – if there are few colored dots, lots of colored dots, or if confused zones are present. The positive result of this test are four or more points for all three zones combined, with a maximum of nine points [16].

LIPCOF test

Small folds parallel to the lower lid margin in the infero-nasal and infero-temporal quadrants of the bulbar conjunctiva are defined as lid-parallel conjunctival folds (LIPCOF), and they were first described by Höh et al. [17]. LIPCOF correlates with reduced mucin production and with epitheliopathy of the eyelid edge. Using the method described by Höh et al., the LIPCOF test graded 0–3, by the slit lamp examination [17]. According to the comparison of the number of conjunctival folds with the height of the normal tear meniscus height there is a scale of grading. In grade 0, no fold appears; in 1, a single small fold appears, smaller than the normal tear film meniscus; in grade 2, multiple folds up to the height of the normal tear meniscus appear; in grade 3, multiple folds higher than the normal tear meniscus appear.

Statistical analysis

Numerical data were presented as an arithmetic mean and median with corresponding measures of variability (standard deviation, minimal and maximal value, range). Categorical data were presented as absolute numbers with frequencies. Differences of the OSDI questionnaire results according to sex were analyzed by the Mann–Whitney U test. Spearman correlation coefficients were calculated to explore the relationship between the LIPCOF test grade and the patient's age. The p-value < 0.05 was considered statistically significant. Statistical analysis was done using the IPSS 1.3 program.

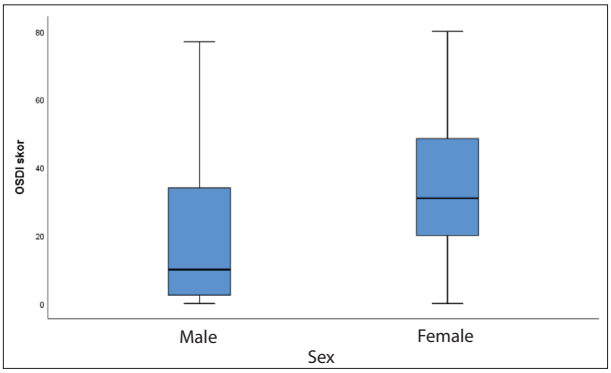

Figure 1. Ocular Surface Disease Index (OSDI) score according to sex

RESULTS

A total of 27 patients, 15 male (55.4%) and 12 female (44.6%), with a mean age of 60 ± 15 years (ranging 22–82 years) were included in the study (Table 1).

Table 1. Demographic characteristics of the patients

Sex, n (%)	
Male	15 (55.4)
Female	12 (44.6)
Age, mean ± SD	60 ± 15

The average value of the OSDI score in our study population was 26.37 ± 23.98, ranging 0–80. The median value of Schirmer I test was 6 for the right eye (ranging 0–12), and 3 for the left eye (ranging 0–10). The median values of the LIPCOF test, the Rose Bengal test, and the TBUT test are presented in Table 2.

The correlations between the OSDI score and age, as well as Schirmer I, LIPCOF, Rose Bengal, and TBUT test results are presented in Table 3. As shown in Table 3, Schirmer I test results for the right and the left eye were positively correlated with the OSDI score: rho = 0.639; p < 0.001 and rho = 0.540, p = 0.004, respectively. Rose Bengal test (OD rho = 0.458, p = 0.016; OS rho = 0.193, p = 0.334), and TBUT test for the left eye (rho = 0.439, p = 0.022) were also positively correlated with the OSDI score (Table 3).

No statistically significant difference was found between the OSDI score and sex (p = 0.136) (Figure 1). Also, no correlation between the age of the respondents and the OSDI score was found (rho = 0.099, p = 0.623).

DISCUSSION

The results of this study showed that the OSDI score was positively correlated with Schirmer I, Rose Bengal and TBUT test results. Also, no statistically significant difference was found between the OSDI score and sex and no correlation between the age of the respondents and the OSDI score. This study is limited by the small group of patients, so further testing within bigger groups is suggested for better validation of the findings.

The core pathophysiological mechanisms of dry eye are lower tear production, higher evaporation, or their combination, with tear film hyperosmolarity and eye surface inflammation [1]. In clinical observation it was found that patients usually do not meet the criteria of making the diagnosis of the disease by all the tests, so more classifications were made, of which the one from Copenhagen is the most popular. This phenomenon is probably a consequence of multifactorial etiology of dry eye. Copenhagen criteria include three main factors – changes in the aqueous layer (Schirmer), higher level of evaporation (TBUT), and eye surface defects (Rose Bengal staining) [3]. By using more tests, the chance of making correct diagnosis is

Table 2. Median test values for Schirmer I, lid-parallel conjunctival folds (LIPCOF), Rose Bengal, and tear break-up time (TBUT) tests

Test	n	mean	SD	median	minimum	maximum
Schirmer OD	27	5.57	3.03	6.0	0	12
Schirmer OS	27	4.09	2.95	3.00	0	10
LIPCOF OD	27	0.7	0.77	1.00	0	2
LIPCOF OS	27	0.59	0.64	1.00	0	2
Rose Bengal OD	27	1.89	2.21	1.00	0	8
Rose Bengal OS	27	1.30	1.2	1.00	0	4
TBUT OD	27	4.81	3.17	5.00	1	10
TBUT OS	27	4.7	3.66	3.00	1	14

OD – right eye; OS – left eye

Table 3. Correlation between Ocular Surface Disease Index (OSDI) score and age, Schirmer I, lid-parallel conjunctival folds (LIPCOF), Rose Bengal, and tear break-up time (TBUT) tests

Variable	OSDI score	
	ρ	p
Age	0.099	0.623
Schirmer OD	0.639	**0.001**
Schirmer OS	0.540	**0.004**
LIPCOF OD	0.114	0.572
LIPCOF OS	-0.130	0.517
Rose Bengal OD	0.458	**0.016**
Rose Bengal OS	0.193	0.334
TBUT OD	-0.064	0.749
TBUT OS	0.439	**0.022**

OD – right eye; OS – left eye

rising, but there is no consensus on which combination, besides best specificity and sensitivity, would cover other aspects, such as severity, quality of life, and follow-up [3]. To overcome this problem, questionnaires like the DEQ-5, McMonnies, and the OSDI are added to the battery of clinical examinations [10].

In this study, in which 27 patients took part, the OSDI score values match OSDI scores of other studies in this field. The mean age of patients in our study is slightly higher than that in other studies [18]. In the literature overview, we found that the significant correlations between the OSDI questionnaire and clinical examinations are common [19]. In general, a positive correlation is most common between the OSDI score and the TBUT test [19, 20, 21]. The positive correlation between these tests is very valuable for clinicians in order to easily establish the dry eye diagnosis and to promptly advise proper therapy.

When we analyzed the LIPCOF clinical test results, we have found that almost 50% of the patients had negative results, and just 20% of the patients were positive to this test, with similar results found by other studies. It is not in correlation to positive findings between the OSDI score and the LIPCOF grade in our study [21]. Further study of etiopathogenic mechanisms, symptoms, and different aspects that any single test evaluates, as well as more patients included, would help in the clarification of these differences.

CONCLUSION

The OSDI questionnaire is a quick, reliable, and inexpensive test, which is a great tool in the evaluation of the first symptoms of dry eye disease. In our study, we have found a correlation between the OSDI score and the most common clinical diagnostic tests, whereas only the LIPCOF test had been without statistical significance. A questionnaire for dry eye disease that could be considered the gold standard still does not exist – therefore, further studies with greater number of participants concerning this topic are needed.

Conflict of interest: None declared.

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Испитивање сувог ока – предности упитника индекса болести предње површине ока са клиничким тестовима

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САЖЕТАК

Увод/Циљ Суво око је болест са инциденцом и до 50% у популацији. То је мултифакторијална болест површине ока где губитак хомеостазе сузног филма прати очне симптоме. Упитник индекса болести површине ока (ИБПО) омогућава брзо постављање дијагнозе.

Циљ овог рада је процена дијагностичке вредности теста ИБПО у болести сувог ока и градацији тежине у односу на налаз релевантних клиничких тестова.

Методе Проспективна, рандомизована и опсервациона студија обављена је на Клиници за очне болести Универзитетског клиничког центра Србије, у периоду од децембра 2018. до фебруара 2019. године. Упитник ИБПО је коришћен ради евалуације симптома и корелације са клиничким тестовима. Клинички тестови примењени у овој студији су Ширмеров тест I, тест прекида сузног филма, тест *Rose Bengal* и тест набора конјунктиве паралелних ивици капка.

Резултати Укупно је било 27 болесника – 15 мушкараца (55,4%) и 12 жена (44,6%), просечне старости 60 ± 15 година. Просечна вредност скора ИБПО у студији била је 26,37 ± 23,98 (0–80). Пронађена је позитивна корелација са скором ИБПО између Ширмеровог I теста и теста *Rose Bengal* за десно и лево око, као и теста прекида сузног филма за лево око (Спирманов коефицијент корелације).

Закључак Упитник ИБПО је брз, поуздан и јефтин тест који добро процењује постојање и тежину сувог ока. У нашој студији пронађена је корелација упитника ИБПО са свим клиничким тестовима у дијагностици сувог ока, изузев теста набора конјунктиве паралелних ивици капка. Тренутно не постоји упитник који би представљао златни стандард у дијагностици сувог ока, те су даља истраживања у овом смеру неопходна.

Кључне речи: суво око; упитник индекса болести површине ока; набори конјунктиве паралелни ивици капка; Ширмеров тест; тест прекида сузног филма; тест *Rose Bengal*

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Psoriatic arthritis and psoriasis severity as metabolic syndrome and insulin resistance predictors

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SUMMARY

Introduction/Objective The aim of this study was to evaluate psoriasis severity and psoriatic arthritis (PsA) as metabolic syndrome (MetS) and insulin resistance (IR) predictors in patients with chronic plaque psoriasis as well as to evaluate if psoriasis severity and PsA are independent predictors for IR regardless of the MetS presence.

Methods This prospective, observational descriptive cross-sectional study was conducted at Dermatovenereological Clinic of the University Clinical Centre of Vojvodina, and included a total of 105 psoriasis patients divided into three groups: group with mild psoriasis (Psoriasis Area Severity Index – PASI score < 10), group with moderate to severe psoriasis (PASI ≥10), and group with PsA diagnosed on the basis of the CASPAR criteria.

Results Percentage of patients who had MetS was higher in the group with the severe form of psoriasis ($p < 0.05$) as well as IR ($p = 0.05$). PsA was also more frequently associated with MetS ($p = 0.05$) and IR ($p < 0.01$). In patients without MetS, no association between psoriasis severity and IR was found ($p = 1.0$), although there was a positive correlation between PASI and index of β-cells secretory capacity % (HOMA B), which shows tendency for IR development. The association between PsA and presence of IR in patients without MetS was statistically significant ($p < 0.05$).

Conclusion MetS and IR prevalence increases in patients with PsA and in patients with the moderate and severe form of chronic plaque psoriasis. Both psoriasis severity and PsA are independent predictors for IR regardless of the MetS presence.

Keywords: psoriasis; psoriasis severity; psoriatic arthritis; insulin resistance; metabolic syndrome

INTRODUCTION

Psoriasis disease spectrum comprises numerous cutaneous, mucosal, and articular manifestations. Psoriasis and psoriatic arthritis (PsA) are frequently associated with obesity, dyslipidemia, insulin resistance (IR), and diabetes, causing psoriasis patients to be susceptible to metabolic syndrome (MetS) and cardiovascular morbidities' development [1].

PsA is an inflammatory type of arthritis characterized by chronic inflammation of the peripheral joints and the axial skeleton and extra-articular manifestations including enthesitis, dactylitis, and skin/nail disease [2, 3]. Several studies have demonstrated the high prevalence of MetS, cardiovascular diseases, and IR in PsA [4–7].

METHODS

This prospective, observational descriptive cross-sectional study was conducted at the Dermatovenereological Clinic of the University Clinical Centre of Vojvodina, and included a total of 105 psoriasis patients organized into three groups, 35 patients in each one. Mild psoriasis patients were with Psoriasis Area Severity Index (PASI) score below 10, the group of moderate to severe psoriasis were with PASI score of 10 and above (in further text 'severe psoriasis'), and the third group were patients with PsA diagnosed on the basis of the Classification Criteria for Psoriatic Arthritis (CASPAR). The study was approved by the Ethical Committee of the University Clinical Centre of Vojvodina.

The inclusion criteria were clinically confirmed diagnosis of psoriasis that lasted for more than six months before the study. The exclusion criteria were patients with secondary hyperlipidemia including hypothyroidism, diabetes, nephrotic syndrome, chronic renal insufficiency, cholestatic liver disease; patients taking beta blockers, thiazides, corticosteroids, antilipemic drugs; patients suffering from malignancies, other autoimmune diseases, systemic connective tissue diseases, pregnant and lactating women.

Measurements of waist circumference, blood pressure, fasting serum glucose, serum insulin, serum triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol, systolic and diastolic blood pressure, rheumatoid factor (RF), radiographic imaging of joints were performed for each patient, in addition to taking demographic data (age, sex, family history of psoriasis). The enzymatic GOD-pap method was used to determine glucose values. Insulin was determined by an

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automated immunometric system (ADVIA Centaur system XP, Siemens Healthcare GmbH, Erlangen, Germany), which is based on direct chemiluminescent immunoassay (CLIA). Cholesterol levels in plasma were determined by enzymatic method, HDL by direct accelerator selective detergent method, triglycerides by glycerol 3-phosphate oxidase method, and LDL was calculated by Friedewald equation. RF testing was performed using a latex-enhanced immunoturbidimetric assay.

The measurement of systolic and diastolic blood pressure was performed using an aneroid sphygmomanometer in the left arm. Blood pressure was measured two times and recorded as the average of two measurements after the patient was sitting for five minutes. To determine the waist circumference, the tape was placed at the level of the uppermost part of the hipbone around the abdomen without causing compression on the skin.

Severity of the disease was determined using PASI. It is a validated psoriasis severity assessment tool [8]. It consists of evaluation and grading of the severity of erythema, thickness and scaling of psoriatic plaques in four regions of the body (head, trunk, arms, and legs). The total score ranges 0–72 [9]. In the present study, mild psoriasis PASI index values are up to 10, while moderate to severe psoriasis has the PASI index score of 10 and above [10].

The diagnosis of PsA was established according to the CASPAR [11].

HOMA2 calculator was downloaded from the official website of the University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and HOMA-S (index of insulin sensitivity %) and HOMA-B (index of β-cells secretory capacity %) were calculated [12].

MetS was diagnosed in the presence of three or more criteria of the National Cholesterol Education Program, Adult Treatment Panel III (NCEP/ATP III): waist circumference ≥ 102 cm in men or ≥ 88 in women; hypertriglyceridemia ≥ 1.7 mmol/l; HDL < 1.03 mmol/l in men or < 1.29 mmol/l in women; blood pressure ≥130/85 mmHg or use of antihypertensive drugs; fasting plasma glucose ≥ 5.6 or diagnosis of diabetes type II [13].

Statistical analyses were performed using IBM SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA). Data was tested for normality using a Kolmogorov–Smirnov test and the Shapiro–Wilk test of normality. The χ^2 test and Fisher's exact test were utilized for the study of the association of categorical variables. Difference in quantitative variables between subject groups were calculated with Mann–Whitney test, Student's t-test or Kruskal–Wallis H test, and for correlation analysis Spearman's correlation was used.

RESULTS

A total of 105 patients were included in this study, 57 (54.3%) of whom were male and 48 (45.7%) were female. The mean age was 50 (± 15.21) years, and the mean time since the diagnosis was 16.2 ± 15 years. There was no significant difference between the groups for age and sex distribution. Waist circumference above European standards was measured in 57.1% patients, 38.1% had hypertriglyceridemia, 29.5% patients had altered fasting blood glucose, 58.1% presented low HDL serum levels, and 61% patients had hypertension. The prevalence of MetS was 46.7% with 49 patients fulfilling at least three criteria.

IR was found in 35 (33%) patients. There was no difference in the prevalence of MetS and IR between men and women.

Differences between groups with and without MetS regarding disease duration were not found (Man–Whitney test, p = 0.72, z = -0.36) (Table 1).

Higher proportion of patients with MetS in the group of patients with severe psoriasis in comparison with mild psoriasis patients is statistically significant ($p < 0.05$, χ^2 test = 5.92) (Table 2).

In patients with PsA, MetS was more frequently detected, in comparison with psoriasis patients without PsA, and this difference is of marginal statistical significance ($p = 0.05$, χ^2 test = 3.75) (Table 3, Figure 1).

Table 1. Disease duration in groups with and without metabolic syndrome (MetS)

MetS presence	Disease duration			
		Min	Max	SD
Absent	15.2	1	50	14.7
Present	17.3	0.6	52	15.6

Table 2. The presence of the metabolic syndrome in relation to the psoriasis severity

Psoriasis severity		Metabolic syndrome presence		Total
		Absent	Present	
Mild psoriasis	n	26	9	35
	% Group	74.3	25.7	100
Moderate/severe psoriasis	n	16	19	35
	% Group	45.7	54.3	100
Total	n	42	28	70
	% Group	60	40	100

Table 3. The presence of metabolic syndrome in relation to the psoriatic arthritis

Presence of arthritis		Metabolic syndrome presence		Total
		Absent	Present	
Absent	n	42	28	70
	%	60%	40	100
Present	n	14	21	35
	%	40%	60	100
Total	n	56	49	105
	%	53.3%	46.7	100

Higher proportion of patients with IR in severe psoriasis in comparison with mild psoriasis patient group was of marginal statistical significance ($p = 0.05$, χ^2 test = 3.81) (Table 4).

In patients with PsA IR was more frequently detected in comparison with psoriasis patients without arthritis ($p < 0.01$, χ^2 test = 7.74) (Table 5, Figure 2).

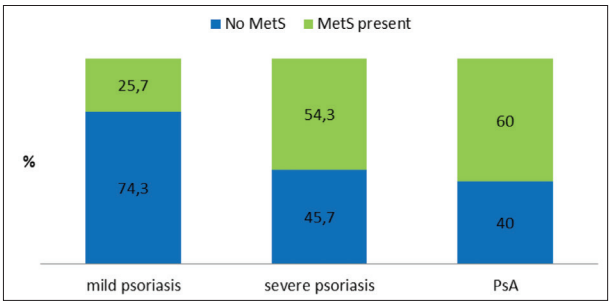

Figure 1. Presence of metabolic syndrome (MetS) in patients with mild psoriasis, severe psoriasis, and psoriatic arthritis (PsA), as percentage of MetS-positive patients in the group

Table 4. The presence of insulin resistance in relation to the psoriasis severity

Psoriasis severity		Insulin resistance presence		Total
		Absent	Present	
Mild psoriasis	n	30	5	35
	% Group	85.7	14.3	100
Severe psoriasis	n	23	12	35
	% Group	65.7	34.3	100
Total	n	53	17	70
	% Group	75.7	24.3	100

Table 5. The presence of insulin resistance in relation to the psoriatic arthritis

Psoriatic arthritis presence		Insulin resistance presence		Total
		Absent	Present	
Absent	n	53	17	70
	%	75.7	24.3	100
Present	n	17	18	35
	%	48.6	51.4	100
Total	n	70	35	105
	%	66.7	33.3	100

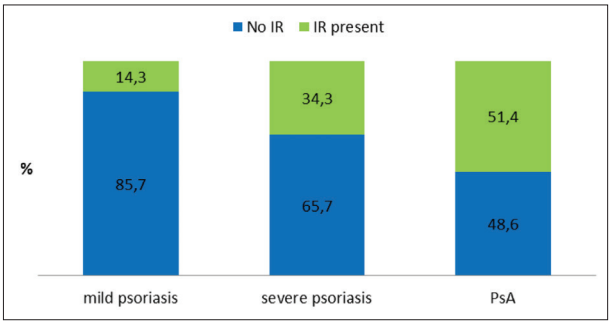

Figure 2. The presence of insulin resistance (IR) in patients with mild or severe psoriasis and in patients with psoriatic arthritis (PsA)

To evaluate psoriasis severity as a risk factor for IR that is independent of MetS, after excluding all patients with MetS, proportions of patients with IR were similar in the rest of the mild and severe psoriasis groups, the difference was not significant ($p = 1.0$, χ^2 test = 5.34) (Table 6).

