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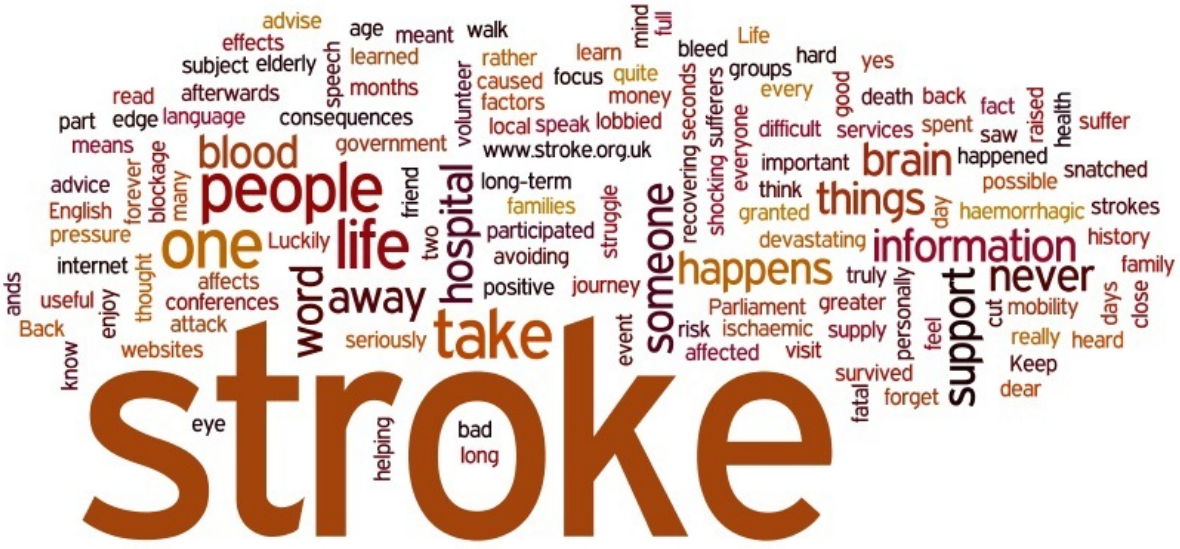

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CASE REPORTS / KAZUISTIKA

Stroke is the third leading cause of death and the leading cause of disability in the world. In Serbia, stroke is the leading cause of shortening life expectancy in the female, and the other one in the male part of the population. In order to promote activities that can improve the prevention and treatment of this disease in Serbia, in 2007, June 9 was designated for the National Stroke Day. This year marks the tenth anniversary of the ceremony in our country. As a contribution to the activities scheduled for that day, in the June issue of the Vojnosanitetski Pregled an article is published on stroke by Gordana Aranđelović Minić "Leucocyte count is an indicator of carotid plaque instability and stroke patients" (pp . 515–25) .

Moždani udar je treći vodeći uzrok smrti i prvi uzrok invaliditeta u svetu. U Srbiji, moždani udar je prvi uzrok skraćenja životnog veka u ženskom, a drugi u muškom delu stanovništva. U cilju promovisanja aktivnosti kojima se može unaprediti prevencija i lečenje ove bolesti u Srbiji je 2007. godine 9. jun određen za Nacionalni dan borbe protiv moždanog udara. Ove godine navršava se desetogodišnjica njegovog obeležavanja u našoj zemlji. Kao doprinos aktivnostima predviđenim za taj dan, u junskom broju Vojnosanitetskog pregleda objavljujemo rad Gordane Aranđelović Minić na temu moždanog udara „Broj leukocita pokazuje nestabilnost karotidnog plaka kod bolesnika sa akutnim infarktom mozga" (str. 515–25).

ORIGINAL ARTICLES

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Leucocyte count indicates carotid plaque instability in stroke patients

Broj leukocita pokazuje nestabilnost karotidnog plaka kod bolesnika sa akutnim infarktom mozga

Gordana Arandjelović Minić

Department of Neurology, Military Hospital, Niš, Serbia

Abstract

Background/Aim. Increasing evidence points to the inflammatory character of atherosclerosis and several parameters of inflammation have been proposed as cerebrovascular risk markers. The objective of the research was to examine the connection of serum inflammatory parameters and ultrasound (US) characteristics of the structure and size of carotid plaque. We assumed that the number of leukocytes (Le) was an indicator of carotid plaque instability and an increased risk of stroke. **Methods.** Serum inflammatory parameters: erythrocyte sedimentation rate in the first (ESR I) and second hour (ESR II), the number of Le, high sensitivity C-reactive protein (hsCRP) and fibrinogen were measured by standard methods. All the subjects (n = 75) were divided into 3 groups (symptomatic, asymptomatic and control). US evaluation of extracranial carotid arteries was performed in a duplex system. Plaques were classified into categories according to stenosis percentage (≥ 50%, < 50%) and pursuant to echomorphological characteristics (Gray-Weale classification). In the subjects with stroke an ischemic lesion was confirmed by computed tomography. **Results.** The average values of biochemical parameters in the symptomatic group were: ESR I 29.57 ± 29.87 cm, ESR II 51.60 ± 36.87 cm, the number of Le 10.10 ± 3.20 × 10^{-9} U/L, hs-CRP 8.15 ± 5.50 mg/L and fibrinogen 4.03 ± 0.70 g/L. The average values of all testing biochemical parameters in symptomatic patients were significantly higher than in the asymptomatic ones and the control group: for ESR I (p < 0.05) and ESR II (p < 0.05); for the number of Le (p < 0.001); for hsCRP (p < 0.001) and fibrinogen (p < 0.001). Category I of echomorphological characteristics in the symptomatic group was present in 66.7% of the cases and it was significantly higher than in the asymptomatic (40.0%; p < 0.05) and the control group (20.0%; p < 0.01). Univariate logistic regression analysis confirmed that all testing biochemical parameters are indicators of stroke risk. Multivariate logistic regression analysis confirmed a statistically significant correlation of the number of Le and stroke risk, while the increase in the value by a unit of measurement was associated with the growth of risk by 3.22 times (from 1.67 to 6.22). **Conclusion.** The number of Le is associated with the phenomenon of carotid plaque instability and may be a useful additional marker of increased risk for developing acute cerebral infarction.

Key words:
stroke; carotid stenosis; plaque, atherosclerotic; leukocyte count.

Apstrakt

Uvod/Cilj. Porast dokaza o inflamatornom karakteru ateroskleroze istakao je više parametara zapaljenja kao pokazatelje cerebrovaskularnog rizika. Cilj istraživanja bio je da se ispita povezanost parametara zapaljenja u serumu i ultrazvučnih (UZ) karakteristika strukture i veličine karotidnog plaka. Pretpostavili smo da je broj leukocita (Le) pokazatelj nestabilnosti karotidnog plaka i povećanog rizika od razvoja akutnog infarkta mozga. **Metode.** Broj Le i ostali serumski parametri zapaljenja [sedimentacija eritrocita u prvom (Se Er I) i drugom satu (Se Er II), visokosenzitivni C-reaktivni protein (hs-CRP) i fibrinogen] mereni su standardnim metodama. Svi ispitanici (n = 75) imali su kompletan klinički pregled i bili podeljeni u tri grupe (simptomatska, asimptomatska i kontrolna). Ultrazvučni (UZ) pregled ekstrakranijalnih karotidnih arterija rađen je u dupleks sistemu. Plakovi su klasifikovani u kategorije prema procentu stenoze (≥ 50%, < 50%) i prema ehomorfološkim karakteristikama (Gray-Weale klasifikacija). Kod ispitanika sa akutnim infarktom mozga ishemijska lezija je potvrđena kompjuterizovanom tomografijom. **Rezultati.** Prosečne vrednosti ispitivanih biohemijskih parametara u simptomatskoj grupi bile su: Se Er I 29,57 ± 29,87 cm, Se Er II 51,60 ± 36,87 cm, broj Le 10,10 ± 3,20 × 10^{-9} U/L, hs-CRP 8,15 ± 5,50 mg/L i fibrinogen 4,03 ± 0,70 g/L. Prosečne vrednosti svih ispitivanih biohemijskih parametara u simptomatskoj bile su značajno više nego u asimptomatskoj i kontrolnoj grupi: za Se Er I (p < 0.05) i Se Er II (p < 0,05); za broj Le (p < 0,001) za hsCRP (p < 0,001) i fibrinogen (p < 0,001). Zastupljenost I kategorije ehomorfoloških karakteristika u simptomatskoj grupi (66,7% ispitanika) bila je značajno viša nego u asimptomatskoj (40,0%; p < 0,05) i kontrolnoj (20,0%; p < 0,01)

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grupi. Univarijantna logistička regresiona analiza potvrdila je da su svi ispitivani parametri indikatori rizika od akutnog infarkta mozga. Multivarijantna logistička regresiona analiza potvrdila je statistički značajnu povezanost broja Le i rizika od akutnog infarkta mozga. Povećanje vrednosti za jednu mernu jedinicu povezano je sa porastom rizika za 3,22 puta (1,67–6,22). **Zaključak.** Broj Le povezan je sa fenomenom nestabilnosti karotidnog plaka i može biti koristan dodatni pokazatelj povećanog rizika od razvoja akutnog infarkta mozga.

Ključne reči:
mozak, infarkt; aa. carotis, stenoza; aterosklerotički plak; leukociti, broj.

Introduction

Ischemic brain disease as the final stage of cerebral atherosclerosis includes the pathological processes on extracranial carotid arteries. Clinical experience of neurologists indicate that acute cerebral infarction is often the first manifestation of the progression of carotid atherosclerosis. Carotid plaque is not a stable lesion in spite of prophylactic drug therapy and carries the risk of definite or transient ischemic complications [1–3].

The scientific research dealing with the vulnerability of atherosclerotic lesions on experimental and human models provided theoretical knowledge that numerous biochemical markers in peripheral blood and modulators and mediators of inflammation in vulnerable atherosclerotic lesions represent a part of complex pathophysiological destabilization mechanisms [2, 3].

The objective of the research was to examine the connection of serum inflammatory parameters, applicable in everyday clinical work, and ultrasound (US) characteristics of the structure and size of carotid plaque. We assumed that the number of leukocytes (Le) could be an indicator of carotid plaque instability and an increased risk of developing acute cerebral infraction.

Methods

The study included 75 subjects of both sexes, 50–70 years of age, processed in hospital and treated in the Intensive Care Unit of the Department of Neurology, Military Hospital in the town of Niš and on outpatient treatment, neurologically monitored in the Section for Neurology of the Military Hospital.

The basic criteria for the inclusion of subjects in this study were the sample structure according to age and sex, with defined traditional risk factors of atherosclerosis [4], ultrasound (US) diagnosed carotid atherosclerosis [5, 6] and multislice scanner (MSCT) diagnosed acute cerebral infarction [4, 7].

The exclusion criteria were: subjects with clinical symptoms and signs of current or recent infection (< 4 weeks), verified by a physical examination, diagnostic assessment by indications (by organ systems) and laboratory confirmation of clinically important infections; patients with diagnosed chronic or specific infections by organ systems; subjects with potentially cardioembolic etiology of acute cerebral infarction after clinical and electrocardiographic/echocardiographic evaluation (intermittent/continuous atrial fibrillation/flutter, recent myocardial infraction < 6 weeks, mitral/aortic stenosis, prolapse, calcification, vegetation or prosthetic valve replacement, aneurysm or left atrial myxoma, thrombosis in the left ventricle, persistent foramen ovale, right-to-left shunt, congestive heart failure, congenital heart disease, endocarditis ...) [8]; subjects diagnosed with kidney failure, based on the clinical stage of disease and laboratory confirmation [9]; patients with diagnosed immunological, malignant diseases and disorders of hemostasis; subjects with trauma or surgery in the past 12 months; subjects on corticosteroids, antioxidant or hormone therapy; subjects with MSCT confirmed alterations of the brain parenchyma not corresponding to acute cerebral infarction by the clinical categorization and pathogenesis of atherothrombosis [7, 8].

Each patient was taken a detailed medical history and subjected to a neurological examination. Additional tests included: laboratory blood test, ultrasound of extracranial carotid arteries and MSCT of endocranium in patients with acute cerebral infarction.

The sample size was calulated on the basis of the results of clinical studies with similar objectives [10] and preliminary results taking into account that $\alpha = 0.05$, and the study power 0.8 according to a flexible statistical power analysis program G* Power 3 [11].

The subjects were divided into three groups: the symptomatic group (30 subjects) comprised subjects with acute cerebral infarction, or with focal or global disturbance of cerebral function, which occured rapidly and lasted longer than 60 minutes and their clinical categorization and pathogenesis which corresponded to atherothrombotic cerebral infarction [4, 7].

The diagnosis of acute cerebral infarction was confirmed by MSCT of endocranium [4]. Carotid atherosclerosis was diagnosed by the ultrasound examination of extracranial carotid arteries and confirmation of the localized atherosclerosis lesion [5, 6].

The asymptomatic group (30 subjects) involved subjects with carotid atheroslerosis and verified hemodynamically significant carotid burification plaque (≥ 50%) [5].

The control group (15 subjects) consisted of subjects with carotid atheroslerosis and verified hemodynamically insignificant carotid burification plaque (< 50%) [5]. The subjects of the asymptomatic and control group, in their medical history, had no anamnestic data on current/prior episodes of rapidly developing focal/global disturbances of cerebral function which lasted longer than 60 minutes, without MSCT confirmed densimetric alterations of the brain parenchyma [7].

In this research we valued and analyzed biochemical, ultrasound, clinical and neuroradiological parameters.

Biochemical parameters. Blood for the required analyses was taken from the medial cubital vein into tubes with a vacuum system. The number of Le was determined on an automated hematology analyzer from a tube with ethylendiamineacetic acid (EDTA) as an anticoagulant; high sensitivity C-reactive protein

(hs-CRP) was determined on a biochemical analyzer from a tube wihout anticoagulants; erytrocyte sedimentation rate (ESR) and fibrinogen were determined from a tube with sodium citrate as an anticoagulant. Ready-made commercial blood tests were used for the analysis. Blood for analyses in the subjects of the symptomatic group was taken on admission in the time frame of "therapeutic window" [12]. Blood for analyses in the subjects of the asymptomatic and control group was taken from 7.30 AM to 8.00 AM, on an empty stomach and before morning administration of the therapy. The listed biochemical analyses were performed in the Clinical Biochemical Laboratory, of the Military Hospital, Niš.

Ultrasound parameters. By the anatomical and morphological depiction of the carotid arteries (B-mode) we analyzed the characteristics of blood vessels: the degree of stenosis expressed by the ratio of the diameter in the stenotic area and the residual lumen diameter according to the following formula – d2-d1/d2x100 [13]; echomorphological plaque characterictics; using a grey-scale median, (GSM) for visual assessment, carotid plaques were divided into 4 types according to the standard classification (Gray-Weale): I – predominantly echolucent plaque with a thin echogenic cap; II – substantially echolucent plaque with small areas of echogenity, < 25%; III – predominantly echogenic plaque with small areas of echolucency, < 25%; IV – uniformly echogenic plague, equivalent to homogenous one [14]. The tested blood vessels were observed in the longitudinal and transverse projection and adequate positions. The ultrasound examination was performed on the appliance HITACHI EUB 5500, Ultrasound Scanner, Japan with a 10 MHz probe. The measurements were taken before the ostium of the internal carotid artery (ICA) and the distal part of the common carotid artery (CCA) in five consecutive sections, and the medium values were used for the statistical analysis of data. Ultrasound examination of the subjects of the symptomatic group was performed on the day of admission, of the subjects of the asymptomatic and the control group after obtaining blood samples for analysis.

Clinical parameters. The degree of neurological deficit in symptomatic patients was assessed by a standardized scale, National Institute of Health Stroke Scale (NIHSS) – admission NIHSS score [4].

Neuroradiological parameters. MSCT of endocranium was performed in symptomatic patients natively or following a contrast agent application. The MSCT was performed on the appliance TOSHIBA AQUILION, 16 multisliced scanner, Japan. The largest diameter (mm) was used in the assessment of the ischemic lesion size. Densimetric alterations which by their clinical categorization and pathogenesis did not correspond to atherothrombotic cerebral infarction were not included in the study [7].

All the subjects gave a written consent to be included in the study after an insight into the written information of the planned research. Those with acute cerebral infarction and altered consciousness were required the consent of the closest family members.

The preliminary design had the authorization of the Ethic Committee of the home institution that it met the professional and ethic criteria, that there was no deviation from the principles stated in the Declaration of Helsinki and that the planned research could be conducted in the home institution.

The assessment of the correlation between the values of different characteristics was performed by correlation analysis. The Friedman's test with (post-hoc) $\chi 2$ to follow or Fisher's test were used for the comparison of the frequency of certain modalities of attributive characteristics. The analysis of variance (ANOVA) with Dunnett's test to follow were used for the comparison of numerical values among the three groups of subjects. The assessment of the influence of certain factors on the degree of stenosis was performed by univariate or multivariate linear regression analysis. The assessment of the significance of certain factors in the prediction of cerebral infarction was done by the application of univariate or multivariate logistic regression analysis, as well as Receiver Operating Characteristic (ROC) analysis.

Results

The average age of all the subjects was 66.21 ± 4.19 years. The average age of the subjects in the symptomatic group was 66.27 ± 4.27 years, and the differences compared to the subjects in the asymptomatic (66.93 ± 4.72 years) and the control group (64.67 ± 2.29 years) were not statistically significant (ANOVA and post-hoc Tukey's test: $p > 0.05$). The symptomatic group included 17 (56.7%) men and 13 (43.3%) women, and the asymptomatic involved 11 (36.7%) men and 19 (63.3%) women, the control group comprised 8 (53.3%) men and 7 (46.7%) women. The distribution according to sex was not homogeneous, but the differences among the compared groups in the structure by sex were not statistically significant ($\chi 2 = 2,62$; $p = 0,27$).

The average value of the erytrocyte sedimentation rate in the first hour (ESR I) in the subjects of the symptomatic group was 29.57 ± 29.87 cm, and in the second hour (ESR II) it was 51.60 ± 36.87 cm (Table 1). The number of leukocytes in the subjects of the symptomatic group was approximately 10.10 ± 3.20 × 10^9/L, the average value of hsCRP was 8.15 ± 5.50 mg/L and fibrinogen was 4.03 ± 0.70 g/L (Table 1). The average value of ESR I in the subjects of the asymptomatic group was 15.73 ± 10.82 cm and ESR II was 32.87 ± 19.61 cm (Table 1). The number of leukocytes in the subjects of the asymptomatic group was 6.59 ± 1.33 × 10^9/L, the average value of hsCRP was (4.12 ± 1.60 mg/L and fibrinogen was 3.43 ± 0.65 g/L (Table 1). The average value of ESR I in the subjects of the control group was 13.60 ± 9.33 cm and ESR II was 28.53 ± 19.09 cm (Table 1). The number of leukocytes in the subjects of the control group was 6.14 ± 1.25 × 10^9/L; the average value of hs-CRP was 3.33 ± 1.25 mg/L and fibrinogen was 3.05 ± 0.80 g/L (Table 1).