Positive correlation between PASI score and HOMA B index in psoriasis patients after excluding patients with MetS is statistically significant (Table 7).

No statistically significant difference between HOMA indices in patients without MetS regarding psoriasis severity was found (Mann–Whitney test) (Table 8).

Table 6. The presence of insulin resistance in relation to the psoriasis severity in patients without metabolic syndrome

Psoriasis severity		Insulin resistance presence		Total
		Absent	Present	
Mild psoriasis	n	23	3	26
	% Group	88.5	11.5	100
Severe psoriasis	n	15	1	16
	% Group	93.8	6.3	100
Total	n	38	4	42
	% Group	90.5	9.5	100

Table 7. Psoriasis Area Severity Index (PASI) score and Homeostatic Model Assessment (HOMA) indices correlation in patients without metabolic syndrome

HOMA		PASI num
HOMA_IR	Correlation coefficient	0.185
	p	0.173
	n	56
HOMA_S%	Correlation coefficient	-0.212
	p	0.117
	n	56
HOMA_B%	Correlation coefficient	0.355**
	p	0.007
	n	56

In patients without MetS, association between IR and PsA was found ($p < 0.05$, χ^2 test = 5.34) (Table 9, Figure 3).

Table 9. The presence of insulin resistance in relation to the psoriatic arthritis in patients without metabolic syndrome

Arthritis presence		Insulin resistance presence		Total
		Absent	Present	
Absent	n	38	4	42
	%	90.5	9.5	100
Present	n	9	5	14
	%	64.3	35.7	100
Total	n	47	9	56
	%	83.9	16.1	100

Table 8. Homeostatic Model Assessment (HOMA) indices in patients without metabolic syndrome regarding psoriasis severity

HOMA	Mild psoriasis group					Moderate/severe psoriasis group					p
	AM	Min	Max	SD	Median	AM	Min	Max	SD	Median	
HOMA_IR	1.5	0.5	6.35	1.09	1.26	1.63	0.67	6.85	1.45	1.28	0.97
HOMA_S%	89	42	200.9	39.4	81.1	82.3	14.6	149.2	34.3	78.7	0.85
HOMA_B%	127.2	64.9	218.1	41.2	119.3	153.3	69.4	373.5	67.5	142.7	0.16

AM – arithmetic mean; SD – standard deviation

Table 10. Homeostatic Model Assessment (HOMA) indices in patients with psoriasis and patients with psoriatic arthritis, after excluding patients with metabolic syndrome

HOMA	Group in relation to arthritis										p
	No arthritis					With arthritis					
	AM	Min	Max	SD	Median	AM	Min	Max	SD	Median	
HOMA_IR	1.55	0.5	6.85	1.22	1.26	1.64	0.37	3.41	0.86	1.7	0.29
HOMA_S%	86.4	14.6	200.9	37.3	81.1	87.5	29.3	267.3	66.7	58.7	0.21
HOMA_B%	137.1	64.9	373.5	53.5	129.7	152.5	56.1	271.6	64.1	161.6	0.33

AM – arithmetic mean; SD – standard deviation

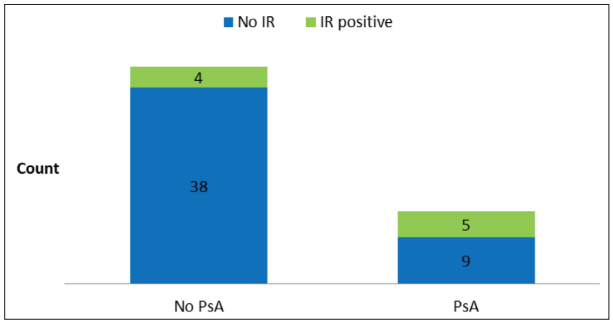

Figure 3. The presence of insulin resistance (IR) in relation to the psoriatic arthritis (PsA) in patients without metabolic syndrome

Differences between HOMA indices in patients with psoriasis (both mild and severe group) and patients with PsA, after excluding patients with MetS, were not statistically significant (Mann–Whitney test) (Table 10).

DISCUSSION

The mean age of psoriatic patients was 50 (± 15.21) years, similar to other previous studies [1, 14, 15].

The prevalence of MetS in our study was 46,7% with 49 of 105 patients fulfilling at least three criteria. Similar results were obtained in a study by Souza et al. [16] conducted in Brazil, in which prevalence of MetS was 50%. Slightly lower prevalence was obtained in a study by Gissondi et al. [17], where prevalence of MetS was 30.1%, in a study by Özkul et al. [2] (36%) conducted in Turkey, and a study by Singh Bhati S et al. [1] (38%) conducted in Central India.

No differences in the prevalence of MetS between man and women was found, which was also the case with the study by Costa et al. [2] and by Özkul et al. [2, 4]. MetS was directly positively correlated with age of the patients, as was as in the study by Gissondi et al. [17].

Differences between groups with and without MetS regarding duration of the disease ($p = 0.72$, $z = -0.36$) were not found, which coinsides with the Singh Bhati et al. [1] study, quite opposite compared to the Gissondi et al. [17] study, in which prevalence of MetS was directly correlated to psoriasis duration.

Dyslipidemia (38.1% had hypertriglyceridemia, 58.1% presented with low HDL serum levels) was the most prevalent comorbidity, followed by hypertension (61%), obesity (57.1%), while 29.5% of the patients had altered fasting blood glucose. The result in the study conducted by Souza et al. [16] were similar – dyslipidemia (74.5%) was the most prevalent comorbidity, followed by hypertension (61.8%), obesity (52.5%), and 30.9% had type 2 diabetes mellitus.

A statistically significant association between severity of psoriasis and MetS was found, as in many other studies [10, 18, 19], in contrast to some other studies where that association was not found [1].

Association between PsA and MetS was statistically significant, as in other previously done studies [4, 6, 20], as well as association between PsA and IR – a higher percentage of patients with PsA had IR, as in the study by Abogamal et al. [21].

IR was found in 35 (33%) patients. There was no difference in the prevalence of IR between men and women.

When comparing the group with mild and more severe form of the disease, there was no statistically significant association between psoriasis severity and IR, although there was a positive correlation between PASI and HOMA B index in patients without MetS, which can be explained as a tendency for IR development, as IR in addition to high β-cell function (HOMA B index increased) is most frequently observed in individuals with prediabetes [22]. Results were similar to a study by Polic et al. [10], conducted in Croatia, in which results suggest that disease severity is an independent factor for IR irrespective of the Mets presence [10].

Association between PsA and IR in patients without MetS was statistically significant, as in the study conducted by Abogamal et al. [21] and these findings explain the role of inflammatory arthritis through inflammatory cytokines on developing IR in PsA patients. However, when comparing association between PsA and HOMA indices in patients without MetS using the Mann–Whitney test, this significance is not proven most likely due to the small number of participants.

Psoriasis promotes IR, which increases the demand for insulin secretion from pancreatic β-cells to maintain the glucose homeostasis and even in normoglycemic patients can lead to atherosclerosis, myocardial dysfunction, and major cardiovascular events [23, 24].

The question is whether all patients with psoriasis and PsA should be tested for IR. It is well known that obesity is one of the major factors, but not all obese patients are insulin resistant so obesity is not the only predictor of increased risk for cardiovascular diseases, as proven in this study.

CONCLUSION

MetS and IR prevalence increases in patients with PsA and in patients with moderate and severe form of chronic plaque psoriasis. Both psoriasis severity and PsA are independent predictors for IR regardless of the MetS presence. These conclusions support the great role of dermatologists in prevention and early diagnosis of cardiovascular diseases in psoriasis patients.

Considering the chronic nature of psoriasis and frequent onset in adolescence, dermatologists are frequently the first healthcare providers in younger patients that do not have established comorbidities yet, and who are suitable for the possible lifestyle intervention aimed for the prevention of MetS and IR, and subsequent cardiovascular diseases.

Study limitations

Our study has several limitations including its cross-sectional design and relatively small number of participants.

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Псоријазни артритис и тежина клиничке слике псоријазе као предиктори метаболичког синдрома и инсулинске резистенције

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САЖЕТАК

Увод/Циљ Циљ ове студије је евалуација тежине клиничке слике псоријазе и присуства псоријазног артритиса као предиктора за развој метаболичког синдрома (МетС) и инсулинске резистенције (ИР) код болесника са хроничном плак псоријазом, као и да се евалуира да ли су тежина клиничке слике и предиктори псоријазног артритиса за развој ИР, независно од присуства МетС-а.

Методе Ова проспективна, опсервационо дескриптивна студија спроведена је на Клиници за кожно-венеричне болести Универзитетског клиничког центра Војводине. У студију је укључено 105 болесника са хроничном плак псоријазом који су подељени у три групе. Група са благим обликом псоријазе (Индекс раширености и тежине псоријазе – *PASI* (*Psoriasis Area Severity Index*) < 10), група са умереним и тешким обликом (*PASI* ≥ 10) и група са псоријазним артритисом, дијагностикованим на основу критеријума *CASPAR*.

Резултати Проценат болесника који су имали МетС је био виши у групи са тежим обликом псоријазе ($p < 0,05$), као и проценат болесника са ИР ($p = 0,05$). Такође, преваленција МетС-а ($p = 0,05$) и ИР ($p < 0,01$) већа је код болесника са псоријазним артритисом. Код болесника без МетС-а није утврђена повезаност између тежине клиничке слике псоријазе и ИР ($p = 1,0$), међутим постојала је позитивна корелација између индекса *PASI* и индекса секреторног капацитета β-ћелија панкреаса % (*HOMA B*), што потврђује тенденцију развијања ИР. Повезаност псоријазног артритиса и ИР код болесника без МетС-а је била статистички значајна ($p < 0,05$).

Закључак Преваленција метаболичког синдрома и инсулинске резистенције је већа код болесника са псоријазним артритисом и код болесника са умереним и тешким обликом хроничне плак псоријазе. Тежина клиничке слике и псоријазни артритис су предиктори инсулинске резистенције, независно од присуства метаболичког синдрома.

Кључне речи: псоријаза; тежина клиничке слике псоријазе; псоријазни артритис; инсулинска резистенција; метаболички синдром

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Differences in anthropometric measures of the orbit between Serbian and Roma population of the Central Serbia

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SUMMARY

Introduction/Objectives The shape and size of the orbital cavity are important parameters in planning surgical interventions and have significance in anthropology and forensic medicine.
The aim of this study was to determine the morphometric characteristics of orbital cavity in Serbian population and to examine if there are differences in examined parameters between Serbs and the Roma Community of Serbia.

Methods Using computer tomography and subsequent multiplanar reconstruction we analyzed orbits from 76 Serbian and 18 Roma healthy volunteers. There was no significant difference in age between the ethnicities.

Results The height and width of the left orbit and the height of the right orbit were significantly higher in Roma group, whereas the width of the right orbit was not statistically different between ethnicities. Orbital indices, however, for both left and right orbit did not significantly differ between Serbian and Roma examinees. Right orbital volume did not differ between the groups, but left orbits had significantly larger volumes in Roma population. Finally, biorbital and interorbital width were both significantly higher in Roma than in Serbian examinees. We could not show differences between sexes except for the biorbital width, which had lower values in Serbian, but not Roma women than in men.

Conclusion Taken together, our results indicate larger orbits, as well as greater distances between the eyes in Roma than in Serbian examinees.

Keywords: ethnicity; sex; multiplanar reconstruction; orbit; Roma; Serbian

INTRODUCTION

The orbit is a craniofacial cavity which contains eyeball, optic nerve and accessory ophthalmic elements including muscles, ligaments, orbital fat body, blood vessels and nerves. The shape of the orbital cavity is determined by geometry of cranial and facial bones and its morphometric characteristics are variable and depend on sex, race, and ethnicity [1–4]. Its anatomical parameters are of great importance for anthropology, reconstructive surgery and forensic medicine [5].

Some studies described the sexual dimorphism of the orbit [6, 7, 8]. Also, its asymmetry was described with the right cavity larger than the left one, as the result of the dominance of the left cerebral hemisphere [7, 9]. The fractures of the orbital cavity, with soft-tissue deviations and severe complications such as double vision, enophthalmos and ocular dystopia, present a complex task in reconstructive surgery [1, 5, 10]. Knowledge of the normal morphometry of the orbital cavity is of great importance in planning the surgical approach to orbital fracture [11], but also in the case of other pathologies such as congenital, neoplastic and Graves' ophthalmopathies [9, 12, 13, 14]. In the adult population, the orbital volume is usually between 20 and 30 cm^3 [1, 10, 13, 15]. Contemporary methods for determination of the orbital measures are based on the analysis of computed tomography (CT) and magnetic resonance imaging scans, although no standardized method for orbital volume measurements exists [16].

Another parameter, often used in facial morphometry is the orbital index. It is defined as the ratio between orbit height and orbit width, multiplied by 100. According to orbital index, three categories of orbital cavities were defined: 1) large (megaseme) – with orbital index 89 and more; 2) intermediate (mesoseme) – with orbital index in the range from 83 to 89; and 3) small (microseme) – with orbital index less than 83 [15, 16, 17]. The orbital index is one of the parameters used in many anthropological studies, because it depends on the shape of the face and varies among the races, regions within the same race, and ethnic groups [18].

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The aim of this study was to determine the morphometric characteristics of orbital cavity in Serbian population and to examine if there are differences in examined parameters between Serbs and the Roma ethnic group of Serbia. Previous anthropometric studies in this population mostly related to the measurement of the body weight, height, body mass index, and nutrition. Studies with orbital measurements have not been conducted so far [19, 20].

METHODS

The study was designed as a retrospective descriptive nonrandomized observational study, that used data from patients' skull images, archived in the hospital system for data archiving. The patients were examined at the Department of Diagnostic Radiology, Clinical Center of Kragujevac in the period from January 2010 to November 2015. The radiological imaging was performed on 64-slice multidetector CT (MDCT) scanner (Aquilion 64, Toshiba, Minato City, Japan). All scans were performed in the axial plane, with subsequent multiplanar reconstruction (MPR). The patient lied on his back, with arms extended downwards, next to the body. The head restraint was used. The study included 94 patients (61 male and 33 female) aged 24–86 years, without pathological changes, who were referred to this examination for various reasons. The ethnicity of the subjects was determined based on the questionnaire form that they filled out before the examination.

The subjects' written consent was obtained according to the Declaration of Helsinki and the study has been approved by the ethic committee and conforms to legal standards.

Orbits were inspected, as part of a broader region examination (whole head, facial mass, paranasal cavities). The radiological imaging was performed on 64-slice MDCT scanner (Aquilion 64, Toshiba, Japan). All scans were performed in the axial plane, with subsequent MPR. The patient lied on his back, with arms extended downwards, next to the body. The head restraint was used. The scan range is determined on the lateral topogram and depends on the region of interest. Scanning parameters were: 120 kVp, 500 mAs, gantry rotation of 0.75 seconds, pitch 0.5 mm, slice thickness of 0.5 mm and 0.4–0.6 mm reconstruction thickness. Analysis of all images and MDCT data is performed on a Vitrea 2 workstation ver.4.1.14.0 (Vital Images, Canon Medical, Minnetonka, MN, USA). All measurements were done by two independent radiologists, using commercially available software (Imaging Software ver.4.1.14.0, Vital Images). For evaluation of inter-observer reliability, the intra-class correlation coefficient was used and intra-class correlation coefficient > 0.8 was considered as excellent agreement. Measurement of orbital morphometric parameters and orbital volume is performed on the same workstation and with the same software on 3D reconstruction of the bone structures obtained by examinations.

Standard anatomical points were determined and used for the measurement of the orbital width, height, biorbital-interorbital diameter and the orbital index. Orbital width – laterally curved distance between the dacryon (the point where frontal, lacrimal and maxillary bones intersect and the medial margin of the orbit is formed) and ectoconchion (the point of intersection of the anterior surface of the lateral limit of the orbit; the line divides the orbit along its axis into two parts) (Figure 1) [8]; orbital height – distance between the superior and inferior orbital margins; it is perpendicular to its width and similarly divides the orbit into two parts (Figure 1) [8]; orbital index – orbital height/orbital width × 100 [21]; biorbital width – distance between left and right ectoconchion (Figure 1) [8]; interorbital width – distance between right and left dacryon (Figure 1) [8]. Measurements were performed on coronal plane using 3D images reconstructed from orbital images. The relation of age and sex with the results was analyzed.

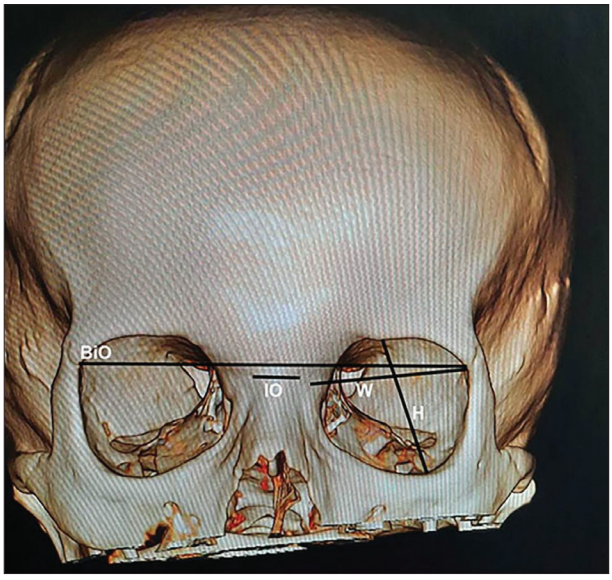

Figure 1. A representative image of reconstructed skull with orbital measurements shown; W – orbital width; H – orbital height; IO – interorbital width; BiO – biorbital width

All data are presented as the mean values ± standard deviation. Statistical analysis was performed using a parametric statistical test, as the data had normal distribution and equal variance. Two-way analysis of variance (ANOVA) with factors "ethnicity" and "sex" was used to compare the groups, followed by Holm-Sidak post-hoc multiple comparisons analysis. Probability value for the rejection of the null hypothesis was set to 0.05.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

RESULTS

We examined the orbits of 76 Serbian (41 male and 25 female) and 18 Roma (10 male and eight female) subjects,

Table 1. Basic demographic data

Examinees	Number of examinees	Mean Age	Age SD	Age span
Serbian male	51	55.9	17.62	24–86
Serbian female	25	58.4	15.68	29–85
Serbian all	76	56.4	17.04	24–86
Roma male	10	50.8	14.06	31–72
Roma female	8	53.5	7.34	44–61
Roma all	18	52	11.35	31–72
All	94	55.6	16.1	24–86

using CT scanner and MPR. The subjects were age-matched, with the average age of 56.4 ± 17 and 52 ± 11.3 for Serbian and Roma groups, respectively ($p = 0.3$; two-way ANOVA). Detailed demographic data is given in Table 1. As sexual dimorphism has been reported for orbital measures, we analyzed our results using two-way ANOVA, with factors "ethnicity" and "sex" [12]. The height of the left orbit (38.05 ± 2.96 mm in Serbian and 39.83 ± 2.28 mm in Roma; $p = 0.012$) was significantly higher in Roma than in Serbian examinees. Similarly, the width of the left orbit (42.12 ± 2.28 mm in Serbian and 44.07 ± 1.95 mm in Roma; $p < 0.001$) was higher in Roma than in Serbs. The height of the right orbit (38.28 ± 3.35 mm in Serbian and 40.12 ± 2.34 mm in Roma; $p = 0.036$) was significantly higher in Roma group, whereas the width of the right orbit (43.69 ± 2.39 mm in Serbian and 44.31 ± 2.01 mm in Roma; $p = 0.088$) was not statistically different between the ethnicities (Figure 2A). Orbital indices, however, for both the left orbit (90.5 ± 6.8 in Serbian and 90.4 ± 3.5 in Roma; $p = 0.78$) and the right orbit (87.7 ± 7.2 in Serbian and 90.5 ± 3.4 in Roma; $p = 0.235$) did not significantly differ between Serbian and Roma examinees (Figure 2B). Right orbital volume (22.49 ± 3.57 mm^3 in Serbian and 23.67 ± 1.22 mm^3 in Roma; $p = 0.053$) did not differ between the groups, although there was a tendency towards higher values in Roma, and the left orbits (21.63 ± 3.14 mm^3 in Serbian and 23.44 ± 1.15 mm^3 in Roma; $p = 0.002$) had significantly larger volumes in Roma population (Figure 2C). As for the distances between the eyes, biorbital width (100.50 ± 4.28 mm in Serbian and 104.65 ± 3.21 mm in Roma; $p < 0.001$) and interorbital width (18.76 ± 3.25 mm in Serbian and 20.15 ± 1.12 mm in Roma; $p = 0.035$) were both significantly higher in Roma than in Serbian examinees (Figure 2D). Of all measured parameters, only biorbital width was significantly different for factor "sex" ($p = 0.004$) and had significant interaction between "sex" and "ethnicity" ($p = 0.003$). Biorbital width was higher in male than in female Serbs (102.37 ± 3.37 mm in males *vs.* 96.88 ± 3.58 mm in females), whereas this difference was not present in the Roma population (104.60 ± 3.65 mm in males *vs.* 104.72 ± 2.82 mm in females). Overall, our results indicate larger orbits and larger distances between them in Roma then in Serbian population.