The average value of ESR I in the subjects of the symptomatic group was significantly higher than in those of the asymptomatic (ANOVA and post-hoc Dunnett's test: $p = 0,030$) and the control group ($p = 0.043$). The average value of ESR II in the subjects of the symptomatic group was significantly higher than in the subjects of the asymptomatic ($p = 0.029$) and the control group ($p = 0.028$). The number of leukocytes in the subjects of the symptomatic group was

Table 1

Serum levels of inflammatory parameters according to the groups

Characteristics	Group			Comparison
	Symptomatic (n = 30)	Asymptomatic (n = 30)	Control (n = 15)	
Erythrocyte sedimentation rate I (cm)	29.57 ± 29.87	15.73 ± 10.82	13.60 ± 9.33	A^{*}, B^{*}
Erythrocyte sedimentation rate II (cm)	51.60 ± 36.87	32.87 ± 19.61	28.53 ± 19.09	A^{*}, B^{*}
Leukocyte count ($\times 10^9$ /L)	10.10 ± 3.20	6.59 ± 1.33	6.14 ± 1.25	$A^{‡}$, $B^{‡}$
hs CRP (mg/L)	8.15 ± 5.50	4.12 ± 1.60	3.33 ± 1.25	$A^{‡}$, $B^{‡}$
hs CRP range, n (%)				
< 3.30 mg/L	0 (0.0)	7 (23.3)	6 (40.0)	$A^{†}$, B^{*}
> 3.30 mg/L	30 (100.0)	23 (76.7)	9 (60.0)	
Fibrinogen (g/L)	4.03 ± 0.70	3.43 ± 0.65	3.05 ± 0.80	$A^{†}$, $B^{‡}$

hsCRP – high sensitivity C-reactive protein; A – symptomatic *vs* asymptomatic; B – symptomatic *vs* control; $^{*}p < 0.05$; $^{†}p < 0.01$; $^{‡}p < 0.001$.

significantly higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$); the average value of hsCRP in the subjects of the symptomatic group was significantly higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$) and the average value of fibrinogen was notably higher in the subjects of the symptomatic than in the subjects of the asymptomatic ($p = 0.005$) and the control group ($p < 0.001$).

All the subjects of the symptomatic group (100.0%) had the measured level of hsCRP higher than 3.3 mg/L, which was a significantly higher incidence than in the subjects of the asymptomatic (76.7%; $\chi^2 = 7.79$; $p = 0.005$) and the control group (60.0%; $\chi^2 = 13{,}54$; $p < 0.01$) (Table 1).

The representation of the category I of echomorphological characteristics (Figure 1) in the subjects of the symptomatic group was present in 20 (66.7%) of the cases and it was significantly higher than in the asymptomatic group where this category was found in 12 (40.0%) of the subjects ($\chi 2 = 4.21$; $p = 0.040$), as well as in the control group where category I of findings was confirmed in 3 (20.0%) of the subjects (($\chi 2 = 8{,}52$; $p = 0{,}003$). The representation of the category III of echomorphological characteristics in the subjects of the symptomatic group was present in 2 (6.7%) of the cases and it was significantly lower than in the control group where this category was found in 7 (46.7%) of the subjects (($\chi 2 = 9.78$; $p = 0.002$).

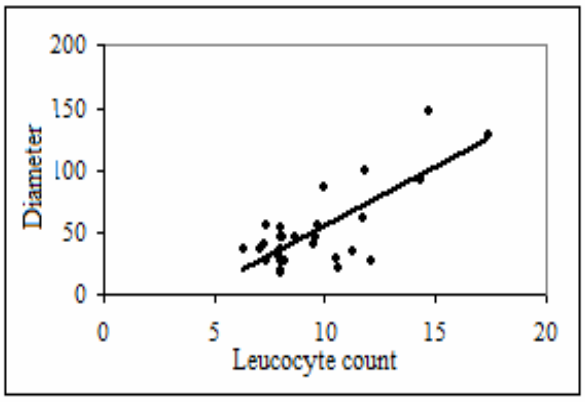

Fig. 1 – Correlation between ischemic cerebral lesion diameter and leukocyte count.

In the echomorphological category I there were 20 (57.1%) of the subjects of the symptomatic group, which was significantly higher than in the category II (8 subjects, 29.6%; $\chi^2 = 4.58$; $p = 0.032$) and the category III (2 subjects, 15.4%; $\chi^2 = 6{,}52$; $p = 0.011$). The echomorphological category III contained 7 (53.8%) of the subjects of the control group, which was a significantly higher incidence than in the category I (3 subjects, 8.6%; $\chi 2 = 12.72$; $p < 0.01$) and the category II (5 subjects, 18.5%; $\chi 2 = 5.08$; $p = 0.024$). $p < 0.05$). The average age of the subjects, as well as the sutructure by sex, were not statistically different in the compared categories of echomorphological characteristics.

The average value of the number of leukocytes in the subjects with the category I of echo findings was 8.82 ± 3.62 × 10^9/L, the average value of fibrinogen was 3.87 ± 0.75 g/L (Table 2). In subjects with the category II of echo findings the average value of the number of leukocytes was 7.27 ± 1.73 × 10^9/L), the average value of fibrinogen was 3.35 ± 0.71 g/L (Table 2). The average value of the number of leukocytes in subjects with the category III of echo findings was 6.76 ± 1.59 × 10^9/L, the average value of fibrinogen was 3.37 ± 0.86 g/L (Table 2).

The average value of the number of leukocytes in subjects with the category I was notably higher than in those with the category II (ANOVA and post-hoc Dunnett's test: $p = 0.043$) and the category III ($p = 0.024$); the average value of fibrinogen in the subjects with the category I was notably higher than in those with the category II ($p = 0{,}022$). There was no significant difference in the values of other biochemical markers in the subjects with the three compared categories.

The percentage of stenosis in the subjects of the asymptomatic group was 57.17 ± 5.47% and it was notably higher than in the subjects of the symptomatic group (32.20 ± 10.69%; $\chi 2 = 51.63$; $p < 0.001$), as well as in the control group (34.73 ± 6.46%; $\chi 2 = 44.00$; $p < 0.001$). In all the 30 (100.0%) subjects of the asymptomatic group the percentage of stenosis was higher than 50%, which was a significantly higher incidence than in those of the symptomatic group (χ2-test: $p < 0.001$) where such findings were confirmed in 2 (6.7%) of the cases, as well as in the subjects of the control group ($p < 0.001$) where such findings were not confirmed in any of the cases. The subjects with 50% stenosis (28–65.1%) belonged to the symptomatic group, and 15 (34.9%)

Table 2

Comparison of serum levels of inflammatory parameters and morphological characteristics

Characteristics	Morphological characteristics			Comparison
	Type I (n = 35)	Type II (n = 27)	Type III (n = 13)	
Erythrocyte sedimentation rate I (cm)	25.97 ± 28.63	17.22 ± 11.37	14.54 ± 10.42	n.s.
Erythrocyte sedimentation rate II (cm)	44.29 ± 35.43	37.41 ± 22.50	30.92 ± 21.33	n.s.
Leukocyte count ($\times 10^9$ /L)	8.82 ± 3.62	7.27 ± 1.73	6.76 ± 1.59	A*, B†
hs CRP (mg/L)	6.92 ± 5.60	4.47 ± 2.05	4.21 ± 1.11	n.s.
hs CRP range, n (%)				
< 3.30 mg/L	7 (20.0)	5 (18.5)	1 (7.7)	n.s.
> 3.30 mg/L	28 (80.0)	22 (81.5)	12 (92.3)	
Fibrinogen (g/L)	3.87 ± 0.75	3.35 ± 0.71	3.37 ± 0.86	A*

hsCRP – high sensitivity C-reactive protein; A – symptomatic *vs* asymptomatic; B – symptomatic *vs* control; ns – no significance; *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

to the control group. The subjects with over 50% stenosis (2–6.3%) belonged to the symptomatic group, and 30 (93.8%) to the asymptomatic group. The difference in the structure of belonging to certain groups among subjects with varying degrees of stenosis was statistically significant ($\chi 2 = 67{,}37$; $p < 0.001$). The results showed no statistically significant sex and age distribution of the percentage of stenosis. The average number of leukocytes was significantly higher in the subjects with 50% stenosis ($8.83 \pm 3.35 : 6.66 \pm 1.32 \times 10^9$/L, ANOVA and post-hoc Dunnett's test: $p = 0.001$) (Table 3). The values of other biochemical parameters were not notably different among groups formed according to the percentage of stenosis (Table 3).

The average value of NIHSS score in the subjects of the symptomatic group was 5.10 ± 2.86, and the ischemic lesion diameter was 52.00 ± 30.83 mm (Figure 2). The correlation analysis showed a very high level of interdependence between the values of NIHSS score and diameter ($r = 0.949$, $p < 0.001$). These two characteristics also show the significant correlation with ESR I (NIHSS score: $r = 0.445$ and $p = 0.014$; diameter: $r = 0.537$ and $p = 0.002$), the number of leukocytes (NIHSS score: $r = 0.822$ and $p < 0.001$; diameter: $r = 0.824$ i $p < 0.001$) (Table 4, Figure 3).

The univariate logistic regression analysis as siginificant predictors of CVI confirmed ESR I, ESR II, the number of leukocytes, hs-CRP, and fibrinogen. The increase in the

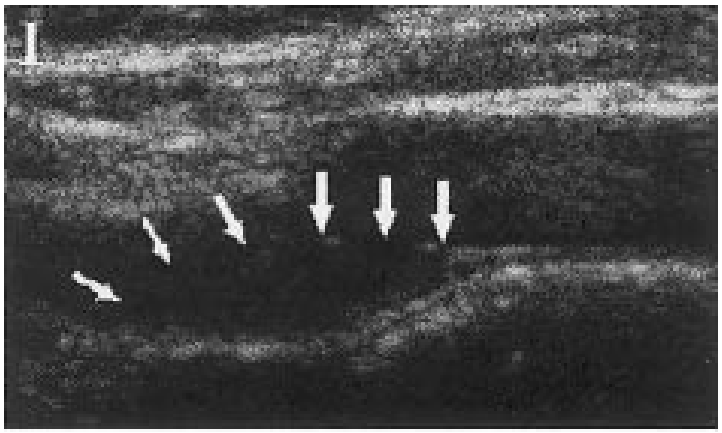

Fig. 2 – Predominantly echolucent plaque with a thin echogenic cap (category I, Gray-Weale classification).

Table 3

Association of serum levels of inflammatory parameters and stenosis

Parameters	Stenosis		Comparison
	≤ 50% (n = 43)	≥ 51% (n = 32)	
Erythrocyte sedimentation rate I (cm), $\bar{x} \pm SD$	23.98 ± 26.33	16.63 ± 11.68	n.s.
Erythrocyte sedimentation rate II (cm), $\bar{x} \pm SD$	43.47 ± 33.45	34.16 ± 21.71	n.s.
Leukocyte count ($\times 10^9$ /L), $\bar{x} \pm SD$	8.83 ± 3.35	6.66 ± 1.32	0.001
hs CRP (mg/L), $\bar{x} \pm SD$	6.22 ± 4.89	4.70 ± 2.92	n.s.
hs CRP range, n (%)			
< 3.30 mg/L	6 (14.0)	7 (21.9)	n.s.
> 3.30 mg/L	37 (86.0)	25 (78.1)	
Fibrinogen (g/L), $\bar{x} \pm SD$	3.69 ± 0.89	3.47 ± 0.64	n.s.

n.s. – no significance; hsCRP – high sensitivity C-reactive protein.

Table 4

Correlation between NIHSS score and serum levels of inflammatory parameters in the symptomatic group

Characteristcs	NIHSS scor		Diameter	
	r	*p*	r	*p*
Erythrocyte sedimentation rate I (cm)	0.445	0.014	0.537	0.002
Erythrocyte sedimentation rate II (cm)	0.254	0.176	0.338	0.068
Leukocyte count (x10^{-9} U/L)	0.822	0.000	0.824	0.000
hs CRP (mg/L)	-0.256	0.172	-0.228	0.225
Fibrinogen (g/L)	0.141	0.458	0.178	0.346

hsCRP – high sensitivity C-reactive protein; NIHSS – National Institute of Health Stroke Scale.

Fig. 3 – Ischemic cerebral lesion.

value of each of the following factors by a unit of measurement was associated with a significant increase in the risk of CVI as follows: ESR I by 5% (95% CI; 1 to 9%; $p = 0.029$), ESR II by 3% (95% CI; 1 to 5%; $p = 0.007$), the number od leukocytes by 3.17 times (95% CI; 1.80–5.56 times; $p =$ 0,001), hsCRP by 91% (95% CI; 33–173%; $p = 0.001$), fibrinogen by 3.86 times (95% CI; 1.83–8.16 times; $p <$ 0.001) (Table 5). On the contrary, the increase in stenosis percentage value by a unit of measurement was associated with a significant decrease in the risk of CVI by 10% (95% CI; 6–15%) (Table 5). In the subjects with category I of echomorphological characeristics the risk of CVI was notably higher than in the subjects with categories II and III by 4 times (95% CI; 1.50–10.66 times) (Table 5).

The multivariate logistic regression analysis as the most important predictor of CVI singled out the number of leukocytes. Each increase in the value of this characteristic by an unit of measurement was associated with a significant increase in the risk of CVI by 3.22 times (1.67– 6.22 times) (Table 6).

The regression model containing these two factors and the regression constant as independent variables, explained even 66% of the variability of the risk of CVI in subjects of the sample (determination coefficient - $R^2 = 0.66$).

Table 5

Stroke risk factors stratified by inflammatory parameters (univariate analysis)

Factors	OR	95% CI bounds		*p*
		Lower	Upper	
Erythrocyte sedimentation rate I (cm)	1.05	1.01	1.09	0.020
Erythrocyte sedimentation rate II (cm)	1.03	1.01	1.05	0.007
Leukocyte count (×10^{-9}/L)	3.17	1.80	5.56	< 0.001
hs CRP (mg/L)	1.91	1.33	2.73	< 0.001
Fibrinogen (g/L)	3.86	1.83	8.16	< 0.001
Stenosis (%)	0.89	0.84	0.94	< 0.001
I category Eho morph	0.82	0.31	2.17	0.695
II category	0.79	0.31	2.05	0.631
III category	1.71	0.59	4.99	0.323

OR – odds ratio; CI – confidence interval; NIHSS – National Institute of Health Stroke Scale. hsCRP –high sensitivity C-reactive protein.

Table 6

Stroke risk factors stratified by inflammatory parameters (multivariate analysis)

Risk factor	OR	95% CI bounds		*p*
		lower	upper	
Leukocyte count (×10^{-9}/L)	3.22	1.67	6.22	< 0.001

OR – odds ratio; CI – confidence interval.

Discussion

Contemporary knowledge about the potential reversibility of ischemic cerebral damage [12] influences the formation of a therapeutic approach in the prevention and treatment of acute cerebral infarction. The results of studies dealing with the morphology of atherosclerotic lesions have provided epidemiological support of the hypothesis that the vulnerability of carotid plaque is an etiopathogenic factor of acute cerebral infarction [15]. Studies on the therapeutic effect of endarterectomy in symptomatic/asymptomatic patients emphasize stenosis as risk factor for cerebrovascular complications [16] in clarifying the mechanisms which associate atheroscletrosis with ischemic cerebral damage. Ultrasound evaluation of the morphological and hemodynamic status of carotid plaque [17, 18] influence the formation of the attitude that the size and structure of atheroma should be considered as separate risk factors of acute cerebral infarction in the assessment of embolic potential of atherosclerotic lesions.

Epidemiological support of the hypothesis that echolucency carries an increased risk of cerebrovascular complications [17–19], the definition of stable/unstable plaque and classification on the basis of echo-morphological characteristics [14] confirm that echomorphological characteristics are an indicator of the vulnerability and risk of future neurological symptomatology [17,18].

The traditional opinion that the size of atherosclerotic lesion is the criterium in the assessment of high-risk changes [20] does not encompass the concept of inflammation as a basic mechanism of atherogenesis [21]. Scientific research on experimental and human models provide theoretical knowledge and assumptions that inflammation can predispose distal embolization [22, 23]. Even though we emphasize the importance of chronic subclinical inflammation in blood vessel wall, precise opinions on the proinflammatory response and the role of inflamatory mechanisms in destabilization of carotide plaque have not been formed. The connection of inflammatory, rheological and coagulation/fibrinolytic processes in the network of complex interactions [21, 22] gives the possibility of considering numerous parameters altered by the biochemical conditions of vulnerable carotid plaque.

By statistical analysis of biochemical markers characteristics it has been determined that the average values od the examined parameters are significantly higher in the subjects of the symptomatic group than in the subjects of the asymptomatic and control group.

The indepedent connection of Er dysfunction and carotide plaque incidence in the results of clinical studies represents a rational basis of the assumption that inflammation and oxidative stress (OS) are the factors which change Er homeostasis through the alteration of the morphology and activity [22, 24]. The influence of plasma proteins fibrinogen, immunoglobulin, lipoprotein, α2 macroglobulin on the ESR and increased aggregation potential under the influence of cytokines [22, 24] confirm that the altered activity of Er is a part of the chronic inflammatory processes of atherogenesis. In estimating the role of the ESR in the progression and stablilty of carotid plaque there were no statistically significant differences in the values of the ESR I and ESR II in the subjects of the three compared categories of echomophological characteristics. The average value of fibrinogen was notably higher in the subjects with category I than in those with category II of echomorphological characteristics ($p < 0.05$). The values of the ESR I, ESR II and fibrinogen were not significantly different among the two groups formed according to the hemodynamic significance of stenosis percentage ($\geq$ 50%, < 50%). The obtained results were in accordance with the opinion formed after the publication of the Norwegian study TROMSO, that the size and structure of carotid plaque should be considered as separate factors in assessing the risk of the development of acute cerebral infarction [17, 18].