DISCUSSION

The morphometric characteristics of the orbital cavity are variable and depend on sex, race and ethnicity, and their estimation is relevant for anatomy, forensic medicine, anthropology and reconstructive and aesthetic surgery [2, 3, 4, 8, 13]. The results of our CT study showed that the height and the width of the left orbit and the height of the right orbit were significantly higher in Roma examinees,

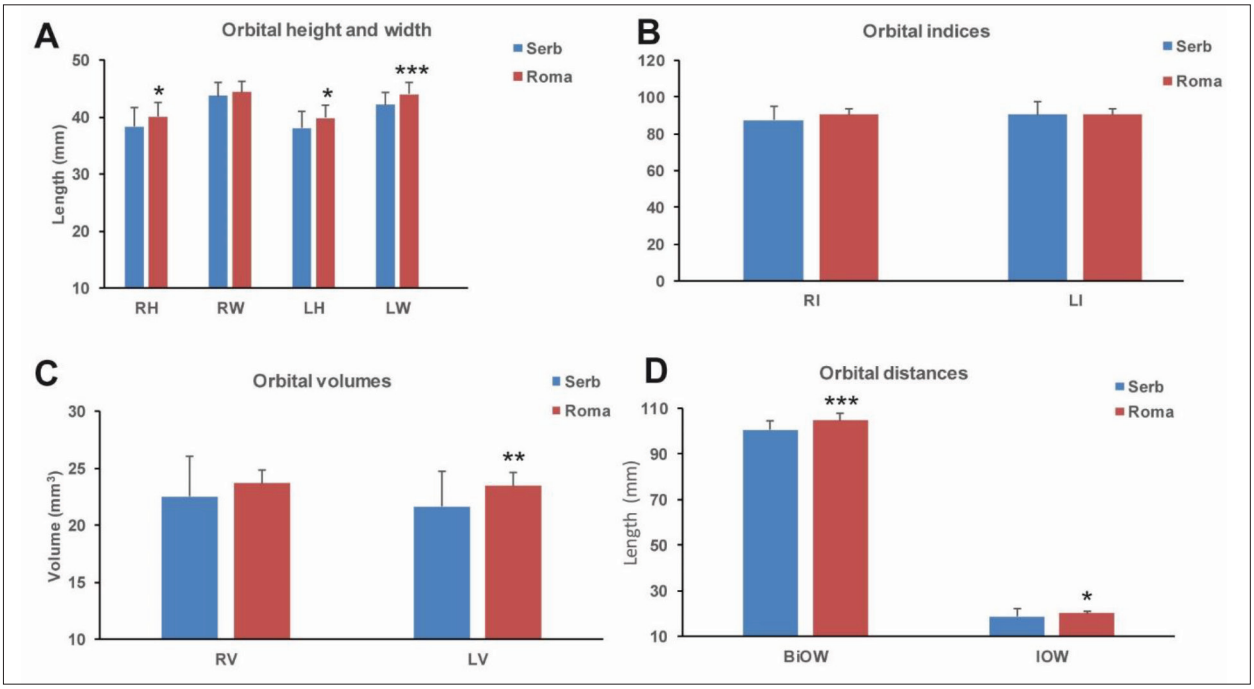

Figure 2. Orbital measurements in Serbian vs. Roma examinees; shown are mean values + SD for orbital height and width (A), orbital indices (B), volumes (C) and distances between the eyes in Serbian (blue bars) and Roma (red bars) examinees; two-way ANOVA with Holm–Sidak post-hoc, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. R (right), L (left) H (height), W (width), I (index), V (volume), BiO (biocular) IO (interocular)

whereas the width of the right orbit was not statistically different between the ethnicities. The obtained results for these parameters were similar to the findings of previous CT studies conducted in Turkish and Indian populations [3, 9, 22]. The sex differences in anatomy of the orbital cavity were confirmed by various studies, with male orbits being significantly larger than female [9, 11, 16, 23]. In comparison to reports that showed sex differences for most of the observed parameters [9, 16], in our study statistically significant sexual dimorphism was found for the biorbital width, which was higher in male than in female Serbs, whereas this difference was not present in Roma population. Thus, it is possible to speculate that the lack of sexual dimorphism in Roma population influenced our results. The results of our study confirmed the earlier findings in variation of orbital dimensions between the left and right side [18, 24]. The observed parameters of the orbital height and width had a tendency to be higher on the right side, leading to the higher values of the left orbital index (right: 88.2 ± 6.7, left: 90.5 ± 6.3; p = 0.019, t-test). This is in accordance with the earlier findings and widely accepted theory that the scull and the face right/left asymmetry with higher values of the right orbital measures are the consequence of the brain asymmetry and the dominance of the left hemisphere [24, 25].

The orbital index is one of the parameters used in many anthropological studies, because it depends on the shape of the face and varies among the races, regions within the same race, and ethnic groups [19]. According to the values of orbital indices (right 86.5 ± 7.2 and left 90.2 ± 6.8), we could conclude that the right orbits in Serbs could be placed in mesoseme category, typical for the European and Caucasian race [24], whereas the left orbits are megaseme. Similar to the data from the Polish population [26], Serbian females had higher orbital indices (right: 90.02 and left: 91.09), which could be classified as megaseme, in comparison to the orbital indices of Serbian males. In both sexes, orbital index had tendency towards higher values on the left side, which was in males significant (p = 0.012, t-test). Orbital indices did not significantly differ between Serbian and Roma examinees. However, Roma examinees had a tendency toward higher values of orbital indices (right 90.5 ± 3.5 and left 90.4 ± 3.6), according to which, their orbits could be classified in megaseme category. Contrary to the findings in Serbian examinees, orbital indices in Roma had a tendency towards slightly higher values on the right side than the left and among males (right: 91.19 ± 3.3 and left: 90.96 ± 3.7) than females (right: 89.75 ± 3.3 and left: 89.69 ± 3.2). These findings demonstrate that ethnicity influences orbital measures.

The volume of the right orbit was slightly higher than the left one, which was also in accordance with the earlier studies and the scull asymmetry [1, 16]. The right orbital volume did not differ between the groups, but the left orbits had significantly larger volumes in Roma population. Finally, biorbital and interorbital width were both significantly higher in Roma than in Serbian examinees. Interorbital width is earlier described as one of the sexually dimorphic morphological parameters of the skull [21], but we did not find statistically significant difference, although it was greater in males. In accordance with similar studies [17], biorbital width was found as sexually dimorphic in Serbian examinees, with higher values among males, while in Roma this sex difference was not statistically significant. A relatively small number of Roma examinees is admittedly a weakness of our study, but it should be considered that ethnic Roma rarely visit doctors' offices and have limited access to the health care system [27, 28].

CONSLUSIONS

Morphological measurements of the orbit are valuable for better practical knowledge and understanding of the difference between sexes, races, and ethnicity. Taken together, our results indicate larger orbits, as well as greater distances between orbits in Roma than in Serbian examinees. This is, to our knowledge, the first study addressing anthropometric parameters of the orbital cavity in Serbian and Roma populations.

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Conflict of interest: None declared.

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Разлике у антропометријским мерама орбите између Срба и Рома у Централној Србији

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САЖЕТАК

Увод/Циљ Облик и величина орбиталне шупљине су битни параметри у планирању хируршких захвата, а имају велики значај и у антропологији и судској медицини.

Циљ овог рада је био да утврди морфометричке карактеристике орбиталне шупљине у српској популацији, као и да утврди да ли постоје разлике између српске и ромске етничке групе.

Методе Коришћењем компјутеризоване топографије и накнадно мултипларне реконструкције анализирали смо орбите 76 српских и 18 ромских здравих волонтера. Није било значајне разлике у годинама између етничких група.

Резултати Висина и ширина леве, као и висина десне орбите биле су значајно веће код Рома него код Срба, док се ширина десне орбите није значајно разликовала. Орбитални индекси за оба ока су, међутим, имали сличне вредности код Срба и Рома. Запремина десне орбите није се разликовала између испитиваних етничких група, али је лева орбита имала значајно већу запремину код ромске популације. Коначно, биорбитална и интерорбитална висина и ширина су биле значајно веће код Рома него код Срба. Нисмо открили разлике међу половима, осим за биорбиталну ширину, која је имала ниже вредности код српских, али не и код ромских жена, него код мушкараца.

Закључак Наши резултати говоре у прилог већим орбитама, као и већем размаку између очију код Рома него код Срба.

Кључне речи: етничка припадност; пол; мултипланарна реконструкција; орбита; Роми; Срби

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Acute respiratory distress syndrome following coronary artery bypass grafting successfully treated with venovenous extracorporeal membrane oxygenation

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SUMMARY

Introduction Acute respiratory distress syndrome (ARDS) is one of the most serious complications during the postoperative period in cardiac surgery. Venovenous extracorporeal membrane oxygenation (VV-ECMO) has proven to be a valuable therapy in ARDS when standard therapy is insufficient. Our aim is to present a case of severe ARDS which was succesfully treated by VV-ECMO.

Case outline A 54-year-old male patient was urgently admitted to our hospital due to anterior wall myocardal infarction. Urgent coronarography was performed, which found severe three-vessel coronary disease. Triple coronary artery bypass grafting (CABG) was performed. After surgery, due to prolonged respiratory insufficiency, the patient was diagnosed with ARDS and VV-ECMO was placed. Nine days later, normal values of gase exchange were achieved and the patient was succesfully weaned from VV-ECMO.

Conclusion We showed that when conventional therapy for ARDS is not effective, use of ECMO should be considered.

Keywords: cardiac surgery; ARDS; VV-ECMO

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is one of the most serious complications in the postoperative period of cardiac surgery patients and it has an extremely high mortality rate. Factors contributing to the development of ARDS are prolonged mechanical ventilation, systemic hypothermia, catecholamine administration, and transient postoperative heart failure [1, 2]. In these patients, the use of extracorporeal circulation (ECC) is also a predisposing factor. ECC leads to a systemic inflammatory response and increased cytokine release, due to the interaction of blood elements with the surface of the ECC machine. Prophylactic measures for ARDS include the use of mechanical ventilatory support (MVS), which will have the least adverse effect on the lungs, adequate fluid intake and early use of neuromuscular blockade [1, 3]. If, in addition to all these measures, ARDS develops, mortality is high because even the MVS cannot provide satisfactory respiratory function [4]. The diagnosis of ARDS is given when the Berlin criteria for ARDS (2012) are met [5].

If a rapid progression of ARDS with life-threatening hypoxemia occurs, a prompt response is required, which includes the use of extracorporeal membrane oxygenation (ECMO) [1]. ECMO provides significant respiratory and circulatory support in patients with advanced acute respiratory and heart failure that are refractory to standard MVS [4]. There are two types of ECMO: venoarterial (VA) and venovenous (VV). VV-ECMO is used in hemodynamically stable patients, when the problem is only in the respiratory function, while VA-ECMO provides both respiratory and hemodynamic support [6]. Therefore, VV-ECMO has proven to be a valuable therapy in ARDS when standard therapy cannot help [1].

CASE REPORT

We present a 54-year-old male patient who was urgently admitted to the hospital due to the anterior wall ST-segment elevation myocardial infarction. The patient complained of feeling pain in the chest and both forearms that started an hour before the admission. Among the risk factors for ischemic heart disease, the patient had unregulated arterial hypertension, obesity (body mass index – BMI of 35.19 kg/m^2), long-term smoking experience as well as positive family history for coronary heart disease. Also he had non-allergic bronchial asthma and cerebrovascular stroke.

On admission, the ST-segment elevation in the anterior leads up to + 3 mm (V1–V4) was registered electrocardiographically, as well as

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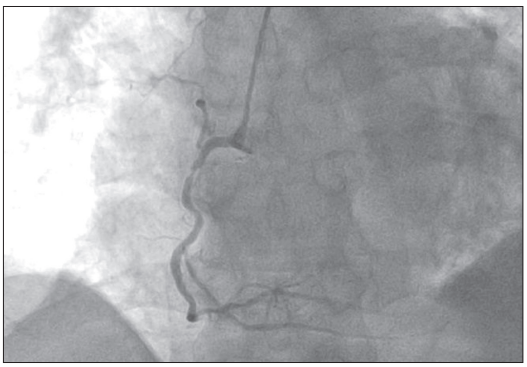

Figure 1. Image from percutaneous coronary intervention

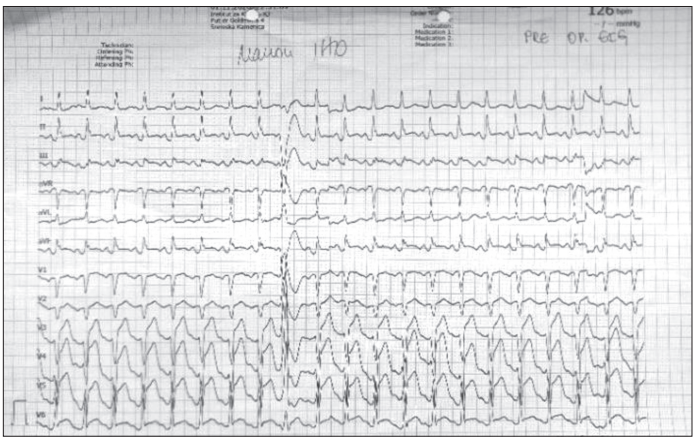

Figure 2. ECG before revascularization

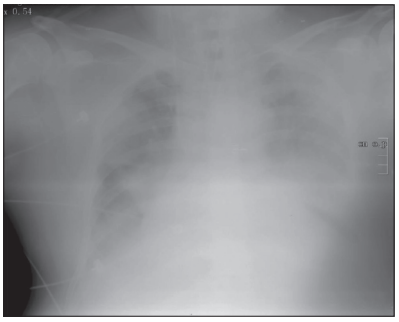

Figure 3. Chest X-ray on the day of venovenous extracorporeal membrane oxygenation placement: decreased transparency of the lung parenchyma completely, primarily of congestive or inflammatory etiology, as well as pleural effusions on both sides

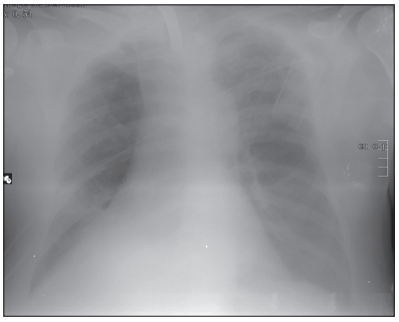

Figure 4. Chest X-ray immediately before weaning from venovenous extracorporeal membrane oxygenation: there is still reduced transparency of pulmonary fields, but improved when compared to the previous image, and there are smaller pleural effusions in the costophrenic angles

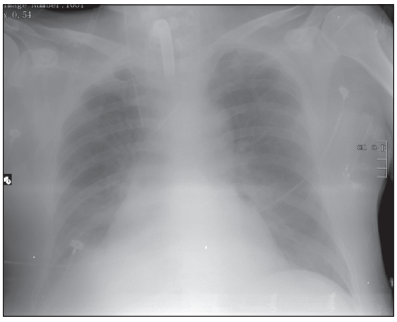

Figure 5. Chest X-ray one week after weaning from venovenous extracorporeal membrane oxygenation: pulmonary parenchyma with significantly better transparency compared to the previous images, a smaller pleural effusion is recorded on the right

biphasic T waves in the lateral leads, while echocardiography registered global left ventricular hypokinesia with decreased left ventricular ejection fraction (40%). Urgent coronarography was performed (Figure 1), which found severe three-vessel coronary disease with the occlusion of the distal segment of the left anterior descending (LAD) artery. Since adequate percutaneous coronary intervention could not be performed, urgent coronary artery bypass grafting (CABG) was indicated. Immediately before the surgical intervention, the progression of electrocardiographic changes in the zone of the septum and lateral wall in the sense consistent acute myocardial ischemia were observed (Figure 2).

The patient underwent surgery on the same day under general endotracheal anesthesia. Revascularization was performed with triple CABG (right coronary artery – posterior descendning artery, ramus intermedius, and LAD). During the operation, the patient was in mild hypothermia, aortic closs-clamp lasted 77 minutes, and the patient was on the ECC for 86 minutes. Upon leaving the operating room, the patient was hemodynamically and rhythmically unstable with frequent single ventricular extrasystoles and on inotropic and vasopressor support with adrenaline and noradrenaline. Gas analyses of the arterial blood at MVS in VC-MMV mode on FiO_2 100% registered respiratory acidosis and type 2 respiratory failure: $PaCO_2$ 58 mmHg, PaO_2 70 mmHg, SaO_2 90%, while pH was 7.27. During the further postoperative course, SaO_2 dropped to 84%. Empiric correction of antibiotics was performed since radiographic finding showed suspected inflammatory process in the lungs. During that period, there was a high rate of SARS-CoV-2 patients, so a possible infection with that virus was suspected. The patient was tested on several occasions, but tracheal aspirate and nasopharyngeal swabs for PCR test were negative. On the second postoperative day the patient developed hypotension and further gas exchange deterioration. SaO_2 decreased to 72% and $PaCO_2$ increased to 67 mmHg with a tendency to worsen. The patient was in severe respiratory acidosis (pH 6.86), and PaO_2 dropped to 40 mmHg. In addition to inadequate pulmonary parameters, an increase in inflammatory markers (leukocytes 24×10^9/l, procalcitonin 1.33 ng/ml) was registered. An increase in urea of 11.6 mmol/l and in creatinine of 228 umol/l with anuria indicated the development of acute kidney injury.

On the third postoperative day, due to prolonged type 2 respiratory failure and worsened radiographic findings of the lungs (Figure 3), high pleateau pressure was observed despite ventilator optimization, which corresponded to the clinical picture of ARDS, and VV-ECMO was placed. VV-ECMO cannulas were placed in the left and right femoral veins. After that, there was a gradual improvement in

the patient's gas exchange, but respiratory failure was still observed ($PaCO_2$ 55 mmHg, PaO_2 42 mmHg, SaO_2 92%, pH 7.5). At the same time, acute kidney injury developed, requiring renal replacement therapy. Continuous veno-venous hemodiafiltration (CVVHDF) was applied via ECMO system.