Fibrinogen as a reactant of the acute phase is an important determinant of the ESR [22, 24–26] which indicates that within a framework of the systemic response, the ESR can be the measure of response of brain alteration at an early stage of ischemia [27].

Significantly higher values of the ESR I and ESR II in the subjects of the symptomatic group compared to the subjects of the asymptomatic and control group represent a rational basis of the conclusion that the ESR can be an acceptable test in the monitoring of chronic inflammatory processes related to atherosclerosis. In assessing the influence of the ESR as risk factor for cerebrovascular ischemic complications, its predictive significance was confirmed by the univariate logistic regression analysis, as well as that the increase in the value of this parameter by a unit of measurement was associated with a significant risk growth. Even though the prognostic significance of appearance of the clinical manifestations of the progression and complications of carotid disease was not confirmed in multivariate logistic regression analysis, in the statistical processing of the interdependence between the values of NIHSS score (ischemic lesion diameter) and examined biochemical markers, a significant correlation during the first hour indicates that the ESR can carry important information for the early prognosis of acute cerebral infarction.

No interindependence has been found between the concentration of fibrinogen, markers of the severity of neurological deficit and the size of ischemic brain lesion, even though there has been confirmation of the association of the average values of fibrinogen with echomorphological characteristics. That is in accordance with the results of immunohistochemical tests that hyperfibrinogenemia is associated with the specific composition of carotid plaque predisposing it to rupture and thrombosis [28, 29]. Fibrinogen is a part of coagulation/fibrinolytic, rheological and inflammatory process and a marker of the progression of atherosclerosis through the mechanisms of the increase in platelet aggregability and blood viscosity, increased generation of fibrinous formations and decrease in fybrinosis [22], which can explain that the calculated average value of fibrinogen in the conducted research was significantly higher in the subjects of the symptomatic group than of the asymptomatic and control group. A connection of fibrinogen concentration and the risk of developing acute cerebral infarction in the this research was confirmed by the univariate logistic regression analysis. Each

increase in the value for an unit of measurement is associated with the risk increase. Similar results have been published by Atherosis Risk in Communites Study (ARIC) and TROMSO studies emphasizing the significance of pharmacological control of carotid atherosclerosis progression [15, 30]. The mechanisms of the pleotropic effect of statin on multi-metabolic disorders encompass, within antiinflammatory effects, also the reduction of the concentration of fibrinogen in the primary and secondary prevention of acute cerebral infarction [31]. As a reactant of the acute phase, a part of the systemic response within the framework of cerebral ischemia can be a purposeful parameter in the assessment of carotid atherosclerosis progression and prediction at early stage of acute cerebral infarction.

Statistical analysis of biochemical parameters shows that the number of Le in the subjects of the symptomatic group is notably higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$). A connection of the number of Le and clinical progression of carotid disease and the influence of the increased number of Le on the risk of developing acute cerebral infarction is reflected in the phenomena of inflammation and infection in the process of atherogenesis [32, 33]. As the study on Risk Factors in Impaired Glucosae Tolerance for Atherosclerosis and Diabetes (RAID) points out, the number of Le is an independent determinant of the initiation and progression of atherosclerotic vascular disease [34]. Low grade inflammation and subclinic infection, expressed through the number of Le, are a more informative indicator of the focal than general endothelial damage [35]. The mechanisms of aggregation, adhesion and migration of Le [32], connection of the number of Le, thickness of intimomedia complexes and atherosclerotic plaque [31] indicate that Le are a part of chronic subclinical inflammation. The rheological significance of Le stems from their size and deformability characteristics and the ability to release biologically active substances such as prostaglandins, leukotrienes, cytokines make them a part of the inflammatory response of the arterial wall [22].

In statistical analysis of the characteristics of biochemical markers, it was determined no statistically significant difference in the average values of Le among the asymptomatic and the control group. The connection of the number of Le and reduction in the blood vessel diameter, an insignificant difference of the number of Le in the subjects with hemodynamically significant carotid plaque (≥ 50%, asymptomatic group) and the subjects with hemodynamically insignificant carotid plaque (< 50%, control group) confirm that the clinical neurologist should include both the size and structure of the atherosclerotic lesion when considering the appropriate treatment. In the conducted research the average value of Le in the subjects with category I of echomorphological characteristics was notably higher than in the subjects with the categories II and III.

The results of an experimental research suggest the mediating and modulatory role of Le in the acute inflammation inside the fibrous cap [34]. The presence of inflammatory and immunocompetent cells in the human atheroma, synthesis and release of the numerous molecules with proinflammatory effects [36] confirm that inflammation is a basic determinant of the vulnerability of atherosclerotic lesions. In defining the hystological criteria of destabilization, we emphasized the ability of Le to dilute tissue by the secretion of protoeolitic enzymes and that they are rarely present in intact plaques [3]. Starting from the premise that the presence of carotid plaque is the risk factor for cerebrovascular ischemic disorders, the results of the Northern Manhattan Stroke Study (NOMASS) give an epidemiological confirmation of the association of the number of Le with the incidence of carotid plaque in persons who did not suffer from acute cerebral infraction [33].

The majority of studies on the connection of carotid disease and acute cerebral ischemia [15, 37, 38] stemmed from the results of the study Aortic Plaque and Risk of Ischemic Stroke (APRIS) suggesting no connection of the increased number of Le, appearance and size of atherosclerotic plaque and risk of acute cerebral infarction [33]. The importance of clinical studies lies in the additional information which influences the formation of attitudes that there is a connection of the number of Le and subclinical atherosclerosis, independent on traditional risk factors [32] and that the connection of the number of Le, carotid ahterosclerosis and acute cerebral infarction confirm that the number of Le can be a significant predictor of cerebrovascular ischemic complications [17, 30]. The altered permeability of the blood-brain barrier, accumulation of Le in the zone of acute ischemia, secretion of the proinflammatory cytokines, increased endothelial permeability and production of reactive oxygen metabolities (ROM), increased expression of the potentially neurotoxic enzymes are a part of physiological changes within a framework of the inflammatory reaction which affects ischemic brain tissue [39]. The reduced flexibility of Le under a reduced pressure in the zone of acute cerebral infraction, adhesion to endothelium, impaired hemodynamics, occlusion of capillaries and altered blood viscosity lead to an increase of the zone of ischemic brain damage [39]. In the conducted research the average number of Le was statistically significanlty higher in the group of the subjects with acute cerebral infarction compared to the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$). Correlation analysis established the values of the numerical characteristics of NIHSS score (ischemic lesion diameter) in a significant connection with the number of Le. The obtained results are in accordance with the attitude that the increased number of Le can be related to the risk of developing acute cerebral infarction [15] and that the degree of Le infiltration in the ischemic zone is in a positive correlation with the size of tissue damage and the disease outcome [39].

Assessing the tested factors influence on the occurence of ischemic cerebral complications by the univariate logistic regression analysis showed that the values of the number of Le in the tested sample represented significant predictors of acute cerebral infarction. The multivariate logistic regression analysis singled out the number of Le as the most significant predictor of acute cerebral infarction.

The increased number of Le in the initial phases of atherogenesis and the increase with the progression of the disease indicate that the number of Le can be an indicator of subclinical inflammation of the arterial walls. Starting from the premise that significantly higher values of the number of Le in subjects with

carotide plaques of lower echogenicity are a part of pathophysiological mechanisms of the change of atherosclerosis lesion phenotype from a structurally vulnerable to functionally unstable form and that there is an increase of the number of Le as a reactant of the acute phase, as well as a positive correlation with the lesion size [40] in the neuroinflammatory response and considering the results of the conducted research, it can be concluded that the number of Le is an informative parameter in the clinical assessment of the stability of carotide plaque and risk of developing acute cerebral infarction.

In the pathophysiological mechanisms of atherogenesis the penetration of hsCRP into the arterial wall at the sites of endothelial dysfunction and the presence of a deposit in the early atherosclerotic lesion, binding to Le, synthesis by monocytes and macrophages, increased platelet aggregation, proliferation of smooth muscle cells and reduced expression of endothelial nitric oxide synthase (e-NOS) [1, 38, 41] confirm the proinflammatory and proatherogenic effects of hsCRP in the initiation and progression of atherosclerotic vascular disease. By measuring numerous mediators of the inflammatory process, scientific studies on inflammation and atherosclerotic vascular disease emphasized that hsCRP as indicator of the risk of cerebrovascular ischemic complications was in correalation with the incidence of acute cerebral infarction [35, 38, 39, 42, 43].

The association of the concentrations of hsCRP with hystological determinants of atherosclerotic lesion vulnerability in symptomatic and asymptomatic patients [23, 44] indicates that hsCRP can be indicator of the change in atheroma phenotype from a stable to unstable form and a significant indicator of the risk of developing acute cerebral infarction [45].

Analysis of the connection of biochemical and ultrasound parameters showed the average values of hsCRP were not significantly different among the groups formed according to the hemodynamic significance and structure of carotid plaque. In the atherogenic profile of subjects the concentration of hsCRP was considered a reactant of the acute phase, from the perspective of neurological practice as a part of the systemic response in acute cerebral ischemia without the presence of the extrahepatic synthesis factor of hsCRP [22].

The average value of hsCRP was notably higher in the symptomatic group compared to the asymptomatic and control ones. All the subjects of the symptomatic group had the level of hsCRP above the reference values. Even though there was no correlation between the concentrations of hsCRP and severity of neurological deficit expressed through NIHSS score, in assessing the influence of the concentrations of hsCRP on the development of acute cerebral infarction by univariate logistic regression analysis it was established that hsCRP was a significant indicator of the possible cerebrovascular ischemic complications. Multivariate logistic regression analysis confirmed no predictive significance of hsCRP. The results of the conducted research and the results of other studies [44, 45] confirm a connection of the increased concentrations of hsCRP, carotid atherosclerosis progression and the incidence of acute cerebral infarction and that hsCRP can be an indicator of the presence of unstable carotid plaque and the risk of ischemic cerebral complications. If we accept the attitude that hsCRP is a part of the process of inflammation in the pathophysiology of cerebral ischemia [18, 46–48] and that the increased concentration is related to the severity of neurological deficit [10], the fact that there are patients with normal values of hsCRP after acute cerebral infarction leads to the conclusion that the relation between hsCRP and brain damage is much more complex within a framework of the acute phase [10, 45, 46]. Clinical trials in which the increase of the concentration of hsCRP in defined time intervals after acute cerebral infarction was compared to the values prior to the disease, confirmed that the significance of hsCRP in the pathogenesis of acute cerebral ischemia is the expression of inflammatory system individual response [10, 44, 45].

Conclusion

The predictive significance of the number of Le confirms that the number of Le is associated with the phenomenon of atherosclerotic plaque vulnerability and may be a useful, additional marker in the clinical practice of neurologists in discovering new pharmacological approaches in the prevention of cerebrovascular complications. It is possible that the risk of developing acute cerebral infarction could be reduced by controlling the carotid plaque stability through the continuous therapeutic influence on the pathogenic mechanisms of destabilization.

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Comparison of cefixime and amoxicillin plus metronidazole in the treatment of chronic periodontitis

Poređenje cefiksima i kombinacije amoksicilina sa metronidazolom u lečenju parodontopatije

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Abstract

Background/Aim. Despite significant advances in current medicine and improvement of overall health education, chronic periodontitis is still a widespread disease. Losing teeth is the most serious complication of this particular illness. The aim of this study was to examine patients with chronic periodontitis in order to evaluate the efficacy of non-surgical therapy and combination of amoxicillin and metronidazole compared with cefixime, which has not been so far used for the treatment of this disease. **Methods.** Adult patients with chronic periodontitis (n = 90) underwent non-surgical periodontal treatment (zero-day) and then randomly divided into three groups. The group I served as a control, the group II was additionally treated with the combination of amoxicillin and metronidazole (for 7 days), while the group III was treated with cefixime (also for 7 days). To assess the condition of periodontium before and seven days after the therapy, four clinical parameters were used: gingival index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL). **Results.** On the day 7 after the beginning of the therapy, we found that all the three groups of patients had statistically significant clinical improvement of three parameters: GI, BOP and PD, but not of the CAL. However, the improvement of PD was only statistically, but not clinically significant. The improvement in the control group of patients on the day 7 was 19% in BOP and 28% in GI; this improvement was statistically highly significant after the addition of amoxicillin plus metronodazole (71% in BOP and 77% in GI) or cefixime (62% in BOP and 82% in GI). Compared to the combination of amoxicillin and metronidazole, cefixim was statistically significantly more effective for GI ($p < 0.05$), while for the other three clinical parameters their effects were equal. **Conclusion**. The conjunction of amoxicillin plus metronidazole or cefixime to the causal treatment of patients with chronic periodontitis led to statistically significant improvement in efficacy in relation to GI and BOP parameters, while cefixime was statistically significantly more efficient than the combination of amoxicillin and metronidazole for GI.

Key words:
periodontal diseases; cephalosporins; amoxicillin; metronidazole; treatment outcome.

Apstrakt

Uvod/Cilj. I pored značajnog napretka u savremenoj medicini i poboljšanja opšteg zdravstvenog obrazovanja**,** parodontopatija je još uvek veoma rasprostranjena. Gubitak zuba je najteža komplikacija ovog oboljenja. Cilj našeg rada bio je da kod bolesnika sa parodontopatijom ispitamo efikasnost kauzalne terapije i kombinacije amoksicilina i metronidazola u poređenju sa cefiksimom, koji do sada nije primenjivan kod te kategorije bolesnika. **Metode.** Odrasli bolesnici sa hroničnom parodontopatijom (n = 90) prvo su podvrgnuti kauzalnoj terapiji (nulti dan), a zatim randomizacijom podeljeni u tri jednake grupe. Prva grupa bila je kontrolna, druga grupa dodatno je lečena kombinacijom amoksicilina i metronidazola (AMO-MET) tokom 7 dana, a treća grupa bolesnika cefiksimom, takođe, tokom 7 dana. Za procenu stanja parodoncijuma na početku lečenja i sedam dana kasnije, primenjena su četiri klinička parametra: gingivalni indeks (*gingival index* – GI), indeks krvarenja gingive (*bleeding on probing* – BOP), dubina parodontalnih džepova (*probing depth* – PD) i nivo pripojnog epitela (*clinical attachment level* – CAL). **Rezultati.** Sedmog dana od početka terapije ustanovljeno je da je kod sve tri grupe bolesnika došlo do statistički visokoznačajnog poboljšanja kliničkog stanja procenjenog parametrima GI, BOP i PD ($p < 0,001$), ali ne i parametrom CAL. Međutim, to poboljšanje PD kod sve tri grupe bolesnika bilo je samo od statističkog, ali ne i kliničkog značaja. Posle kauzalne terapije, poboljšanja GI i BOP sedmog dana u odnosu na nulti dan iznosila su 28% i 19%, a nakon primene AMO-MET 71% (BOP) i 77% (GI), odnosno 62% (BOP) i 82% (IG) posle primene cefiksima, što je bilo statistički visokoznačajno bolje u odnosu na kauzalnu terapiju (u oba slučaja $p <$

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0,001). Poređeni međusobno, cefiksim je u odnosu na kombinaciju amoksicilina i metronidazola bio statistički značajno efikasniji za GI ($p < 0,05$). **Zaključak**. Dodatna primena amoksicilina u kombinaciji sa metronidazolom ili cefiksima kauzalnoj terapiji kod bolesnika sa parodontopatijom dovela je do statistički visokoznačajnog poboljšanja parametara GI i BOP, dok je cefiksim bio statistički značajno efikasniji od kombinacije amoksicilin-metronidazol u odnosu na parametar GI.

Ključne reči:
periodontalne bolesti; cefalosporini; amoksicilin; metronidazol; lečenje, ishod.

Introduction

Periodontitis is a severe illness with clinical features manifesting in gingival inflammation, gum recession, formation of periodontal pockets with corresponding pathological content and appearance of subgingival concrements, teeth loosening and pathological teeth migration. Untimely treated, periodontitis leads to teeth loss as the major complication of the illness [1].

Formerly, periodontitis was predominantly the illness of the elderly. However, in the past few decades, there has been an increase in the number of diseased children and adolescents [2]. The main etiological cause of periodontitis is bacterial infection. Until the introduction of antibiotics, the therapy was only based on scaling and root planning. Afterwards, a widespread use of antibiotics has begun in dentistry, too. In the meantime the range of used antibiotics has been most commonly reduced to the combination of amoxicillin (AMO), broad spectrum antibiotic, and narrow spectrum metronidazole (MET) acting on anaerobes as the main co-causative agents of the infection.

Related to antibiotic therapy, Zandbergen et al. [3] stated that the efficacy of AMO-MET combination with and without non-surgical periodontal treatment was the topic of examination in 526 publications. Their statement is in accordance with meta-analysis data of Villagrana et al. [4] and Sgolastra et al. [5], as well as with recent systematic review of Kolakovic et al. [6]. According to them, combination of these two antibiotics has significantly improved the efficacy of non-surgical periodontal treatment proclaimed as "gold standard". Nevertheless, according to a systematic review and meta-analysis, Sgolastra et al. [5] are of the opinion that additional studies are needed to confirm these results.

As a representative of the third generation of cephalosporins – cefixime (CEF), which is an antibiotic with wide spectrum of effect on Gram-positive and Gram-negative bacteria, was used in cases of oral infections [7, 8], but not in the treatment of periodontitis. In studies *in vitro* on 178 bacterial strains isolated from 74 patients with pyogenic infections of odontogenic origin, some strain isolates were fairly susceptible, with CEF MIC ranging from 8 to 16 μg/mL. Therefore, the aim of our study was to examine the efficacy of CEF in comparison with AMO-MET as the most common combination of antibiotics, and in both cases after the conduction of non-surgical therapy.

Methods

The clinical study was conducted at the Department of Periodontology, Clinic for Dentistry of the Military Medical Academy in Belgrade. It involved 90 patients with the progressed form of periodontitis (35 females and 55 males, the mean age 49.3 years). Criteria for including patients in the study entailed newly, previously untreated and systemic healthy patients, selected on the basis of clinical inspection, whereby a prerequisite was to have at least 23 natural teeth, four of which were first molars and each of the examinees had minimally three teeth in each quadrant, with periodontal pocket depth of 5 mm and larger and also gingival bleeding after periodontal probing.

All the participants underwent a clinical examination of the oral cavity as well as periodontal examination. Adequate clinical and anamnestic data were taken for all the participants and also personal data, general medical and dental anamnesis. All the patients first underwent non-surgical periodontal treatment (day zero) and then they were randomly divided into three equal groups. The first group served as a control, the second one was additionally treated by the combination of AMO and MET, and the third one by CEF.