Due to the prolonged MVS, on the seventh postoperative day, a percutaneous tracheostomy was placed to prevent complications caused by the prolonged endotracheal intubation. On the same day, bronchoscopy was performed for aspiration of airway secretions. The conditions were met for gradual liberation from VV-ECMO. At the same time, the radiographic pulmonary infiltrates improved (Figure 4). Nine days after the placement of VV-ECMO, the patient was assessed as ready for weaning from ECMO.

After the deoxy and CO_2 challenge test, the patient was successfully weaned from ECMO. Subsequently, transient deterioration of respiratory function was observed, which still required high values of FiO_2 on the ventilator (90–100%). Impaired gas exchange with the values of $PaCO_2$ 51 mmHg, PaO_2 52 mmHg, SaO_2 80%, and pH 7.26 can be explained by the abolition of hypoxic vasoconstriction during the ECMO support. After the stabilization of respiratory function, the conditions for re-reduction of FiO_2 were met. On the 14th postoperative day, a high fever appeared, followed by an increase in markers of inflammation (leukocytes 26.41 × 10^9/l, procalcitonin 0.42 ng/ml), due to which antibiotic therapy was corrected and the bronchoscopy was performed again. The normal flora of the upper respiratory tract was obtained.

After the gas exchange stabilization and improvement of the patient's general condition, as well as radiographic findings (Figure 5), the conditions to separate the patient from the MVS were obtained, so the decannulation of tracheostomy was performed. In the meantime, CVVHDF was performed on two more occasions, after which the renal function recovered.

The patient was discharged on the 39th postoperative day, in good general condition, hemodynamically and rhythmically stable. At a regular check-up after three months, the patient appeared in an improved clinical condition with the presence of sternum instability, due to which the sternal resuture was indicated.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research comimittee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

DISSCUSION

Coronary artery surgery is a high-risk procedure and is associated with 30-day morbidity of up to 14% and a mortality rate of up to 2% (in the USA) [7]. Many complications can occur during the intervention, especially when it comes to an emergency procedure. The most common are bleeding, operative site infections, cardiac arrhythmias, myocardial infarction, acute kidney injury, cerebrovascular stroke, as well as respiratory complications, primarily pneumonia and ARDS [7].

Perioperatively, chest trauma and postoperative pain limit the expansion of the operated patient's chest, impede normal lung function and expectoration, and carry an increased risk of respiratory complications [7]. In addition, the reduction of lung function can be related to respiratory muscle dysfunction, because the synthesis of proteins needed for skeletal chest muscles is reduced immediately after surgery leading to muscle weakness and atrophy in the first weeks after surgery. It should be emphasized that during the first week after the operation, there is a reduction of vital capacity by 30–60%, which remains reduced by 12% even one year after the surgery [8]. Since our patient had bronchial asthma as an associated disease, as well as long-lasting history of smoking, his lung function was certainly impaired, which also contributed to the appearance of ARDS. Obesity can lead to disorders of the mechanics of the respiratory organs, obstruction of the airways, and disruption of normal gas exchange, and therefore is also classified as a risk factor for the development of ARDS. Since our patient had a BMI of 35.19 kg/m^2, this certainly had an impact on the lung function impairment and contributed to the development of ARDS [9].

ARDS is characterized by acute, diffuse, inflammatory lung damage that leads to increased permeability of alveolar-capillary membranes and loss of functional lung tissue. It is clinically manifested by hypoxemia with bilateral radiographic consolidation of the lung parenchyma, functionally reduced compliance, and increased dead space in the lungs [10]. The onset of ARDS is abrupt and usually occurs in the first few hours after surgery [11]. The treatment of ARDS still mostly remains only on the application of the supportive measures with the MVS. In the past two years, high mortality from this syndrome has been registered in hospitalized patients – around 40%. Those who survive may suffer consequences in the form of permanent psychological and neurological morbidity that affect the quality of life of these patients, even up to five years after recovery [12, 10]. In this case report, the diagnosis of ARDS was made on the third postoperative day after a radical worsening of the patient's clinical condition, persistent hypoxemia and severe hypercapnia with respiratory acidosis, and a characteristic radiographic finding showing reduced parenchymal transparency with pleural effusion on both sides.

According to the recommendations of the Extracorpreal Life Support Organization, the indications for the use of VV-ECMO are as follows:

1. It should be considered in hypoxic respiratory failure due to any cause when a predicted risk of death is > 50% and is indicated when the risk of mortality is > 80%;
2. CO_2 retention at MVS regardless of high Pplat (> 30 cm H_2O);
3. Severe air leak syndromes;
4. Need for intubation in a patient on lung transplant list;
5. Immediate respiratory collapse [13].

We applied the femoro-femoral VV-ECMO to our patient. High blood flow (4–8 l/minute) and diffusion of gases between the blood and the sweep gas passing through the membranes supply the blood with oxygen and remove carbon dioxide directly from the blood, which allows the use of more protective MVS and thus reduce the frequency of ventilation-induced lung injury [12]. One of the more important roles of ECMO is that, by improving tissue oxygenation, it reduces damage to other organs, primarily reduces respiratory acidosis, and reduces neurocognitive sequelae [12].

In a study conducted by Song et al. [1], 13 out of 2234 patients underwent VV-ECMO after cardiac surgery procedure. The mean time of VV-ECMO placement was about 7.5 days after surgery, which coincides with the time of placement in our patient. In their study, separation from ECMO was successful in nine of 13 patients (69%) and the duration of VV-ECMO averaged 7.2 days, while in our patient, the removal was done on the ninth day of placement, and the patient was on the ECMO treatment for seven days. When the patient is weaned from ECMO, transient hypoxemia may occur. This happened with our patient, and it can be elucidated by the abolition of hypoxic vasoconstriction during ECMO support [14]. In patients who undergo the ECMO procedure, an extended stay in the intensive care unit (ICU) and longer hospital treatment are observed, which is confirmed in our patient, who was hospitalized for 39 days. Although the long-term prognosis after the ECMO administration in ARDS has not yet been sufficiently studied, the patients who underwent ECMO in the Conventional Ventilation or ECMO for Severe Adult Respiratory Failure (CESAR) study had almost the same or better quality of life than those who had ARDS and were treated with conventional therapy. However, in 84 patients who survived the first six months after ECMO, long-term psychological and emotional problems were observed [12].

In March 2020, the World Health Organization declared a pandemic of SARS-CoV-2, which resulted in a globally devastating effect, with over 180 million people being affected and about six million deaths [15]. It is necessary to mention that at the time of the patient's admission to the hospital, there was a large number of patients with the SARS-CoV-2 in our country. Since one of the possible manifestations of this viral infection is the sudden appearance of ARDS, it was suspected that it was a SARS-CoV-2 infection. Therefore, the PCR testing was repeated twice, and the results were negative. Also, it should be emphasized that ARDS caused by infection with this virus has some unique features, but much of the experience with severe respiratory failure in SARS-CoV-2 patients is not different from other forms of ARDS, and it is one of the indications for the VV-ECMO use [16]. Recent studies have shown that in patients infected with the SARS-CoV-2 and treated with ECMO the mortality in the first 60 days was about 31%, and a very similar result was obtained in the ECMO to Rescue Lung Injury in Severe ARDS (EOLIA) study (35% in 60 days) [12]. Although the patient has been PCR- and antigen-tested on several occasions, the SARS-CoV-2 etiology of ARDS could not be ruled out with certainty to date.

In cardiac surgery patients in whom conventional therapy is not effective in ARDS, the use of ECMO should be considered. Although still insufficiently studied, VV-ECMO shows great potential and provides a chance of survival to patients who have a severe form of ARDS, regardless of the etiology.

Conflict of interest: None declared.

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Успешно лечење синдрома акутног респираторног дистреса после хируршке реваскуларизације миокарда применом веновенске екстракорпоралне мембранске оксигенације

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САЖЕТАК

Увод Синдром акутног респираторног дистреса (САРД) једна је од најозбиљнијих компликација у постоперативном периоду кардиохируршких болесника. Веновенска екстракорпорална мембранска оксигенација (ВВ-ЕКМО) показала се као драгоцена терапија код САРД-а када стандардна терапија не може да помогне.

Наш циљ је да прикажемо случај тешког облика САРД-а који је успешно лечен применом ВВ-ЕКМО.

Приказ болесника Болесник стар 54 године хитно је примљен у болницу због инфаркта миокарда антериорне регије. Ургентном коронарографијом нађена је тешка тросудовна коронарна болест, те је начињена хируршка реваскуларизација миокарда троструким аортокоронарним бајпасом. Постоперативно се уочава продужена глобална респираторна инсуфицијенција, те је болеснику постављена дијагноза САРД-а, због чега је пласиран ВВ-ЕКМО. Девет дана после пласирања постигнута је адекватна гасна размена, те је болесник успешно одвојен од ВВ-ЕКМО.

Закључак Код кардиохируршких болесника код којих конвенционална терапија није делотворна за САРД, неопходно је размотрити примену ВВ-ЕКМО.

Кључне речи: кардиохирургија; синдром акутног респираторног дистреса; веновенска екстракорпорална мембранска оксигенација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Giant spleen as a surgical challenge – case report and literature review

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SUMMARY

Introduction According to the guidelines of the European Association of Endoscopic Surgery, any case where the maximum craniocaudal splenic diameter exceeds 20 cm is considered massive splenomegaly. In addition to metabolic, hematological, and hemodynamic problems, enlarged spleen may cause mechanical difficulties due to the pressure to surrounding organs and vascular structures. The aim of this paper is to present the surgical challenges and technique applied in massive splenomegaly, in a patient who had neglected the importance of regular medical checkups.

Case outline We present a 62-year-old male patient who was admitted to hospital for treatment of previously clinically and radiologically verified splenomegaly but who neglected the importance of regular checkups and medical treatment. Splenectomy was performed with a splenic specimen 38 cm in its maximal diameter.

Conclusion Taking into consideration all the possible benefits and possible complications of surgical treatment, including the quality of life of splenectomized patients, comprehensive preoperative assessment should be made, and surgical treatment selectively applied.

Keywords: splenomegaly; massive splenomegaly; splenectomy

INTRODUCTION

Massive splenomegaly is an enlargement of the spleen that can occur in a wide array of diseases and conditions. According to the guidelines of the European Association of Endoscopic Surgery (EAES), any case where the maximum craniocaudal splenic diameter exceeds 20 cm is considered massive splenomegaly [1].

In addition to metabolic, hematological, and hemodynamic problems, an enlarged spleen may also cause mechanical difficulties. Due to the pressure to surrounding organs and vascular structures, patients often complain of a sense of heaviness in the abdomen, frequent perspiration, nausea, indigestion, etc. [2].

The diagnostics in splenomegaly, depending on the cause, entails a series of procedures, starting with a physical exam, as well as abdominal ultrasonography, computerized tomography (CT), magnetic resonance imaging, laboratory analyses, and finally splenectomy. A definitive diagnosis is established after a histopathological examination [2, 3, 4].

Splenomegaly treatment may be nonsurgical, which includes the treatment of the basic cause of spleen enlargement, as well as surgical. Surgical treatment involves the removal of the spleen, necessitated by the difficulties that its size causes, as well as by rupture or imminent risk of rupture. Also, when preoperative diagnostics does not offer reliable data, splenectomy is performed for diagnostic purposes [5].

The aim of our paper is to present the surgical technique applied in massive splenomegaly, in a patient who had neglected to observe the importance of regular checkups and medical treatment.

CASE REPORT

We present a 62-year-old male patient who was admitted to hospital for treatment of previously clinically and radiologically verified splenomegaly. Abdominal CT detected an enlarged spleen of enormous size – 39.3 cm (Figure 1). At admission, we learned from the patient that he had been diagnosed with an enlarged spleen a number of years before, that he had had bone marrow biopsy several times, and that the results of the biopsies had been normal. We also found out that he had not been keeping up with regular check-ups and examinations during the previous several years. Medical records revealed that he had undergone urinary bladder surgery several years before, due to calculosis, as well as surgery of bilateral inguinal hernia, two years earlier.

Two months before admission to our hospital, the patient visited his doctor complaining of abdominal pain, the sensation of heaviness, discomfort, and increased perspiration. After complete radiological diagnostics had been performed, the patient was examined and tested by a hematologist. Immunophenotyping of

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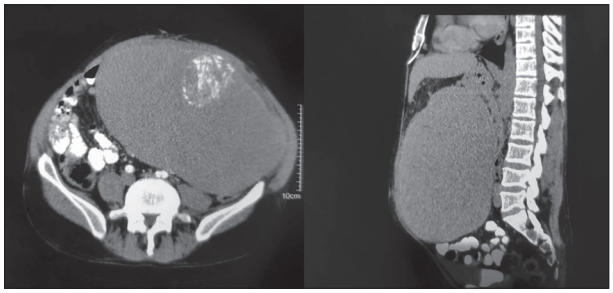

Figure 1. Abdominal computed tomography detected an enlarged spleen of enormous size, 39.5 cm in diameter

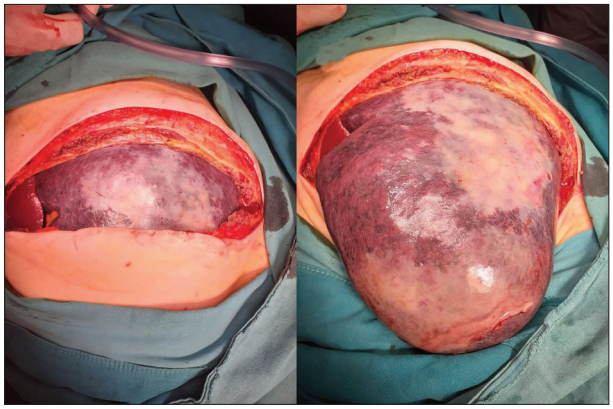

Figure 2. Intraoperative photo shows the enlarged spleen that occupied most of the abdominal cavity

peripheral blood was performed and it was determined that the patient had the B monoclinic population which was Pan B+, CD5-, CD10-, FMC7+ hetero, CD23+ hetero, CD38+ hetero, with IgM/kappa+, with a HILL score of 1. In other laboratory results, there was a marked monoclinic IgM kappa paraprotein in low concentration.

Upon the completion of hematological diagnostics and preparation, further surgical treatment was decided on during a clinical case conference. After preoperative preparations were performed, the patient was placed under general anesthesia and the abdomen was opened with midline laparotomy. The abdomen revealed an enlarged spleen interspersed with white patches (Figure 2), which was pressing the surrounding organs and vascular structures to the opposite side of the abdomen. The next step was the mobilization of the spleen with the aid of the LigaSure device (SurgRx, Redwood City, CA, USA), for severing splenic ligaments. We first identified the lienal

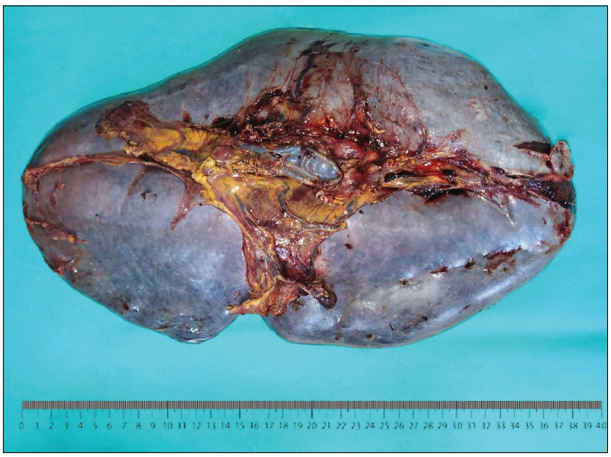

Figure 3. Splenic specimen

artery and vein, which were then, through careful preparation, ligated and severed. Then, we dealt with the short gastric blood vessels, also with the use of LigaSure. After this, splenectomy was completed without incident and the spleen was removed from the abdomen in its entirety. After hemostasis was established, an abdominal drain was placed in the left subphrenic space, and the abdomen was closed by anatomical layers.

The splenic tissue specimen taken after the removal of the spleen from the abdomen (Figure 3) was sent for definitive histopathological examination, which confirmed hairy cell leukemia.

The patient was discharged from hospital on the sixth postoperative day with prescribed antibiotic prophylaxis and a recommendation for post-splenectomy immunization. In addition, the patient was referred for further treatment and follow-up with a hematologist.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

DISCUSSION

Clinically, massive splenomegaly represents a finding corresponding to a palpable mass in the abdomen below the

Table 1. Causes of splenomegaly

Liver disease	cirrhosis, hepatitis
Hematologic malignancies	lymphomas, leukemias, myeloproliferative disorders
Venous thrombosis	portal or hepatic vein thrombosis
Splenic congestion	venous thrombosis, portal hypertension
Cytopenias	immune thrombocytopenic purpura, autoimmune hemolytic anemia, immune-mediated neutropenia, Felty syndrome
Splenic sequestration	pediatric sickle cell disease, hemolytic anemias, thalassemias
Acute or chronic infection	bacterial endocarditis, infectious mononucleosis, HIV, malaria, tuberculosis, histiocytosis, abscess
Connective tissue diseases	systemic lupus erythematosus, rheumatoid arthritis, adult-onset Still's disease, and some familial autoinflammatory syndromes
Infiltrative disorders	sarcoidosis, amyloidosis, glycogen storage disease
Focal lesions	hemangiomas, abscess, cysts, metastasis

left costal margin at a distance > 8 cm towards the superior iliac spine. The recommendation of the EAES is to use metric units for defining splenomegaly in preoperative imaging diagnostics. The golden standard in diagnosing and determining massive splenomegaly is calculating the splenic volume; however, this procedure requires more time than standard radiological methods [5, 6].

An enlarged spleen can occur within numerous infectious diseases, with hereditary diseases, immunological diseases, as well as in hematological diseases. The most frequent causes of splenomegaly are shown in Table 1 [7].

Pronounced symptoms are mostly present in massive splenic enlargement, which is why patients commonly complain of symptoms manifesting after food intake, of the inability to adequately empty their bladders, of obstructive complaints, as well as of the inability to flex their trunk [8, 9, 10].

In the patient we are presenting, the symptoms were the main reason for his visiting the doctor again. It is our belief, based on insight into the medical records, that bilateral inguinal hernia was directly caused by splenic enlargement and increased pressure within the abdomen.

Splenomegaly can generally be treated with medication, with reductive therapy (radiation therapy), although in massive splenomegaly, potential complications related to this condition should always be taken into consideration – this is why, in this sense, surgical treatment should not be delayed. As a treatment modality in potential rupture, preoperative embolization of the splenic artery should be considered, when there are technical conditions for this procedure, since, in this way, the possibility of bleeding is reduced and partial reduction of the volume of the spleen is achieved, which further facilitates surgical treatment [11, 12, 13].

Since the diagnosis had been established preoperatively and since other treatment options had been exhausted, splenectomy was performed in our patient as a form of palliative treatment, i.e., debulking.

Surgical treatment is a second, sometimes a third therapeutic option, in treating splenomegaly. Taking into consideration all the possible benefits and possible complications of surgical treatment, including the quality of life of splenectomized patients, preoperative assessment should be made, and surgical treatment should be selectively applied, bearing in mind that massive splenomegaly is a great surgical challenge.

Conflict of interest: None declared.

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Масивна спленомегалија као хируршки изазов – приказ болесника и преглед литературе

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САЖЕТАК

Увод Према критеријумима Европског удружења за ендоскопску хирургију масивна спленомегалија подразумева случајеве у којима максимални краниокаудални дијаметар слезине прелази 20 *cm*. Осим метаболичких, хематолошких и хемодинамских поремећаја, значајно увећана слезина може испољавати непожељан компресивни ефекат на суседне органе и васкуларне структуре.

Циљ овог рада је да се прикажу изазови и технике хируршког лечења масивне спленомегалије.