In order to assess the condition of periodontium, we used the level of gingival inflammation which is expressed through gingival index (GI) according to Löe and Silness [9], bleeding on probe (BOP), according to Mühlemann and Son [10], probing depth (PD) and clinical attachment level (CAL). As a part of periodontal examination, we performed measuring on all permanent teeth. Measurement was done by the graduated periodontal probe (CPITN:US, WILLIAMS; Pro-Dentec, Batesville, Ark) by the routine method. All listed clinical parameters were noted at the beginning as well as seven days after the treatment.

AMO (500 mg) and MET (400 mg) were given perorally, three times a day, and CEF (400 mg) perorally, once a day. Antibiotics were administered over a period of seven days and immediately after the causal treatments of periodontium had been performed. The patients were advised to observe any adverse reaction during the use of antibiotics. All three antibiotics are registered in Serbia in the form of oral use [8].

Statistical analysis was performed by Student's *t*-test for differences between the groups, with statistically significant results for $p < 0.05$.

Results

The results of the study are shown in Table 1 and Figure 1.

Table 1 shows that the values of three (GI, BOP and PD) out of four examined clinical parameters were statistically significantly improved on the seventh day since the beginning of non-surgical therapy. Nevertheless, the fourth clinical parameter (CAL) showed only negligible improvement after seven days and it did not reach statistical significance in relation to the control value.

Table 1

The influence of the type of treatment on clinical periodontal parameters

Groups (before/after) treatment	GI	BOP	PPD	CAL
	$\bar{x} \pm SD$			
Control				
Day 0	1.950 ± 0.437	0.850 ± 0.140	4.296 ± 0.179	3.607 ± 0.918
Day 7	1.408 ± 0.502	0.616 ± 0.260	4.290 ± 0.174	3.389 ± 1.171
p_1	< 0.001	< 0.001	< 0.001	n.s.
AMO-MET				
Day 0	1.783 ± 0.618	0.750 ± 0.254	4.269 ± 0.162	3.579 ± 1.128
Day 7	0.525 ± 0.427	0.175 ± 0.209	4.241 ± 0.153	3.201 ± 1.013
p_1	< 0.001	< 0.001	< 0.001	n.s.
p_2	< 0.001	< 0.001	n.s.	n.s.
Cefixime				
Day 0	1.700 ± 0.680	0.816 ± 0.404	4.309 ± 0.125	3.543 ± 0.992
Day 7	0.316 ± 0.346	0.133 ± 0.182	4.268 ± 0.175	3.199 ± 1.115
p_1	< 0.001	< 0.001	< 0.001	n.s.
p_2	< 0.001	< 0.001	n.s.	n.s.
p_3	< 0.05	n.s.	n.s.	n.s.

p_1 – significance within the groups before and after the treatment; p_2 – significance between the antibiotics and control group after treatment; p_3 – significance between the antibiotics groups after the treatment; GI – gingival index; BOP – bleeding on probing; PD – probing depth; CAL – clinical attachment level; AMO-MET – amoxicillin-metronidazole.

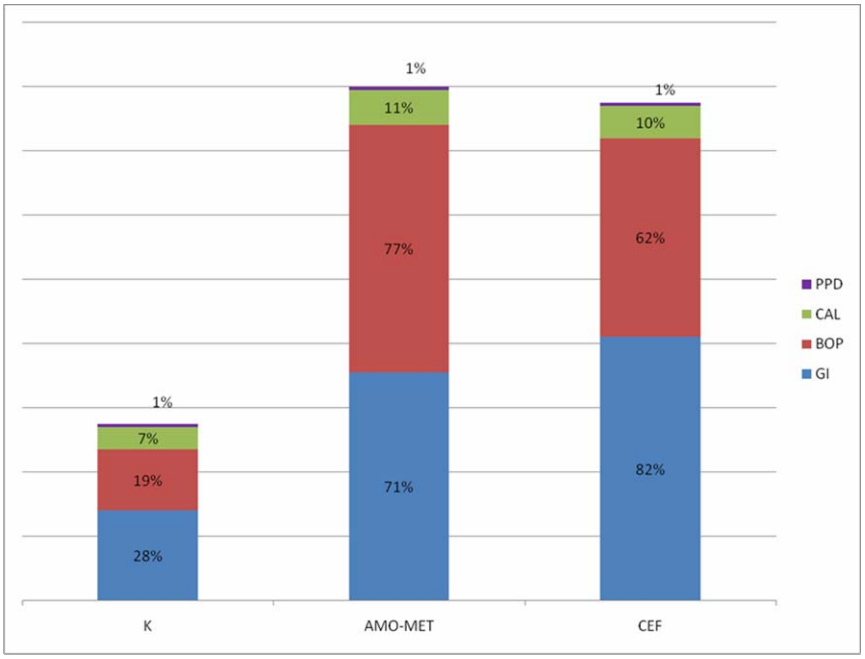

Fig. 1 – The percentage of relative values of clinical parameters improvement based on the results shown in Table 1 at the end of the treatment (Day 7) in relation to the initial values (Day 0) taken as 0%.
Each column represents one of the three tested groups. The values of the tested parameters at day 0 were represented as 0%, and those of day 7 were calculated as the increase of the corresponding percentage for each of the four tested clinical parameters shown in Figure 1. PPD – periodontal probing depth; CAL – clinical attachment level; BOP – bleeding on probing; GI – gingival index.

When antibiotics AMO-MET or CEF were added to non-surgical therapy, the treatment results of GI and BOP showed statistically significantly higher improvement compared to the control values on the zero day. Simultaneously, concerning two other clinical parameters, there was a statistically significant improvement in the PD, but not in the CAL. On the other hand, when the effects of the antibiotics were compared to the values of the control group on the day zero (P2), then a statistically significant improvement was registered only in values of GI and BOP. However, contrary to the administration of antibiotics, differences of other two indexes (PD) and (CAL) were not statistically significant compared to their control values on the day zero. Comparing the results between the two groups of antibiotics (P3), it was found that CEF was statistically significantly more efficient than the combination of AMO and MET for GI. The other three parameters (BOP, PD, CAL) were not statistically significantly different comparing two antibiotic regimes.

Figure 1 shows that in respect of the periodontal pocket depth in the control group of patients there was practically no relative improvement (1%); a relatively better improvement

was registered in CAL (7%), and the best improvement was noted in BOP (19.9%) and GI (28%).

In the group of patients treated with AMO-MET combination, there was practically no relative improvement in PD (1%). A somewhat greater improvement was registered in CAL (11%), while the improvements in GI and BOP were statistically significant (71% and 77% respectively).

In the group of patients treated with CEF, there was practically no improvement regarding the periodontal pocket depth (1%), while it was somewhat higher in CAL (10%). Unlike those clinical parameters, there was a statistically significant improvement in clinical condition of GI (82%) and BOP (62%).

Reported side-effects were rare, mild and transient in nature. They mainly manifested as nausea, dizziness and/or anorexia and appeared in both groups of patients treated with antibiotics.

Discussion

The results of our study show that administration of AMO-MET or CEF statistically highly significantly improve the efficacy of non-surgical therapy concerning GI and POB, while all the three types of treatment are practically ineffective concerning PD and CAL. Furthermore, CEF is significantly more efficient than AMO-MET concerning GI. Since each of the three patient groups represented separate entities, the discussion is accordingly divided in that way.

Non-surgical therapy

Although non-surgical therapy was introduced long ago, it still represents the basis of periodontitis treatment [11]. This kind of therapy removes biofilm (dental plaque), supragingival and subgingival concrements, which are the source of infection and the cause of illness, curettes periodontal pockets and removes all factors that contribute piling plaque and decreases the overall resistance of bacteria [1].

From that point of view, the results of our study show that non-surgical therapy effectively improves GI and POB, has slight significance on PD and is practically ineffective concerning CAL. Our results are in accordance with the majority of authors who have also show that, seven days after the beginning of non-surgical therapy, a significant improvement in clinical condition in the values of GI and POB is achieved [12–14]. Furthermore, hitherto clinical experiences also show that the improvement in clinical condition in PD and in CAL, after three, six and twelve months also ensued [15–19]. We did not follow-up our patients longer than seven days as, according to our previous experience, after the improvement of clinical condition, they rarely regularly return for control examinations, making thus maintaining the initial number of patients in the formed groups difficult.

However, with non-surgical therapy it is impossible to remove all bacteria deposits. For example bacteria from deep unapproachable periodontal pockets or microorganisms from other areas of oral cavity, which are beyond the scope of non-surgical therapy, make results of this therapy to be unpredictable and dependent upon many different factors [20].

AMO-MET

At first, antibiotics were administered as monotherapy which was not enough effective due to the existence of mixed aerobic-anaerobic flora [7]. For this reason, a dual therapy consisting of AMO-MET was introduced. At the same time, this combination is considered to be the "golden standard" of antibiotic therapy [16].

The results of our study show that the combination of AMO-MET has statistically significantly improved clinical efficacy of non-surgical therapy concerning two indexes (GI, BOP), but not the two others (PD, CAL). Our results are in accordance with the findings of other authors [5, 6, 21], as well as meta-analyses [4, 22]. However, the mentioned benefit of AMO-MET treatment has to be balanced against their possible adverse reactions [23, 24].

CEF

Our results with CEF show significant improvement of non-surgical periodontal treatment in values of two clinical parameters (GI, BOP), while for the other two parameters (PD, CAL) it was practically ineffective.