Приказ болесника Болесник стар 62 године са раније дијагностикованом спленомегалијом, који је занемарио значај редовних контролних прегледа, примљен је на клинику због тегоба узрокованих масивном спленомегалијом. После пажљиво спроведене преоперативне припреме одстрањена је слезина краниокаудалног дијаметра 38 *cm*.

Закључак Узимајући у обзир све могуће добробити али и компликације хируршког лечења болесника са масивном спленомегалијом, неопходна је свеобухватна преоперативна хируршка процена и припрема, а хируршко лечење се планира у пажљиво изабраним случајевима.

Кључне речи: спленомегалија; масивна спленомегалија; спленектомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Association of recurrent fever and anemia with infective endocarditis in a 13-year-old girl with bicuspid aortic valve

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SUMMARY
Introduction Infective endocarditis is relatively rare in pediatric population, but can result in significant morbidity and mortality. Children with bicuspid aortic valve are at higher risk of developing infective endocarditis as compared to the general population. Our objective is to emphasize the importance of rapid diagnosis and proper treatment of infective endocarditis in patients with bicuspid aortic valve with the aim of preventing serious adverse events.
Case outline We report a case of a 13-year-old girl with a newly diagnosed bicuspid aortic valve who developed infective endocarditis with severe complications and underwent cardiac surgery. Recurrent fever and anemia, as well as cardiac murmur, were present for six months prior to diagnosing infective endocarditis. During the course of illness, only one of many blood cultures taken was positive for *Streptococcus sanguinis*.
Conclusion Patients with bicuspid aortic valve require careful evaluation for infective endocarditis, especially if recurrent fever associated with anemia is present. Delayed diagnosis of infective endocarditis is associated with serious complications.
Keywords: endocarditis; congenital heart defect; children; case report

INTRODUCTION

Infective endocarditis (IE) is a rare and life-threatening disease in the pediatric population. The predominant underlying condition of IE in children nowadays is congenital heart disease, of which bicuspid aortic valve (BAV) is common. Bicuspid aortic valve occurs predominantly in men, and currently is considered an intermediate-risk factor for IE. The presentation of IE in children may be fulminant, but more often has slow progress, with prolonged low-grade fever, and a variety of somatic complaints. Consequently, diagnosing IE in children is challenging and frequently delayed. However, the presence of a new murmur or a change in the nature of a preexisting one is significant [1, 2].

We report a case of a 13-year-old girl with newly diagnosed BAV, who developed IE with severe complications and underwent cardiac surgery. Recurrent fever and anemia, as well as cardiac murmur, were present six months prior to IE diagnosis. During that period, the girl was hospitalized three times and received six courses of antibiotic therapy. Numerous blood cultures were taken, but only one was positive for *Streptococcus sanguinis*. Our objective is to emphasize the importance of rapid diagnosis and proper treatment of IE in BAV patient with the aim to prevent serious adverse events.

CASE REPORT

A 13-year-old girl without significant medical history appeared at a sports preparticipation screening at a primary care center with grade 2/6 systolic heart murmur. Electrocardiogram and routine laboratory tests were normal with the exception of slightly lower hemoglobin concentration and hematocrit levels (Table 1). On the cardiologist's evaluation one month later, the transthoracic echocardiogram (TTE) showed BAV with aortic insufficiency grade II. The diameter of the aortic annulus was normal, with normal flow rate and an eccentric insufficiency jet. The cardiologist advised next examination in six months, with permitted recreational sport activities.

During the next three months, the girl had three episodes of upper respiratory tract infection with fever, associated with iron-deficiency anemia (Fe 3.9 μmol/l). In every episode, oral antibiotic treatment (azithromycin, amoxicillin, and amoxicillin/clavulanic acid, respectively) improved the symptoms, but the patient was

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Table 1. Laboratory results during the course of illness

Variable	Sports Exam	Febrile illness after sports exam					First Hosp.	First Discharge	Second Hosp.	Third Hosp.
		A month after	Two months after	Three months after	Four months after	Four months after				
SE (mm/h)	-	-	-	-	46	46	55	65	-	-
Hgb (g/l)	111	103	93	90	84	80	85	91	84	111
Hct (l/l)	0.35	0.32	0.297	0.291	0.272	0.26	0.257	0.30	0.27	0.36
Er ($\times 10^{12}$/l)	4.46	4.2	4.09	4.07	3.91	3.77	4.01	4.3	4.03	5.05
MCV (fl)	78.5	76.7	72.5	71.5	69.7	68.9	67.8	-	67.0	-
Le ($\times 10^9$/l)	6.2	6.8	8.0	5.5	7.8	7.3	12.5	11.7	10.36	12.2
Tr ($\times 10^9$/l)	231	313	280	218	187	222	360	362	210	274
CRP (mg/l)	-	12	48	12	> 96	> 96	59.7	42.4	133.1	149.2

SE – erythrocytes sedimentation rate during first hour; HgB – hemoglobin; Hct – hematocrit; Er – erythrocytes; MCV – mean corpuscular volume; Le – leucocytes; Tr – thrombocytes; CRP – C-reactive protein

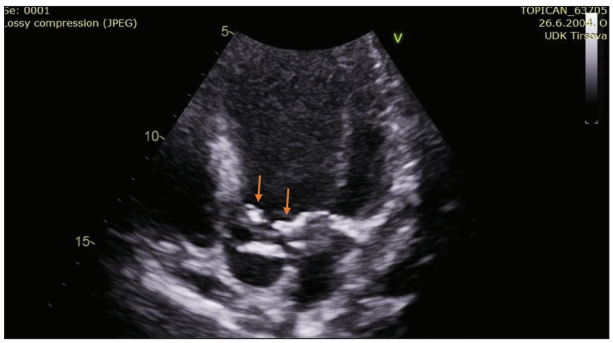

Figure 1. Five-chamber transesophageal echocardiography view of the patient with a bicuspid aortic valve showing vegetations in the left ventricular outflow tract (indicated by the arrows)

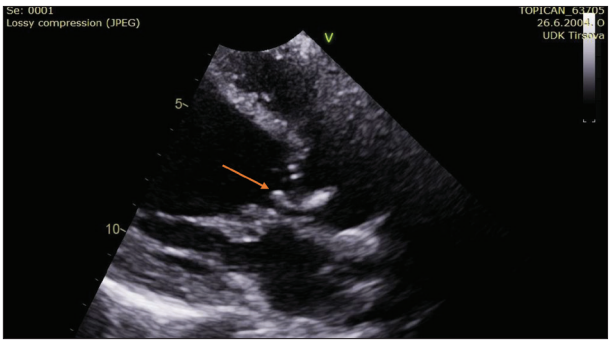

Figure 2. Parasternal long axis transesophageal echocardiography view of the patient with a bicuspid aortic valve showing vegetation in the left ventricular outflow tract (indicated by the arrow)

unresponsive to iron supplementation (Table 1). Peripheral blood smear showed hypochromic red blood cells with anisocytosis.

Three months after diagnosing BAV, the girl was hospitalized due to five-day fever (> 38°C) with nausea, vomiting, dizziness, weakness, and leg pain. Inflammatory markers were elevated and anemia got worse (Table 1). All blood cultures were negative and the TTE cardiac findings were unchanged as compared to the baseline. The girl was treated with an oral antibiotic (cefpodoxime) and discharged home in good general condition.

A few weeks following the first hospitalization, the girl presented to hospital again with a four-day fever (39°C) and right thigh pain. Inflammatory markers were elevated and anemia present (Table 1). On auscultation, a diastolic murmur was heard. Abdominal computerized tomography demonstrated splenomegaly (131 × 52 mm) and TTE suspected verruca on the anterior mitral valve leaflet with mild mitral and aortic regurgitation. Infective endocarditis was suspected and empirical intravenous (IV) antibiotic therapy initiated (linezolid and gentamicin for 14 days). Out of several blood cultures taken, only one was positive for *Streptococcus sanguinis* and the antibiotics were changed to IV penicillin G and gentamicin for 14 days. The girl's condition slowly improved and she was discharged home after five weeks of hospitalization.

Two weeks after the second hospitalization, the girl presented at a tertiary hospital reporting three-day fever (up to 38.8°C), acute onset of severe headache, and right leg pain that made walking difficulties. On admission, the patient was febrile (> 38°C), had low blood pressure with a wide pulse pressure (100/20 mmHg), and a diastolic murmur was present. Inflammatory markers were elevated (Table 1). One major and three minor modified Duke criteria for IE were established. Transesophageal echocardiogram (TOE) showed a circular formation (14 × 9 mm) on the anterior mitral valve leaflet (Figures 1 and 2). In addition, a suspected rupture of BAV coronary leaflet as well as significant mitral and moderate aortic regurgitation were present. The left ventricle was mildly dilated with preserved ejection fraction of 70%. Doppler ultrasound of the legs as well as computerized tomography of the head were normal. Abdominal magnetic resonance imaging confirmed splenomegaly (140 × 47 × 67 mm). N-terminal pro-brain natriuretic peptide was 2672 pg/ml. The serial blood cultures were negative and empirical antibiotic therapy for blood culture-negative IE was initiated (IV ampicillin and gentamicin). Fever persisted for three weeks and repeated TOE showed no reduction in vegetation. Antibiotic therapy was changed to IV penicillin G and amikacin. On the 30th day of hospitalization, N-terminal pro-brain natriuretic peptide was doubled (5217 pg/ml) and TOE showed suspected perforation of the aortic and mitral valve. The finding was confirmed by multislice detector cardiac computerized tomography, which showed an anterior–posterior BAV without raphe, thickened coronary cusp (2.5 mm), 4.3 mm leaflet perforation, and two aortic valve aneurysms (4.8 × 5 mm and 11.5 × 12 mm).

Additionally, a periannular abscess (19.8 × 6.2 × 14.6 mm) was present along the anterior wall of the aortic root. The anterior mitral valve leaflet was thickened (2.5 mm) with an aneurysm (11 × 13 mm) at the site of previous vegetation and a medial cusp perforation (2 mm in diameter).

On the 58th day of hospitalization, the patient underwent aortic valve replacement with 19 mm bileaflet mechanical prosthesis (St Jude Medical, St Paul, MN, USA), along with aortic root augmentation and anterior mitral leaflet reconstruction. No vegetations were present at surgery. Antibiotic prophylaxis for bacterial endocarditis (IV cefazolin, amikacin, vancomycin) was administered following the operation. Subsequent laboratory tests and electrocardiogram were normal. The patient recovered uneventfully and was discharged asymptomatic on the 18th postoperative day.

At one-year follow-up, the girl was asymptomatic, and TTE showed significantly lower size of the left ventricle, normal function of the mechanical valve and residual moderate regurgitation at the place of the anterior mitral leaflet reconstruction.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient's parent/guardian for the publication of this case report and any accompanying images.

DISCUSSION

Despite improvements in diagnostics and management, IE remains associated with significant morbidity and mortality. Congenital heart diseases predispose to the development of IE. Bicuspid aortic valve is the most common form, with a prevalence of 0.5–2% in the general population and is currently considered intermediate-risk cardiac condition for IE [1, 2]. Some studies showed that the risk of IE was markedly greater for BAV than tricuspid aortic valve patients [3]. Patients with IE and BAV were also significantly younger and had similar rates of intracardiac complications as high-risk patients [4]. As of recently, we must also consider that COVID-19 infection and acute endocarditis may present similarly, yet with very different treatments [5].

The presence of non-specific febrile illness, irrespective of the duration of the fever, fever pattern, or the resolution of fever with antipyretics, in children with congenital heart disease should be considered as suspected IE [6]. Routine laboratory findings in IE are non-specific, such as elevated inflammatory markers and anemia, usually normocytic and normochromic, which reveals the disease activity and is well known as anemia of inflammation [7]. Our patient had hypochromic and anisocytic anemia, but also slightly lower hemoglobin concentration and hematocrit levels present six months prior to IE. Although anemia was mild, it cannot be ruled out it was associated with the development of IE. One analysis has shown that iron-deficiency anemia changed oral microbiota by decreasing overall bacterial diversity and altered taxonomic composition but it did not identify whether iron deficiency anemia can raise the risk of IE [8]. A new-onset systolic murmur was also discovered prior to BAV diagnosis in our patient. Nevertheless, the typical murmur of aortic insufficiency, revealed on TTE one month afterwards, is diastolic. Mitral and aortic regurgitation were developed later in the course of illness alongside with the diastolic murmur. The abovementioned limits us from drawing a firm conclusion on the association of the new-onset cardiac murmur with the beginning of IE. A study by N'Guyen et al. [9] showed that the time interval between IE first symptoms and the diagnosis is closely related to the IE clinical presentation, patient characteristics, and the causative microorganism.

Infective endocarditis in BAV patients is mostly community acquired with oral cavity viridans group streptococci as the most common causative microorganisms. Our patient denied any dental procedures; however, even routine daily dental hygiene could cause oral bacteria enter the bloodstream [1, 2, 10]. History of excessive antibiotic use in our patient might have been the reason for only one *Streptococcus sanguinis*-positive blood culture out of numerous taken. Other possible reasons for negative blood cultures may include infections with highly fastidious bacteria or IE caused by a virus or a fungus. Culture-negative IE is described in patients with clinical and echocardiographic evidence of IE, with blood cultures yielding no organisms [11]. In our patient, no vegetations were found during the cardiac surgery and pathological examination of the resected valvular tissue was not done. Nevertheless, histological diagnosis of IE remains the gold standard according to the guidelines [11]. Our patient's IE diagnosis is based on one major (echocardiogram positive for IE) and three minor modified Duke criteria (fever, predisposing heart condition, positive blood culture).

Surgical treatment is used in approximately half of patients with IE due to severe complications. Heart failure is the most frequent complication of IE, observed in 42–60% of native valve endocarditis cases and represents the most common indication for surgery. It is more often present when IE affects the aortic rather than the mitral valve [11]. Patients with BAV IE have a high risk of perivalvular abscesses and thus prompt diagnosis and timely surgery might be required [12]. Even though antibiotic therapy for IE was administered appropriately for the age, dose, and duration, our patient underwent cardiac surgery due to significant insufficiency of both the aortic and the mitral valve, as well as other intracardiac complications.

It is worth mentioning that according to the Sievers classification, our patient had the anterior–posterior BAV type 0 with no raphes, which is rare [13]. A large multicenter study showed that the presence of raphe is a risk factor significant for both aortic stenosis and regurgitation and subsequent aortic valve and aortic surgery [14]. Considering BAV phenotypes according to the fusion of leaflets, our patient had a fusion of the right and the left coronary cusp (coronary cusp fusion). All other types of BAV (mixed cusp fusion) are considered to be one of the risk factors for the occurrence of aortic stenosis and associated aortopathy, which could result in significant hemodynamic changes [2].

Diagnosing IE may be difficult due to non-specific symptoms. However, the presence of a cluster of symptoms in a patient with BAV requires careful evaluation for IE. If recurrent fever and anemia are present in children with BAV, IE should always be suspected. Late IE diagnosis is associated with a high risk of serious complications and higher rates of surgical interventions.

Conflict of interest: None declared.

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Повезаност рекурентне температуре и анемије са инфективним ендокардитисом код тринаестогодишње девојчице са бикуспидном аортном валвулом

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САЖЕТАК

Увод Инфективни ендокардитис је редак у педијатријској популацији, али узрокује значајано оболевање и смртност. Деца са бикуспидном аортном валвулом имају већи ризик од развоја инфективног ендокардитиса у односу на општу популацију. Наш циљ је да истакнемо важност брзе дијагнозе и правилног лечења инфективног ендокардитиса код болесника с бикуспидном аортном валвулом, у циљу спречавања озбиљних нежељених догађаја.

Приказ болесника Приказујемо случај тринаестогодишње девојчице са новодијагностикованом бикуспидном аортном валвулом која је развила инфективни ендокардитис са тешким компликацијама и била подвргнута кардиохируршкој операцији. Рекурентна температура и анемија, као и срчани шум, били су присутни шест месеци пре постављања дијагнозе инфективног ендокардитиса. Током болести, само једна од многобројних узетих хемокултура била је позитивна на *Streptococcus sanguinis*.

Закључак Болесници са бикуспидном аортном валвулом захтевају пажљиву процену у погледу инфективног ендокардитиса, посебно ако је присутна рекурентна температура удружена са анемијом. Одложено постављање дијагнозе инфективног ендокардитиса је повезано са озбиљним компликацијама.

Кључне речи: ендокардитис; урођена срчана мана; деца; приказ болесника

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Exercise-induced Valsalva retinopathy – a case report and literature review

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SUMMARY

Introduction Valsalva retinopathy is an uncommon disorder that usually presents with acute onset of unilateral, or less frequently, bilateral visual impairment varying from subtle scotoma to total vision loss. It occurs as a result of Valsalva's maneuver. In the vast majority of cases, hemorrhage is preretinal although subretinal, intraretinal and vitreous hemorrhage can be found. Valsalva retinopathy often develops due to numerous triggering activities such as vomiting, coughing, heavy weight lifting, intense aerobic exercise, labor, and general anesthesia. Management options are either clinical observation or invasive techniques. We report a case of premacular hemorrhage due to Valsalva retinopathy induced by gym training.

Case outline A 34-year-old woman was referred to the Eye Clinic, University Clinical Center of Serbia, complaining of sudden and painless unilateral decrease in vision occurred during intense physical activity. Best corrected visual acuity was measured as counting fingers at five meters distance. Dilated fundoscopy demonstrated a large, well demarcated premacular subhyaloid hemorrhage with visible rupture of the retinal vein branch. The patient was treated conservatively. Three months after the onset of symptoms, hemorrhage absorbed and best corrected visual acuity was 20/20.

Conclusion Valsalva retinopathy, although a rare condition, should not be omitted as a differential diagnosis of retinal and vitreous hemorrhages. Standard, observational treatment is generally sufficient for complete vision recovery; however, literature suggests that an individualized approach to each patient is required.

Keywords: Valsalva retinopathy; Valsalva's maneuver; preretinal hemorrhage; vitreous hemorrhage

INTRODUCTION

Valsalva retinopathy (VR) is an uncommon disorder that usually presents with acute onset of unilateral or, less frequently, bilateral visual impairment varying from subtle scotoma over blurry vision to total vision loss [1, 2]. It occurs as a result of Valsalva's maneuver, forced expiratory effort against a closed airway, which causes increase in venous intrathoracic pressure [3]. This rapid rise in venous pressure is transmitted to the retinal capillaries causing their rupture, typically at the macular area [1, 3, 4]. In the vast majority of cases, hemorrhage is preretinal (sub-internal limiting membrane (sub-ILM) or subhyaloid), although subretinal, intraretinal and vitreous hemorrhage can be found [5].

VR often develops in young adults with no medical history, due to numerous triggering activities such as vomiting, coughing, heavy weight lifting, intense aerobic exercise, labor, or general anesthesia [6, 7]. It is most commonly found in adolescent males [8].

Diagnosis can be challenging considering that premacular hemorrhage may also be seen in systemic and many other ocular conditions including proliferative diabetic retinopathy, vein occlusion, macroaneurysm, Terson's syndrome, shaken adult syndrome, and blunt trauma [9, 10].

Management options are either clinical observation or invasive techniques [9], such as neodymium-doped yttrium aluminum garnet (Nd: YAG) laser hyaloidotomy, green argon laser, pneumatic relocation of the hemorrhage with intravitreal gas and pars plana vitrectomy (PPV) [6]. Generally, the blood resorbs spontaneously with full recovery of visual acuity, however, prolonged presence of preretinal hemorrhage can lead to pigmentary macular damage or development of epiretinal membrane. Moreover, continuous exposure to hemoglobin and iron may cause toxic retinal damage which can be irreversible [6, 11]. When choosing therapeutic option, thickness and amount of blood should be considered as well as anatomical location of the hemorrhage [12].

In this paper, we report a case of premacular hemorrhage due to VR induced by a gym training.

CASE REPORT

A 34-year-old woman was referred to the Eye Clinic, University Clinical Center of Serbia, complaining of a sudden and painless unilateral decrease in vision which occurred during intense physical activity in gym. Initially, she spotted "sparkles" in front of both eyes, then an hour later, while heavy weight lifting, she

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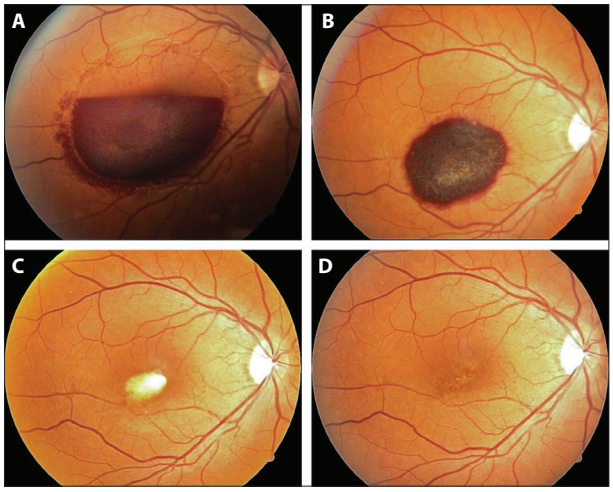

Figure 1. Color photo of the affected eye; A) on admission – large, well demarcated premacular subhyaloid hemorrhage more than 4-disc diameter in size; B) three weeks after discharge – the blood had started to resolve; C) two months after the accident – significantly smaller, dehemoglobinized hemorrhage in the macular area; D) at the third follow-up, three months after the onset of symptoms – complete resolution of the hemorrhage

noticed blurry vision and a black spot in her right eye. There was no medical history, however positive family history of abdominal aneurysm and cerebrovascular insult was present. Ocular anamnesis was negative and she denied history of eye trauma. A complete ophthalmological examination was performed. Best corrected visual acuity (BCVA) was measured as counting fingers at five meters distance in her right eye and 20/20 in her left eye. The intraocular pressures, pupillary light reflexes and eye motility tests were normal. There was no evidence of abnormalities of the both anterior segments. Dilated fundoscopy demonstrated a large, well demarcated premacular subhyaloid hemorrhage with visible rupture of the retinal vein branch in the right eye (Figure 1A). Left eye fundoscopy was unremarkable. The patient was admitted to the hospital for further clinical observation and treatment. All laboratory parameters (complete blood work, hemostasis, and biochemical profile) were within normal limits. During hospitalization an optical coherence tomography (OCT) and fundus photography were performed. The OCT showed localized hyperreflective lesion causing shadowing of the underlying retinal layers while central macular thickness was 779 μm (Figure 2A). Considering the patient's anamnesis and appearance of the hemorrhage on clinical examination and OCT, VR diagnosis was established. The patient was treated conservatively with topical glucocorticoid, nonsteroidal anti-inflammatory, anticholinergic drugs and oral antihemorrhagic agents. After several days of follow-up, the patient was discharged home with recommendation for close regular follow-up. At the first follow-up, three weeks after discharge from hospital, clinical examination revealed smaller hemorrhage in the macular area (Figures 1B and 2B), suggesting that blood started to resolve spontaneously, although there was no improvement in visual acuity. Second follow-up, two months after the accident, showed significantly smaller, dehemoglobinized blood

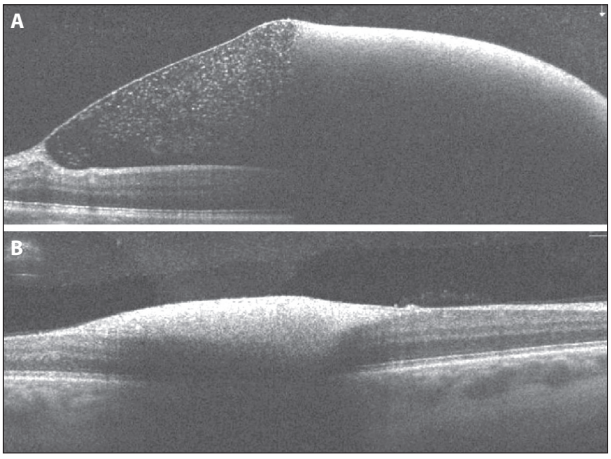

Figure 2. Optical coherence tomography of the affected eye; A) on admission – localized, hyperreflective lesion and shadowing of the underlying retinal layers; B) three weeks after discharge from the hospital

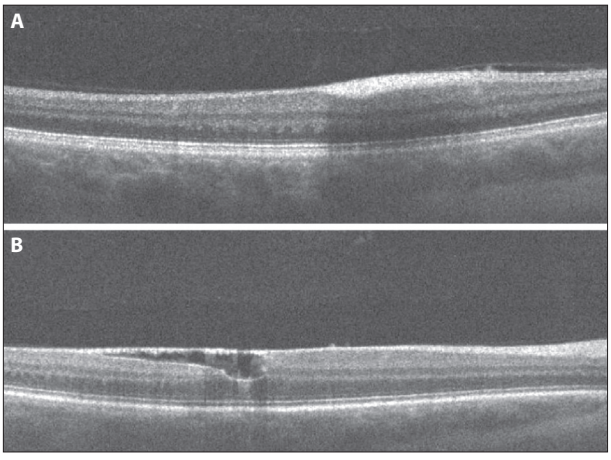

Figure 3. Optical coherence tomography of the affected eye; A) two months after the accident; B) at the third follow-up, three months after the onset of symptoms – epiretinal membrane formation

(Figures 1C and 3A). BCVA on the affected eye improved to 20/100. The hemorrhage was still in the macular area which is the reason why visual acuity was not fully recovered. At the third follow-up, three months after the onset of symptoms, hemorrhage absorbed and BCVA was 20/20, yet the patient reported that visual quality was slightly worse compared to the unaffected eye, which was presumably due to epiretinal membrane formation discovered by OCT (Figures 1D and 3B).

This care report was done in accordance with the institutional standards on Ethics and principles of the Declaration of Helsinki.

DISCUSSION

VR was first described by Duane [13]. It arises secondary to physical activities corresponding to Valsalva's maneuver. The absence of functional valves in the venous system of head and neck allows transmission of the elevated systemic venous pressure to the eye resulting in rupture of the superficial retinal vessels. Localization of preretinal hemorrhage is most commonly on the posterior pole probably

due to lose bonds between internal limiting membrane and retina right peripheral to the fovea [2].

Patients with VR are often young or middle-aged people who present complaining of sudden unilateral floaters, scotoma, or even complete loss of vision [1]. Good medical history and thorough examination of both eyes are essential in diagnosis since the differential diagnosis for the unilateral retinal hemorrhages includes multiple conditions. Systemic diseases, such as diabetes mellitus, hypertension, hematologic disorders, should be excluded. The patient should be asked about recent head or eye injury to rule out blunt trauma [2, 10]. Valsalva-induced and spontaneous sub-ILM and vitreous hemorrhages due to temporarily dysfunctional coagulation status in patients with SARS-CoV-2 infection were also described in recent years [14, 15, 16]. Another possible cause is Terson's syndrome, which arises secondary to subarachnoid hemorrhage. Pathogenesis of this syndrome is similar as in VR – rapid rise in intracranial pressure is transferred to retinal vein system leading to retinal or vitreous bleeding [17]. Therefore, neurological signs and symptoms should be observed to exclude Terson's syndrome [2, 10]. Considering the fact that our patient had positive family history of abdominal aneurysm, we involved the rupture of retinal aneurysm as differential diagnosis, although this condition is usually seen in older population as an outcome of hypertension and arteriosclerosis [18]. OCT is useful in determining the exact location of the hemorrhage and fundus imaging is very significant in monitoring the progression/regression of the disease [12]. In our patient, through careful anamnesis, laboratory analyses and detailed examination of both affected and unaffected eye, diagnosis of VR was made with certainty.

There is no clear protocol for treating VR. However, literature data suggests that standard treatment is observation and in the majority of patients vision is fully recovered over a period of few months [19, 20]. The main issue regarding conservative approach is the extended exposure of the retina to phototoxic blood products (hemoglobin and iron) which can lead to permanent vision damage or formation of an epiretinal membrane [20]. According to one retrospective study conducted on 24 eyes with VR, preretinal hemorrhage size 4-disc diameter or less could be conservatively treated. In the same study, all of their 13 patients who were observed for spontaneous resorption of the hemorrhage recovered visual acuity of 20/20 over the next six months [12]. Many other studies reported resolution of the blood and good visual outcome after observation period without any additional treatment [1, 2, 5]. If conservative treatment is chosen, patient should be advised to rest and not to involve in any physical activity. Sleeping in half-sitting position is also recommended to accelerate blood settling [12]. Nd: YAG laser was successfully used in management of VR. This procedure involves laser perforation of the posterior hyaloid membrane or ILM resulting in drainage and inferior settlement of the blood into the vitreous cavity [20]. Nd: YAG laser is considered to be relatively safe, affordable and effective option in treatment of this condition, yet several complications including macular hole, epiretinal membrane and retinal detachment were reported [19, 21]. There are remaining questions about patient selection, timing of laser treatment and energy levels needed. Most of the studies indicate that Nd: YAG laser can be used in patients with non-clotted preretinal hemorrhage greater than 3-disc diameter in size and no longer than three weeks of duration. Energy levels should be as low as needed to perforate hyaloid/internal limiting membrane, not crossing 9 mj [9, 12, 19, 22, 23]. In a case series presented by Kuruvilla et al. [9] visual acuity was improved in three out of four patients who underwent laser treatment. Dulger et al. [12] performed Nd: YAG laser hyaloidotomy in 10 eyes, all of which restored good vision in the first week after treatment. One recent case report, showed that laser treatment can be effective even when done more than three weeks after the onset of symptoms, yet treatment had to be repeated on two separate occasions in order to achieve a favorable result [22]. In contrary to previously mentioned studies, García Fernández et al. [6] reported two unsuccessful Nd: YAG laser treatments due to coagulated blood, therefore they decide to perform PPV resulting in full visual recovery one day postoperatively in both cases.

When there is insufficient spontaneous resorption of the preretinal hemorrhage and Nd: YAG laser is not indicated or failed due to coagulated blood, PPV can be performed. It is an invasive surgical technique with well-known complications, for instance cataract and macular hole formation [20]. García Fernández et al. [6] managed five of their patient's performing vitrectomy, all with excellent postoperative visual acuity, yet one of the patients developed cataract and retinal break.

In conclusion, VR although a rare condition, should not be omitted as a differential diagnosis of retinal and vitreous hemorrhages. Standard, observational treatment is generally sufficient for complete recovery of vision; however, literature suggests that an individualized approach to each patient is required.

Conflict of interest: None declared.

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Валсалвина ретинопатија изазвана вежбањем – приказ болесника и преглед литературе

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САЖЕТАК

Увод Валсалвина ретинопатија је ретко обољење које се најчешће испољава једностраним, ређе обостраним, оштећењем вида које варира од дискретног скотома до потпуног губитка вида. Јавља се као резултат Валсалвиног маневра. У највећем броју случајева крварење је преретинално, иако се могу пронаћи и субретиналне, интраретиналне и витреалне хеморагије. Валсалвина ретинопатија се често развија услед бројних окидачких активности као што су повраћање, кашљање, дизање тешког терета, порођај, интензивни аеробни тренинг и општа анестезија. Терапијске опције укључују праћење или инвазивне методе. Приказујемо случај премакуларног крварења услед Валсалвине ретинопатије изазване тренингом у теретани.

Приказ болесника Жена стара 34 године јавила се у Клинику за очне болести Универзитетског клиничког центра Србије жалећи се на изненадно, једнострано и безболно ослабљење вида које се догодило током интензивне физичке активности. Најбоље коригована видна оштрина била је бројање прстију на пет метара удаљености. Преглед очног дна у мидријази показао је велико, јасно ограничено, премакуларно, субхијалоидно крварење са видљивом руптуром гране ретиналне вене. Пацијенткиња је конзервативно лечена. Три месеца после појаве симптома крварење се апсорбовало, а најбоље коригована видна оштрина била је 20/20.

Закључак Валсалвина ретинопатија, иако ретко обољење, не треба да буде диференцијално дијагностички занемарена код случајева ретиналних и витреалних крварења. Стандардни третман је праћење и он је углавном довољан за потпуни опоравак вида; међутим, подаци из литературе указују да је потребан индивидуални приступ сваком пацијенту.

Кључне речи: Валсалвина ретинопатија; Валсалвин маневар; преретинално крварење; витреално крварење

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Prehospital care of cardiac arrest in COVID-19 patients

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SUMMARY
During the COVID-19 pandemic, there was an urgent need to revise the existing cardiopulmonary resuscitation (CPR) guidelines published in 2015. The coronavirus pandemic increased the rate of cardiac arrests, and the need for CPR. International resuscitation associations proposed updated resuscitation guidelines during the COVID-19 pandemic. Although there is a clear consensus in most recommendations, there are also disparities. Their implementation in everyday clinical practice would alleviate the fear of health workers at the prehospital level and reduce the indecision to apply CPR in such patients as well.
Keywords: cardiopulmonary resuscitation; COVID-19; recommendations; prehospital care

INTRODUCTION

The year 2020 will be remembered as the year when the WHO declared the pandemic of the COVID-19 disease [1]. Thus, guidelines for cardiopulmonary resuscitation (CPR) published in the same year were adapted to this disease [2].

Cardiac arrest (CA) is the leading cause of death in the world, with around 700,000 people dying annually in Europe alone. It is most common at the out-of-hospital level with an incidence of 67–170 per 100,000 inhabitants [3]. The ongoing COVID-19 pandemic has dramatically altered the landscape of pre-hospital response to out-of-hospital CA (OHCA). Research exploring the incidence of OHCA events during the pandemic has been mixed. Parisian study indicated an increasing number of OHCA associated with an escalating COVID-19 case burden [4]. Italy's research demonstrated no appreciable change in OHCA incidence during the early pandemic period [5]. However, a meta-analysis published in December 2020 reported a 120% increase in incidence since the start of the pandemic, as well as higher mortality rates [6].

OHCA is potentially curable if early CPR is initiated [2]. The main and most significant difference between non-COVID and COVID-19 patients is in the second link of the chain, where early application of basic life support (BLS) in COVID-19 patients requires the application of a protective mask for rescuer and casualty, and that CPR is performed only by chest compressions [2].

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BASIC LIFE SUPPORT ADAPTED FOR COVID-19 PATIENTS

BLS can be performed by: lay persons, trained nonprofessionals, health care workers (HCWs) outside the workplace and without professional equipment, until the arrival of the emergency medical team (EMT) [7] or HCWs (both in- and out-of-hospital).

Emphasis is placed on: early identification of suspected or positive COVID-19 patients, the importance of safety (self, bystander, the patient) and application of personal protective equipment (PPE) in case of a decision to perform CPR [2].

In COVID-19 patients, CA is identified if a person is unresponsive and not breathing normally [2, 8]. In the current COVID-19 circumstances, to check breathing by the look-listen-feel method [9] is declared invalid and is replaced by a modified method look-feel for no longer than 10 seconds [7]. Do not open the airway (lifting the chin and tilting back the forehead) and do not check breathing by leaning over the persons' mouth/nose because of possible aerosol transmission [8].

If there are no signs of life and no breathing, or if there is any doubt about it, immediately perform CPR, call the emergency number 194 [8], and inform the dispatcher about the suspected or confirmed infection with the COVID-19 virus, so that a EMT can be sent with complete PPE.

Lay rescuers start and conduct compression-only CPR until the arrival of the EMT. There is evidence that compression-only CPR was better than no attempt at CPR [7]. Lay rescuers should consider placing a facemask or cloth/towel over the person's mouth and nose before performing chest compressions and public-access defibrillation. This may reduce the risk of spreading the virus during chest compressions.

Medical PPE, according to the European Resuscitation Council, is divided into two groups: minimum droplet-precaution PPE (gloves, short-sleeved apron, surgical mask, eye and face protection: fluid resistant surgical

mask with integrated visor or full-face shield/visor or polycarbonate safety glasses or equivalent) and minimum airborne-precaution PPE (double gloves, scaphander or long-sleeved apron, filtering facepiece FFP3/N99 mask/respirator (FFP2/N95 if FFP3 not available), eye and face protection: full-face shield/visor or polycarbonate safety glasses or equivalent) [2].

Without PPE, the use of BLS is not recommended for anyone [7], except in the case when the rescuer himself is infected or has contracted COVID-19.

HCWs perform CPR with chest compressions and ventilation with a bag-valve-mask (BVM) and oxygen at a 30:2 ratio [2]. In order to achieve effective chest compressions, apply pressure quickly, strongly, and without interruption: on the lower half of the sternum ('in the center of the chest'), depth of 5–6 cm, at a rate of 100–120/minute with as few interruptions as possible and allow the chest to recoil completely after each compression [8].

If a BVM not available, perform compression-only CPR. If a BVM available, use special high-efficiency particulate air (HEPA) filters or bacteria/virus filters during ventilation that reduces aerosol generation during CPR.

Instead of the usual method of ventilation with a BVM in non-COVID patients, the method of **one rescuer and two hands** (so-called C-E grip): one hand covers the mask-face seal, and the other presses the Ambu bag), in COVID-19 patients ventilation is performed by the method of **two hands and two rescuers** (so-called V-E grip): one rescuer covers the mask-face seal with both hands i.e., the thumbs and thenar eminence are placed over each side of the mask while the second through fifth digits pull the jaw upward, another rescuer presses the Ambu bag twice after 30 compressions) [10].

Early use of automated external defibrillator significantly increases the chances of survival and does not increase the risk of COVID-19 infection [2].

ADVANCED LIFE SUPPORT ADAPTED FOR COVID-19 PATIENTS

Guarantee a safe environment. Before starting CPR, all EMT members must make use of the recommended PPE, following the established fitting and removal standards. In COVID-19 era, CPR, due to some components being high aerosol-generating procedures [11], has become high-risk procedure for the HCWs. Instead of "*Primum non nocere*" (first do no harm), we are forced to change to "*Primum non nocere ad te*" (first do no harm to yourself) [12].

The following devices have been designed around the world to reduce COVID-19 virus contamination: Polycarbonate barrier box in which ventilation is performed by a technique involving two persons: one person holds a mask and the other ventilates on an Ambu bag, and protective aerosol box on intubation [13].

Once adequately protected, the presence of CA is to be confirmed, assessing the patient response to stimuli and the presence of spontaneous ventilation and pulse [14]. The quality chest compression should be started as soon as possible. Until the available defibrillator or until advanced airway management occurred [ideally with the use of endotracheal intubation (ETI)], compression-only CPR are performing [8]. Providing compression-only CPR and initial passive oxygenation through a "high oxygen concentration with low flow" mask system coupled with a HEPA filter or a nonrebreather face mask (covered with a surgical mask) is an acceptable alternative to active BVM ventilation during the initial phase of CPR [15]. Once a BVM device arrives, proceed with a compression: ventilation ratio of 30:2. The person doing compressions can pause to squeeze the bag [2].

Defibrillate as soon as indicated; without delay for application of PPE. In the witnessed shockable CA, and situations where a defibrillator can be applied immediately for defibrillation and the resuscitator has not been able to put on the corresponding PPE to start CPR, it is reasonable to follow the recommendation of applying three consecutive discharges without previous chest compression or compression between discharges [14]. There is no scientific evidence that direct current shock delivery leads to aerosol generation, so it can be delivered safely. Whenever possible, use self-adhesive electrodes instead of handheld defibrillator electrodes. If a defibrillator is not available, one team member will begin only the external massage until the defibrillator is delivered and prepared.

If not yet, place an oxygen mask and give oxygen. Leave the mask on the patient until a BVM device arrives [2], and then, proceed CPR with a compression: ventilation ratio of 30:2. Manual ventilation with a BVM with high-flow 100% oxygen (target: SaO_2 of 94–98%), should be performed only by experienced staff using a 2-person technique (V-E grip), because an ill-fitting mask/poor seal will generate an aerosol [2].

Establishment of an advanced airway in COVID-19 patients due to the high degree of aerosol contagion is one of the riskiest procedures in the treatment of these patients [16]. Provide airway by using ETI or supraglottic device or with mandatory placement of HEPA filters on the Ambu bag [17], as well as on the ventilator hoses. It is recommended that the ETI be performed by an experienced physician so that the procedure can be done in one act, which reduces the exposure to the virus, as well as to use an endotracheal tube with a balloon cuff [18]. If ETI is delayed, consider ventilation with a self-inflating balloon and/or the insertion of a supraglottic device, both with HEPA filters. The position of the placed endotracheal tube should not be checked auscultatory (transmission of infection), but only by capnography [9, 18] or observation of chest movements during ventilation with an Ambu bag. After ETI, perform chest compressions with 10 ventilations per minute. If feasible, one person performs ETI, another assists, and a third administers medication and sets up electrocardiogram monitoring [18]. Shao et al. [19] suggested frequent changes (every minute) of persons performing chest compressions, since performing chest compressions while wearing PPE causes rapid fatigue.

Provide a venous route for drug administration, giving preference to the intraosseous approach.

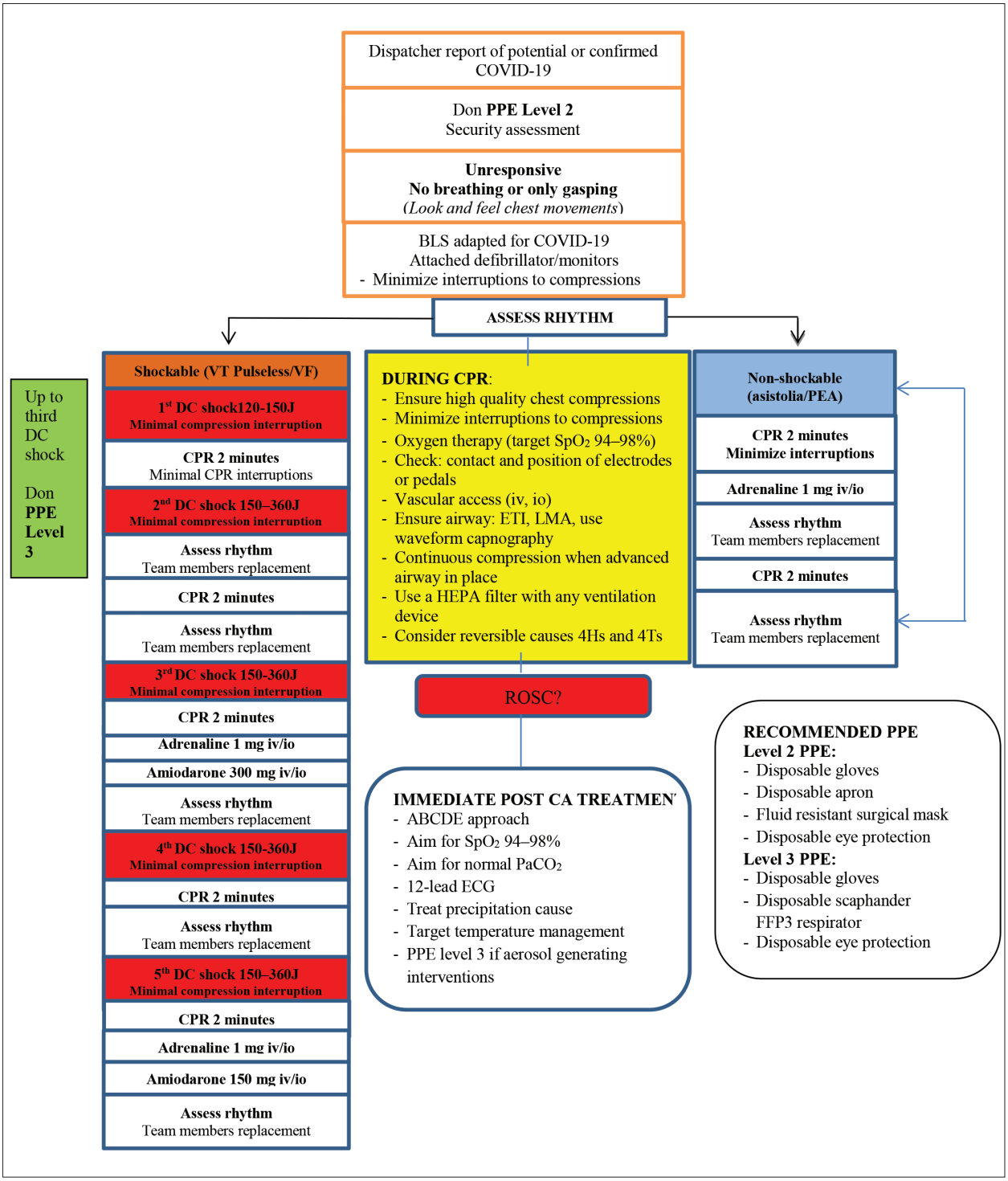

Figure 1. Advanced Life Support Algorithm for COVID 19 adult patients. Adapted for Resusci-tation Council UK guidance, available from: https://firstaidforlife.org.uk/giving-cpr-during-the-pandemic-resuscitation-council-uk-guidance/ [20]; PPE – personal protective equipment; BLS – basic life support; VT – ventricular tachycardia; VF – ventricular fibrillation; DC – direct current; CPR – cardiopulmonary resuscitation; ETI – endotracheal intubation; LMA – laryngeal mask air-way; HEPA – high-efficiency particulate air; ROSC – return of spontaneous circulation; CA – cardiac arrest; ECG – electrocardiogram; IV – intravenous; IO – intraosseous; PEA – pulseless electrical activity

All other algorithmic procedures during the management of CA (shockable or nonshockable), reversible causes of arrest, post-resuscitation care and the use of sophisticated medical equipment remain unchanged compared to the recommendations from 2021.

Figure 1 presents the Universal Advanced Life Support Algorithm adapted for COVID 19 patients [20].

TRANSPORT OF A SUCCESSFULLY RESUSCITATED PERSON

For easier memorization, the transport of a successfully resuscitated person is subject to the acronym **STOP COVID** [21]:

S (secure) – secure airway,

T (team building) – team griefing,

O (organize) –competence, team,

P (prepare) – prepare all the equipment,

C (checklist) – checklist for equipment and sequence of procedures,

O (optimize) – optimization of hemodynamic status and oxygenation,

V (vigilated) – careful dressing/undressing of protective equipment,

I (invasive) – invasive airways evaluation,

D (debriefing) – oral and written report at the time of patient handover.

After transport, completely and thoroughly disinfect (with chlorine-based solution) the ambulance and used medical apparatus and equipment, before the vehicle is re-included in the normal work process.

CONCLUSION

This paper presents a sublimation of relevant recommendations during the COVID-19 disease pandemic. Their implementation in everyday clinical practice would alleviate the fear of health workers at the prehospital level and reduce the indecision to apply CPR in such patients as well.

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The authors are familiar with the ethical standards of the journal. The work was done in accordance with these standards, as well as with the standards of the institutional committee on ethics.

Conflict of interest: None declared.

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Прехоспитално збрињавање срчаног застоја код оболелих од ковида 19

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САЖЕТАК

Током пандемије ковида 19 указала се хитна потреба за ревидирањем постојећих смерница за кардиопулмоналну реанимацију (КПР), објављених 2015. године. Пандемија ковида 19 је повећала инциденцу срчаног застоја и потребу за КПР. Међународна удружења за КПР предложила су модификоване препоруке за КПР током пандемије ковида 19. Иако постоји јасан консензус у већини препорука, постоје и диспаритети. Њихова примена у свакодневној клиничкој пракси ублажила би страх здравствених радника на прехоспиталном нивоу и смањила неодлучност да примене КПР и код оваквих болесника.

Кључне речи: кардиопулмонална реанимација; ковид 19; препоруке; прехоспитално збрињавање

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Comprehensive evaluation of risk factors for the development and complications of chemotherapy-induced febrile neutropenia

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SUMMARY
Febrile neutropenia is a serious adverse effect of chemotherapy. It can lead to complications and death, as well as delays in treatment, chemotherapy dose reductions, compromised treatment efficacy, and reduced survival. The assessment of the patient-related risk factors plays a significant role in the prevention of febrile neutropenia and its complications. In the case of intermediate-risk chemotherapy, the patient-related factors contribute to the estimation of an overall febrile neutropenia risk as well as to timely planning of primary prophylaxis using growth factors. Patients presenting with febrile neutropenia undergo a detailed initial risk assessment for serious complications so that an appropriate treatment can be selected. Recommendations given by the guidelines outline the classification of and risk factors for febrile neutropenia complications. The use of patient-related factors and validated tools for the risk assessment of complications makes it possible to optimize the treatment for each patient and to reduce the risk of morbidity and mortality due to febrile neutropenia.
Keywords: febrile neutropenia; patient-related risk factors; risk assessment

INTRODUCTION

Febrile neutropenia (FN) is an oncology emergency and one of the most frequent and most serious complications of chemotherapy treatment [1]. It is a significant cause of morbidity, mortality, and burden to healthcare services [2]. The incidence of FN in patients receiving chemotherapy for solid tumors is 10–50%, while for hematological malignancies it is up to 80% [1, 3]. Around 20–30% of patients with FN will present with complications requiring hospitalization with an overall mortality of 10% [1].

FN is defined as a fever (oral temperature of > 38.3°C or two consecutive readings of > 38°C, one hour apart) in patients with severe neutropenia (absolute neutrophil count of $< 0.5 \times 10^9$/l, or expected to fall below 0.5×10^9/l)[1, 3, 4]. In the majority of patients with FN, symptoms and signs of infection are absent. Bacteriaemia is documented in 20% of FN patients [1]. In the past, there used to be a prevalence of Gram- (G) negative bacteriemia among patients with FN, but in the last few decades the shift has occurred towards G-positive bacteriemia and at the present time the ratio between G-positive and G-negative bacteria is 60:40 [5]. Patients with FN and proven bacteriemia have poorer prognosis, with a mortality rate of 18% (G-negative) and 5% (G-positive) [1]. The most common isolated G-positive bacteria are *Staphylococcus spp.,* enterococci, and viridans streptococci, while among G-negative bacteria the most common are *Escherichia coli, Klebsiella spp.*, and *Pseudomonas aeuruginosa* [5]. Fungal and viral infections in patients with FN are rarely an initial type of infection and are related to prolonged severe neutropenia induced with high-dose chemotherapy regimens such as in hematological malignancies.

RISK FACTORS FOR THE DEVELOPMENT OF FEBRILE NEUTROPENIA

There is a clear relationship between the severity of neutropenia and the dose-intensity of chemotherapy [1]. According to the risk to induce FN, all chemotherapy regimens are classified as high risk (incidence of FN > 20%), intermediate risk (incidence of FN of 10–20%), or low risk ones (incidence of FN < 10%). The majority of high-risk regimens are high-dose chemotherapy regimens for the treatment of lymphomas, leukemias, osteo- and soft tissue sarcomas, and certain regimens for the treatment of colorectal, pancreatic, and breast cancer [6].

It has been shown that several factors, other than chemotherapy itself, are responsible for increasing the risk of FN and its complications, which is of special importance in the case of intermediate-risk chemotherapy regimens. These patient-related factors augment the risk produced by chemotherapy and create an overall risk for developing FN. The overall FN risk is high if one or more patient-related factors

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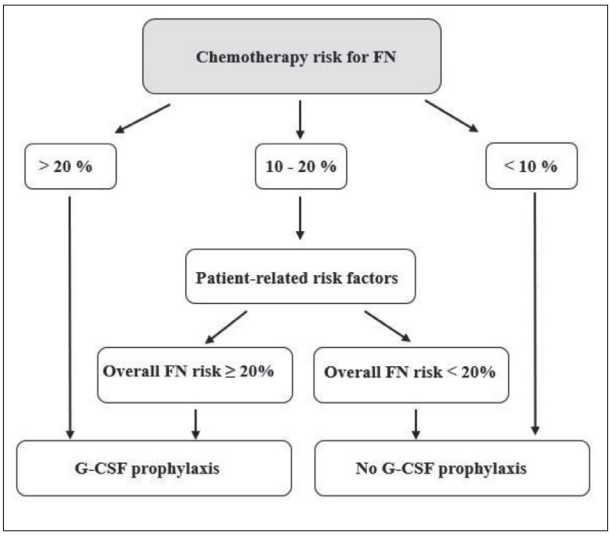

Figure 1. Decision making algorithm regarding the use of the granulocyte colony-stimulating factor (G-CSF) in primary prophylaxis of febrile neutropenia (FN)

are present. In everyday clinical practice, the majority of standard-dose chemotherapy protocols with the intermediate risk for FN are used for the treatment of various types of solid tumors [6]. Assessment of patient-related factors is of importance in order to prevent occurrence of FN and, consequently, morbidity, mortality, and burden to health care services. On the other hand, assessment of patient-related factors in order to prevent FN results in better prevention of chemotherapy dose delays and dose reductions that may affect overall survival.

Several meta-analyses have shown that primary prophylaxis with the granulocyte colony-stimulating factor (G-CSF) reduces the risk of FN by at least 50% in patients with solid tumors and lymphomas as well as early mortality during chemotherapy and infection-induced mortality. [7, 8, 9]. Most guidelines recommend the use of the G-CSF prophylactically if the risk of FN is > 20% for all planned cycles of treatment [1, 3, 6]. For patients with an intermediate risk, it is important to consider patient-related factors, as already mentioned (Figure 1) [1, 3, 6]. With most chemotherapy used for the treatment of common malignancies, the risk of FN is maximal during the first course of chemotherapy [4]. Thus, for patients at risk, primary prophylaxis of FN is recommended from the first cycle of therapy.

Data from the guidelines regarding patient-related risk factors are heterogenous (Table 1) [1, 3, 6].

Patient age is one of the most important patient-related risk factors for FN and the only one that all the guidelines agree upon. Advanced disease, comorbidities, poor performance status, as well as nutritional status, are equally important. The presence of malnutrition increases treatment-related toxicities in cancer patients receiving chemotherapy [10]. It is estimated that in 10–20% of patients, death is caused by malnutrition-related adverse events and not by the tumor itself; therefore, early assessment for malnutrition and adequate nutritional interventions before the start of the treatment are recommended [10]. Before the diagnosis of malnutrition is considered, it is mandatory to assess patients for being "at risk" of malnutrition by any validated risk screening tool (e.g. Malnutrition Universal Screening Tool, MUST) [11].There are several criteria that should be addressed in order to diagnose malnutrition: weight loss, anorexia, body composition (e.g. fat-free mass index, FFMI), anthropometry (e.g. body-mass index, BMI), and biochemical markers (albumin levels, C-reactive protein levels). The proposed criteria for the diagnosis of malnutrition are as follows: unintentional weight loss > 10% indefinite of time, or > 5% over the last three months combined with either BMI < 20 kg/m^2 (< 70 years), or < 22 kg/m^2 (≥ 70 years), or FFMI < 15 and 17 kg/m^2 in women and men, respectively [11].

In general, careful assessment of patient-related risk factors in patients scheduled to receive chemotherapy of intermediate risk for FN enables adequate estimation of an overall FN risk and, consequently, timely planning of primary prophylaxis with the G-CSF in order to prevent FN and its complications.

RISK FACTORS FOR THE COMPLICATIONS OF FEBRILE NEUTROPENIA

As mentioned before, FN is one of the most serious complications of chemotherapy treatment. However, not all the patients with FN will have complications or require hospitalizations. For example, a worse prognosis is expected in high-risk FN with the case of proven bacteriemia or the presence of a focal site of presumed infection (e.g. pneumonia, cellulitis) [1].

Multiple randomized control trials have demonstrated that outpatient treatment is safe and feasible in patients with low-risk FN, with associated savings in resources and improved patient's quality of life [12].

Table 1. Patient-related factors considered by the guidelines as risk factors for febrile neutropenia

NCCN	ASCO	ESMO
Prior ChT or RT	Age > 65 years	Age
Persistent neutropenia	ECOG performance status	Advanced disease
Bone marrow involvement by tumor	Nutritional status	History of prior FN
Recent surgery and/or open wounds	Comorbidities	No antibiotic prophylaxis or G-CSF use
Liver disfunction (bilirubin > 2)	History of prior FN	Mucositis
Renal disfunction (creatinine clearance < 50)		Poor performance status
Age > 65 years receiving full dose chemotherapy		Cardiovascular disease

NCCN – National Comprehensive Cancer Network; ASCO – American Society of Clinical Oncology; ESMO – European Society for Medical Oncology; ChT – chemotherapy; RT – radiotherapy; ECOG – Eastern Cooperative Oncology Group; G-CSF – granulocyte colony-stimulating factor; FN – febrile neutropenia

Table 2. Multinational Association for Supportive Care in Cancer tool for risk stratification in febrile neutropenia

Burden of illness	Severe symptoms Moderate symptoms No or mild symptoms	0 3 5
No hypotension (systolic blood pressure > 90 mmHg)		5
No chronic obstructive pulmonary disease		4
Solid tumor or hematological malignancy with no previous fungal infection		4
No dehydration requiring parenteral fluids		3
Outpatient at presentation		3
Age < 60 years		2

Table 3. Clinical Index of Stable Febrile Neutropenia score for risk stratification in febrile neutropenia

ECOG performance status ≥ 2	2
Stress-induced hyperglycemia ≥ 6.7 mmol/L or ≥ 13.9 mmol/L in diabetics or if on steroids	2
Chronic obstructive pulmonary disease	1
Cardiovascular disease	1
NCI mucositis ≥ 2	1
Monocytes < 200/μl	1

ECOG – Eastern Cooperative Oncology Group; NCI – National Cancer Institute

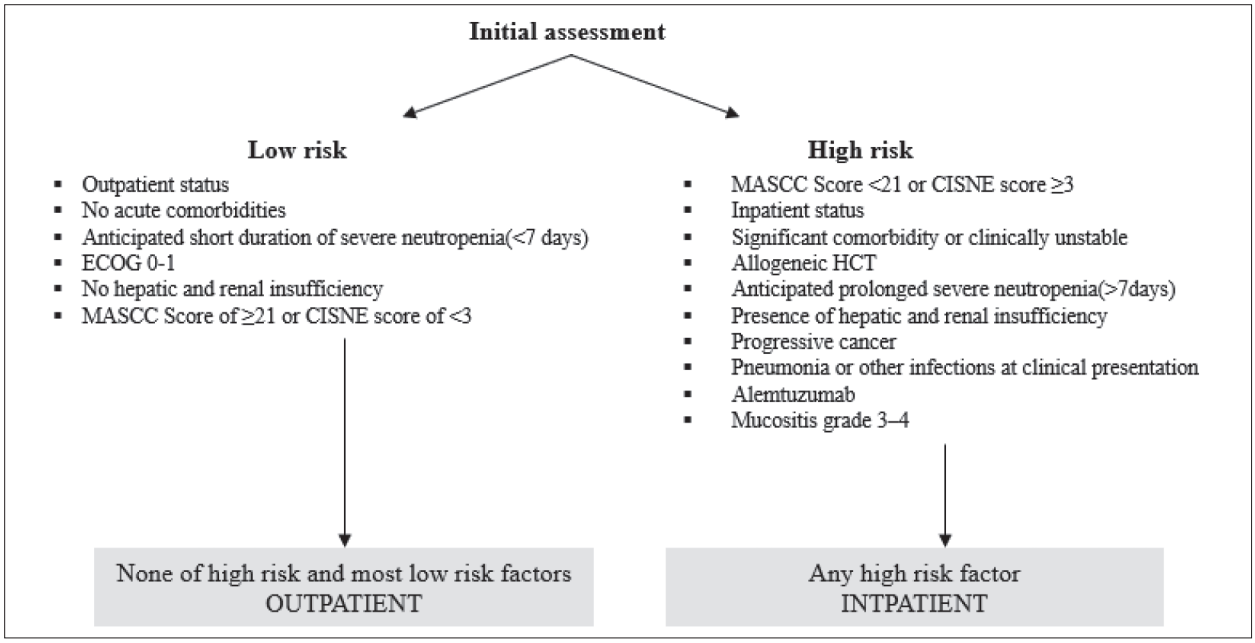

Figure 2. National Comprehensive Cancer Network; (NCCN) initial risk assessment algorithm for FN patients;
ECOG – Eastern Cooperative Oncology Group; MASCC – Multinational Association for Supportive Care in Cancer; CISNE – Clinical Index of Stable Febrile Neutropenia

Considering that the rate of complications from FN is still high, it is crucial to accurately stratify patients who can safely be treated on an outpatient basis. Several tools have been proposed in order to recognize patients with high-risk FN. One of the most common used tools for risk stratification is the Multinational Association for Supportive Care in Cancer (MASCC) tool (Table 2) [13].

An MASCC score of 21 or more identifies low-risk patients eligible for outpatient care with a positive predictive value of 91%, a specificity of 68%, and a sensitivity of 71% [12]. Another commonly used risk stratification tool is the Clinical Index of Stable Febrile Neutropenia (CISNE) score (Table 3) [13].

It was validated to predict major complications in FN patients who are assigned a score ≥ 3 (high risk). Due to the validation study design, the CISNE can only be applied to patients with solid tumors treated with standard-dose chemotherapy) [13].

Although these scores are validated and no-time consuming tools for the prediction of complications in FN patients, it is not clear whether they could be applied to all FN patients. In a recent paper published in the Journal of Oncology Practice, the authors deem that one tool cannot fit all the patients with FN [14]. In this paper, it is stated that the treatment of FN should be personalized and that several patient-related, treatment-related, and logistic factors should be taken into account in order to decide whether to treat the FN patient as an inpatient or as an outpatient. It is discussed that an ideal tool to help decision-making in this regard probably should be a system that accommodates all components of patient care and patient-related factors: type of cancer, expected prognosis, intent of cancer treatment and type of chemotherapy regimen, expected severity and duration of neutropenia, patient's comorbidities, patient's performance status, hemodynamic stability, adherence to oral antibiotics, patient's compliance to close monitoring, and availability of emergency health care services. Once again, the focus is on the patient-related factors.

The current American Society of Clinical Oncology (ASCO)/Infectious Diseases Society of America (IDSA) guideline recommends the use of MASCC score and clinical criteria to identify patients with high-risk FN [3]. In the ASCO guideline, Taplitz et al. [3] based clinical criteria

on various patient-specific and organ-specific symptoms, signs, and conditions. Patients with an MASCC score < 21 and the presence of clinical criteria are candidates for inpatient treatment. In the case of an MASCC score ≥ 21 and the absence of clinical criteria, patients with FN should be treated as outpatients. This guideline also recommends the use of the CISNE score in the case of clinically stable low-risk FN patients with solid tumors treated with mild-to-moderate intensity chemotherapy, as already mentioned [3]. The current ESMO guideline recommends the use of the MASCC score to identify low-risk and high-risk FN patients [1]. The current National Comprehensive Cancer Network guideline recommends the use of these tools (MASCC or CISNE) together with several additional patient-related factors (Figure 2) [4].

CONCLUSION

Chemotherapy-induced FN may lead to serious complications and represents a burden to healthcare services. A careful and comprehensive assessment of risks for FN development and its complications plays a key role in determining whether the G-CSF should be initiated for primary prophylaxis or not. In the case of developed FN, it is crucial to perform a careful risk assessment for complications with validated tools to determine whether the FN management should be inpatient or outpatient. Besides the validated tools, the guidelines suggest the use of clinical criteria in order to make a treatment of FN more personalized and to reduce the incidence of its complications including death.

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Свеобухватна процена фактора ризика за развој и компликације фебрилне неутропеније изазване хемиотерапијом

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САЖЕТАК

Фебрилна неутропенија је озбиљно нежељено дејство хемиотерапије. Може довести до појаве компликација и смрти, као и до кашњења у примени хемиотерапије, до смањења доза антинеопластичних лекова, што може утицати на ефикасност онколошког лечења и скраћење преживљавања. Процена фактора ризика порекла болесника игра значајну улогу у превенцији фебрилне неутропеније и њених компликација. У случају хемиотерапије умерене мијелосупресивности, фактори порекла болесника морају да се узму у обзир јер повећавају укупан ризик за фебрилну неутропенију. Адекватна процена укупног ризика за фебрилну неутропенију омогућава правовремено планирање примарне профилаксе применом фактора раста. Код болесника који развију фебрилну неутропенију детаљно се процењује ризик од озбиљних компликација, укључујући ту и смртни исход, како би се одредио одговарајући приступ у лечењу. У водичима су дате препоруке за процену ризика за компликације на терену фебрилне неутропеније. Коришћењем валидираних помагала за процену компликација и адекватном проценом фактора ризика порекла болесника могуће је прилагодити лечење фебрилне неутропеније сваком болеснику и смањити ризик од компликација и смрти.

Кључне речи: фебрилна неутропенија; фактори ризика порекла болесника; процена ризика

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Development of bariatric/metabolic surgery in Vojvodina

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SUMMARY
The paper presents the chronological development of bariatric/metabolic surgery in Vojvodina, the region with the largest incidence of obesity in Serbia, and in which 13.2% of the population suffers from diabetes with a mortality of 15.7/100,000, which is one of the highest rates in Europe.
Bariatric surgery began in the 1950s in the United States, with the consecutive development of various restrictive, malabsorptive, and combined procedures, which are intensified by the adoption of laparoscopic techniques.
After initial, European knowledge and preparation of obese patients for surgery at the end of the 1970s, the first laparoscopic bariatric/metabolic interventions started in Vojvodina at the Clinical Center in Novi Sad in 2006. Next year, the multidisciplinary team for bariatric surgery was prepared in Slovenia and Austria, and the first interventions were performed in 2008 at the Clinic for Thoracic Surgery of the Institute in Sremska Kamenica, in the first-place laparoscopic sleeve gastrectomy. Since then, bariatric/metabolic surgery at this Institute have continuously been performed. By establishing international cooperation, three courses were held with demonstration operations in bariatric/metabolic surgery and the participation of eminent surgeons and other experts from the country and abroad. Several bariatric surgeries were performed on children (at the Children's Surgery Clinic in Novi Sad), as well as in Sremska Mitrovica. Their own experience in this field has been published in the international literature and through several regional meetings dedicated to metabolic surgery.
Keywords: bariatric surgery; laparoscopic sleeve gastrectomy; mini gastric bypass

INTRODUCTION

Bariatric surgery or weight loss surgery is considered different operative procedures with the aim of treating obesity. The word bariatric has origin from the Greek root of the two words which mean weight and treatment [1]. Obesity is a serious public health problem [2]. People who have elevated body mass index (BMI) have a significantly increased risk of developing cardiovascular, endocrine and infectious diseases, diabetes mellitus, cancer and asthma, as well as sleep apnea, musculoskeletal disease and sudden death [3, 4, 5]. In addition, obesity affects the social, economic and psychological function of a person in society. Although conservative treatment is first in the treatment of obesity and includes a diet, exercise, lifestyle change, and anti-obesity drugs, it is not successful in a long way [6]. Today, the surgical treatment of obesity and associated "metabolic syndrome" is recommended by the World Health Organization, but also by endocrine and other international associations [7, 8]. Due to the effects on metabolism, primarily on the metabolism of glucose and fat, bariatric surgery is also called metabolic surgery. Vojvodina is, according to the epidemiological and social medical indicators of the regions in Serbia and Europe, severely affected by obesity and associated metabolic syndrome, primarily diabetes and hypertension. According to the Institute of Public Health Institute of Serbia report from 2016, 13.2% of the adult population suffers from type 2 diabetes mellitus, with a mortality rate of 15.7 / 100,000, which represents the highest rate in Europe. Vojvodina and its "districts" (Bačka, Central Banat, and South Banat district) have the highest incidence of diabetes, over 160:100,000 [9, 10]. In the past decades sporadically and relatively constant in the last ten years, bariatric/metabolic surgery in Vojvodina has been performed.

The aim of the paper is to present the development of bariatric/metabolic surgery in Vojvodina.

THE HISTORY OF BARIATRIC/METABOLIC SURGERY IN THE WORLD

The development of open bariatric surgery began in the fifties of the last century, using the intestinal bypass [11]. In this procedure, the upper part of the intestine (jejunum) merged with the lower part of the small intestine (ileum), which left a large part of the small intestine to be excluded from digestion and caused weight loss by malabsorption [12]. Thereafter in the 1960s, a jejuno-colic bypass was made: the proximal small intestine is transected and anastomosed to the colon [13]. In 1967, on the basis of experiments

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in the laboratory, the "father" of bariatric surgery Edward Mason developed the original gastric bypass [14]. In 1977, following the work of Alden (who made gastro-jejunostomy – a surgical procedure in which an anastomosis is created between the stomach and the proximal loop of the jejunum), Griffen introduced Roux-en-Y anastomosis into bariatric surgery and this procedure became a gold standard in bariatric/metabolic surgery [11, 15].

As a combined restrictive and malabsorptive procedure, the Roux-en-Y gastric bypass was followed by dumping syndrome, iron deficiency and vitamin B12 deficiency. In the 1970s and 1980s, purely restrictive surgery, gastroplasty, was brought into bariatric surgery [16]. The procedures on the stomach and its reduction were followed by the set of rings or staples [17]. These operations showed fewer complications but were accompanied by minor weight loss.

The modern era in bariatric surgery begins with the operation of the biliopancreatic diversion performed by the Italian surgeon Nicola Scopinaro. The first report on 18 patients was published in 1979 [18]. He performed a horizontal hemi-gastrectomy with pouch drainage by a Roux limb of at least 250 cm in length, joining a long biliopancreatic limb to form 50 cm long common ileal segment, or channel. This procedure was converted by Marceau et al. [19] in Canada to a vertical sleeve gastrectomy with cross-stapling of the duodenum and an approximately 100 cm-long common channel.

In 1998, Hess and Hess [20] in the USA made a duodenal switch, by performing a sleeve gastrectomy, with pyloric preservation, duodenal division, a proximal duodeno-ileostomy, and a common channel of approximately 100 cm. In this version of the biliopancreatic diversion called "biliopancreatic diversion with duodenal separation" (BPD-DS), which began to be performed laparoscopic, the volume of the stomach was reduced to about 200 milliliters, drawing the future sleeve gastrectomy.

Kuzmak [21] introduced a silicone tape in 1986 that could be filled through the subcutaneous chamber and "adjusted" with clamping around the cardia area, preventing excessive filling of the stomach. Forsell et al. [22] first set up a laparoscopic "adjustable gastric band" in 1993.

Laparoscopic sleeve gastrectomy (LSG) or vertical gastric resection was first performed by Michel Gagner in 1999 within the BPD-DS. In 2003, the same surgeon did the LSG as the first stage of two-stage Roux-en-Y gastric bypass (RYGB) surgery in patients at high risk and BMI higher than 50 kg / m^2. Shortly after this description, the articles followed in which the LSG was performed as an independent and sufficient procedure for the treatment of obesity and metabolic disorders due to good results [23, 24]. Today, this operation is the most commonly performed procedure in metabolic surgery in the whole world [25].

DEVELOPMENT OF BARIATRIC/METABOLIC SURGERY IN VOJVODINA

The first theoretical knowledge on bariatric surgery and the first preparation of patients were done in Novi Sad by the associate professor of surgery Borislav Savić, who worked in the Surgical Clinic from 1979 to 1981. He came from Germany as an affiliated surgeon and author of the book "Septic Surgery", published in Stuttgart [26]. The cooperation of surgeons and internist-endocrinologists was done on the Faculty of Medicine in Novi Sad. The sudden death of Professor Savić stopped the realization of the first bariatric interventions, but the idea continued to live in the followed generations of surgeons and internists. The first laparoscopic interventions on biliary tract were carried out in 1996 in Sremska Kamenica at the Clinic for Cardiovascular Surgery by Professor Branislav Daničić and Dr Miroslav Ilić, who were abdominal surgeons (equipment for laparoscopic procedures was only available to this institution). After the introduction of laparoscopic methods in the Clinical Center of Vojvodina at the Clinic for Abdominal and Endocrine Surgery and the complete experience in this surgery (laparoscopic adrenalectomy, laparoscopic reparation of hiatal hernia, laparoscopic appendectomy), the first operation of setting up a Swedish adjustable gastric band (SAGB – Johnson & Johnson®) was performed by Professor Radovan Cvijanović with his team in 2006. He continued this procedure to work in Parks Dr Dragi Special Surgical Hospital in Novi Sad. In 2007, after obtaining the necessary equipment for bariatric procedures at the Institute for Lung Diseases at the Clinic for Thoracic Surgery, the multidisciplinary team (surgeons, psychologist, nutritionist, anesthesiologist, nurses) was in Slovenj Gradec (Slovenia) for conducting bariatric operations under the management of Dr Brane Breznikar.

Immediately afterward, courtesy of Johnson & Johnson® medical company, Dr Miroslav Ilić was at the Clinic for Bariatric Surgery under the mentorship of Associate Professor Karl Miller (Head of the Surgical Department, Hallein Clinic, Austria), where he was educated primarily for the SAGB procedure and Roux-en-Y gastric bypass.

The first bariatric/metabolic laparoscopic procedures were performed at the Clinic for Thoracic Surgery of the Institute of Pulmonary Diseases of Vojvodina on October 31, 2008, with the guidance of Dr Miller, two SAGB procedures and one LSG on a mega-obese patient (BMI 70 kg/m^2) [27]. At the same time, Professor Miller held a public lecture about bariatric medicine and surgery in front of representatives of provincial authorities in order to point out the financial advantages of early treatment of obesity and metabolic syndrome.

The first accredited two-day multidisciplinary Alma Mons course (Continuing Medical Education) was held in October 2013 with lecturers from Serbia, Bosnia and Herzegovina, Croatia, Romania and Turkey, with live performance and monitoring with the possibility of asking questions to surgeons during the procedure. The lecturers were: internist endocrinologists Professor Edita Stokić (Novi Sad, Serbia), Dr Snežana Polovina (Belgrade, Serbia), internist pulmonologist Assistant Professor Ivan Kopitović, anesthesiologists Dr Milana Komarčević (Sremska Kamenica, Serbia) and surgeons Professor Miroslav Bekavac-Beslin (Zagreb, Croatia), Assistant Professor Fuad Pašić (Tuzla, Bosnia and Herzegovina), Professor Catalin

Figure 1. Dr Robert Rutlage and Professor Miroslav Ilić

Copăescu (Bucharest, Romania), Professor Alper Celik (Istanbul, Turkey), Assistant Professor Miloš Koledin and Professor Miroslav Ilic (Sremska Kamenica, Serbia). The following operations were performed: laparoscopic "resleeve" gastrectomy and laparoscopic ileal interposition. Both of these operations were first performed in Serbia.

The second Alma Mons course was held in November 2014 with the participation of surgeons from the Netherlands, Croatia and Serbia. The lecturers were Dr Arlond van de Laar, Head of Bariatric Surgery at the Slotervaart Hospital in Amsterdam (the Netherlands), the European Center of Excellence for Bariatric Surgery, with associates Dr Yair Acherman and Dr M. Hoen, whose focus was on Roux-en-Y gastric bypass, then Professor Davor Štimac (Rijeka Clinical Hospital Center, Croatia) – about intragastric balloons. Cooperation and meeting with Professor Bešlin-Bekavac (Sestre Milosrdnice University Hospital Center, Zagreb, Croatia) and Assistant Professor F. Pašić (Tuzla University Clinical Center, Tuzla, Bosnia and Herzegovina). The third Alma Mons course was dedicated to only one surgeon and surgery: Dr Robert Rutlegde (Center for Excellence in Laparoscopic Obesity Surgery, Henderson, USA) and his metabolic laparoscopic procedure – "mini-gastric bypass" (MGB/OAGB). This course was held on May 30, 2016, with the theoretical part and the performance of two MGB/OAGB operations carried out by Dr Robert Rutlage and Professor Miroslav Ilić (Figure 1). These interventions were performed for the first time in the region of Southeast Europe.

Since 2008, bariatric/metabolic surgeries are continuously performed at the Clinic for Thoracic Surgery in Sremska Kamenica under the leadership of Professor Miroslav Ilić [28].

According to the data from the operational protocols and electronic patient records, in the period from 2008 to 2020, about 500 bariatric procedures were performed.

The most commonly performed procedure is LSG with 380 operations. Complications included three postoperative bleeding, four anastomotic leaks, two chronic fistulas and one neofundus that were resolved surgically and one fistula that was treated conservatively.

Other bariatric procedures have been performed: 18 SAGBs, nine laparoscopic mini-gastric bypass, five Roux-en-Y gastric bypass, three laparoscopic "resleeve" gastrectomy, one laparoscopic ileal interposition, two single anastomosis sleeve ileal bypass, one more open LSG, and one mini-gastric bypass.

Professor Radoica Jokić, a pediatric and abdominal surgeon, has performed several bariatric interventions on children (LSG), with his colleague Professor Dragan Kravarušić (Schneider Children's Medical Center, Israel), with excellent results at the Children's Surgery Clinic in Novi Sad. Attempts to carry out the SAGB bariatric procedure were also recorded at the General Hospital of Sremska Mitrovica by Dr Goran Ivić in 2010 but without further continuity.

Dr Srđan Putnik (Vršac General Hospital) has presented the results of his own experiences and guest appearances abroad in several publications, as well as doctoral theses, in which the general surgeon from Vršac was very diligent [27, 28, 29].

Apart from Vojvodina, bariatric surgery is also performed at the University Clinical Center of Serbia. Thanks to Academician Professor Dragan Micić, the Multidisciplinary Center for Obesity Treatment was opened in 2010. Of the bariatric procedures, RYGB and LSG are the most common. According to the data from the literature, about 100 Roux-en-Y gastric bypass were performed [30].

Other centers in Serbia are also showing great interest in this type of increasingly popular surgery.

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Развој баријатријске/метаболичке хирургије у Војводини

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САЖЕТАК

У раду је дат хронолошки развој баријатријске/метаболичке хирургије у АП Војводини, регији која има највећу инциденцу гојазних особа и у којој 13,2% популације болује од шећерне болести са морталитетом од 15,7/100.000 становника, што је једна од највиших стопа у Европи.

Баријатријска хирургија почиње педесетих година прошлог века у САД, са консекутивним развојем различитих рестриктивних, малапсорптивних и комбинованих захвата који се интензивирају усвајањем лапароскопских техника.

Након почетних, европских сазнања и припреме гојазних болесника за операцију крајем седамдесетих година, прве лапароскопске баријатријске/метаболичке интервенције започињу у АП Војводини у Клиничком центру у Новом Саду 2006. године постављањем „шведске прилагодљиве траке". Следеће године мултидисциплинарни тим за баријатријску хирургију припрема се у Словенији и Аустрији, те се прве интервенције лапароскопске рукавне ресекције желуца изводе 2008. године на Клиници за грудну хирургију Института у Сремској Каменици. Од тада се баријатријска/метаболичка хирургија на овом месту изводи у континуитету. Успостављајући међународну сарадњу, одржана су три курса са показним операцијама из баријатријске/метаболичке хирургије и учешћем еминентних хирурга и других експерата из земље и иностранства. Неколико баријатријских операција изведено је у Сремској Митровици, као и код деце на Клиници за дечју хирургију у Новом Саду.

Сопствена искуства из ове области публикована су у међународној литератури и представљена на великом броју регионалних састанка посвећених метаболичкој хирургији.

Кључне речи: баријатријска хирургија; лапароскопска рукавна ресекција желуца; мини желудачно премошћавање

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

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