CEF belongs to the third generation of cefalosporins with a broad spectrum of antibacterial activity. So far, it was studied only *in vitro* in relation to bacterial strains isolated from patients with pyogenic infections of odontogenic origin [25], but not in patients with periodontitis. CEF significantly improved the clinical condition of GI and in this respect was statistically significantly more effective than the AMO-MET combination. According to the data from Eusterman [7], CEF is very effective against many oral infections. CEF also has several advantages over AMO-MET combination like: taking only one antibiotic instead of two, its dosage is much more comfortable (once instead of three times a day), and it could be more tolerant than the AMO-MET combination.

That is why we consider that whenever additional antibiotic therapy is needed in non-surgical therapy CEF might be useful alternative. Certainly, it should be pointed out that for definitive attitude, clinical experience with CEF on larger number of patients is needed.

Conclusion

Co-administration of amoxicilline plus metronidazole or cefixime in adult patients with periodontal disease significantly increases therapeutic effect of causal therapy concerning gingival bleeding and bleeding on probing indexes, but not probing depth and clinical attachment level. Between the two groups of antibiotics, the improvement of gingival index was significantly better in the group of patients treated with cefixime. Side effects of antibiotics were rare, mild and transient by nature.

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Patterns of prescription antihypertensive drug utilization and adherence to treatment guidelines in the city of Novi Sad

Praksa propisivanja lekova protiv povišenog krvnog pritiska u Novom Sadu i usklađenost sa farmakoterapijskim smernicama

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Abstract

Background/Aim. Hypertension is one of the leading causes of cardiovascular morbidity and mortality and more than a half of all health insurance expenditures for reimbursed medicines are allocated to antihypertensive drugs in Serbia. The aim of this study was to identify the antihypertensive drug utilization patterns among hypertensive outpatients in the city of Novi Sad, Serbia, determine the adherence to clinical guidelines and address the economic aspects of current prescribing practices. **Methods.** This retrospective observational study was conducted in Novi Sad over a period of six months. The data on the number of packages, size their, and retail price of antihypertensives issued on prescription in outpatients with the diagnosis of essential arterial hypertension was collected from all state-owned pharmacies in Novi Sad. Drug consumption was analyzed using the Anatomical Therapeutic Chemical (ATC)/ defined daily dose (DDD) methodology. **Results.** Total consumption of antihypertensives issued on prescription over a 6-month period in the city of Novi sad, Serbia was 283.48 DDD *per* 1,000 inhabitans *per* day (DID). Angiotensin converting enzyme inhibitors (ACEi) were most commonly prescribed drugs, and were used 3 times more often than calcium channel blockers and 5 times more than beta-blockers. The consumption of diuretics and angiotensin receptor antagonists was low within all the groups of outpatients. Both national and international guidelines state superiority and effectiveness of diuretics in treatment of hypertension in the elderly, but their consumption was unreasonable low despite the fact that over 70% of all antihypertensive drugs in the city of Novi Sad were dispensed in people aged > 60. The use of more expensive ACEi was observed despite the guidelines deeming all the drugs of this class equally effective in treatment of hypertension. **Conclusion.** Large differences in utilization of different groups of antihypertensive agents were noted in this study. Underutilization of valuable, efficacious, and cost-effective thiazide diuretics and overuse of expensive ACE inhibitors is unjustifiable. There is a potential for large savings with switching to low-price ACEi, modeling the practice of Scandinavian countries.

Key words:
antihypertensive agents; serbia; cost-benefit analysis; economics, pharmaceutical; drug utilization review.

Apstrakt

Uvod/Cilj. Hipertenzija je jedan od vodećih uzroka kardiovaskularnog morbiditeta i mortaliteta u Srbiji, te više od polovine troškova za lekove na teret osiguranja odlazi na antihipertenzivne lekove. Cilj ove studije bio je analiza strukture upotrebe antihipertenzivnih lekova kod hipertenzivnih vanbolničkih pacijenata u Novom Sadu, usklađenosti sa farmakoterapijskim uputstvima i ekonomskih aspekta trenutne prakse propisivanja. **Metode.** Podaci o prometu i potrošnji antihipertenzivnih lekova propisanih kod vanbolničkih pacijenata sa dijagnozom esencijalne arterijske hipertenzije u šestomesečnom periodu prikupljeni su u državnim apotekama u Novom Sadu. Lekovi su klasifikovani prema anatomsko-terapijsko-hemijskoj klasifikaciji lekova i izračunate definisane dnevne doze (DID) na hiljadu stanovnika na dan. **Rezultati.** Ukupna potrošnja antihipertenzivnih lekova izdatih na recept u periodu od šest meseci u Novom Sadu bila je 283,48 DID. Najčešće korištena grupa lekova bili su inhibitori angiotenzin konvertujućeg enzima (ACEi), koji su propisivani tri puta češće od kalcijumskih antagonista i pet puta više od beta blokatora. Iako uputstva za lečenje hipertenzije navode da su svi ACEi jednako efikasni, primećena je upotreba skupljih ACEi. Upotreba diuretika i antagonista receptora angiotenzina bila je niska u posma-

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tranom periodu. Farmakoterapijska uputstva naglašavaju prednost i efikasnost diuretika u lečenju hipertenzije kod starijih osoba, ali njihova potrošnja bila je neopravdano niska, uprkos činjenici da je preko 70% svih antihipertenzivnih lekova u Novom Sadu propisana pacijentima starijim od 60 godina. **Zaključak.** Ovo ispitivanje pokazalo je velike razlike u propisivanju različitih grupa antihipertenzivnih lekova. Nedovoljno korišćenje tiazidnih diuretika, lekova sa najboljim odnosom koristi i troškova i upotreba skupih ACE inhibitora je neopravdana. Postoji mogućnost za značajne uštede sa racionalnijom upotrebom ACEi, po uzoru na skandinavske zemlje.

Ključne reči:
antihipertenzivi; srbija; troškovi-korist, analiza; farmakoekonomika; lekovi, korišćenje, izveštaji.

Introduction

Cardiovascular diseases (CVD) are a leading cause of morbidity and mortality in the Republic of Serbia [1]. Hypertension has been recognized for decades as a major risk factor for heart failure, coronary heart disease, myocardial infarction and stroke [2]. However, many other factors contributing to CVD such as obesity, smoking, physical inactivity and high cholesterol levels are also widely present among Serbian people with over a half of population having some of the risk factors listed above [3, 4]. Therefore, it is not surprising that CVD accounted for 53.7% of deaths in Serbia in 2012 [4]. According to the Institute of Public Health of Serbia reports, the overall prevalence of hypertension in Serbia is 33% [5]. Reducing blood pressure is one of the most cost-effective methods to reduce CVD morbidity and mortality [6]. Despite the life style changes, treatment of hypertension relies mostly on medications, and cardiovascular drug utilization therapy consumes a huge amount of financial resources [7]. In Serbia, 55.5% of all health insurance expenditures for reimbursed medicines are allocated to antihypertensive drugs [8]. Previous studies showed that there may be certain inadequacies in the treatment of arterial hypertension in Serbia. Inappropriate use of drug resources exacerbates the problem of plummeting drug expenditure, often without contributing to improved patient outcomes [9]. Research conducted in 2006 showed that among patients being treated for hypertension in the city of Novi Sad only 20.9% had blood pressure within the desired range [10] which points to the need for evaluation of prescribing practices among Serbian physicians.

This study was designed to identify the antihypertensive drug utilization patterns among outpatients in the city of Novi Sad, Serbia, with essential arterial hypertension, to determine the adherence to clinical guidelines, address the economic aspects of current prescribing practices and to estimate the potential for drug-cost savings if more rational prescribing practices were employed. Rational prescribing would in this case mean using less expensive angiotensin converting enzyme inhibitors (ACEi) as in Scandinavian countries, with developed pharmacotherapeutic practice.

Methods

This retrospective observational study was conducted in the city of Novi Sad (estimated population 350,000) from September 2011 to February 2012. The data on the number of packages, size of packages, and retail price of antihypertensive drugs issued on prescription were collected from all state-owned pharmacies in the city of Novi Sad. A report from state-owned pharmacies includes data on diagnosis, age and sex of the patients and price of drug package (in dinars - DIN). Conversion of prices to euros was done according to the National Bank of Serbia exchange rates on February 29, 2012 (EUR = 110.2 DIN). Since antihypertensive drugs are also used in treatment of other cardiovascular diseases, only drugs issued on International Classification of Diseases (ICD) code I10 (essential arterial hypertension) were included in this study. Drug consumption was analyzed using the Anatomical Therapeutic Chemical (ATC) classification of World Health Organization (WHO) Collaborating Center for Drug Statistics and Methodology [11]. Utilization rates were calculated as defined daily dose (DDD) *per* 1,000 inhabitants *per* day (DID). Drug utilization (DU90%) methodology was also used. The DU90% profile is a simple method that reflects the number of drugs that account for 90% of drug prescriptions. High quality of prescribing is associated with the use of a relatively limited number of pharmaceutical products within a drug group. The number of products in the DU90% segment and adherence to prescription guidelines serve as general quality indicators [12]. The price *per* DDD for each specific drug utilized and the mean total price *per* DDD were also calculated. The impact of adjusting the patterns of ACE inhibitors use to that of Scandinavian countries and direct drug costs of current and of the adjusted pattern were calculated. Possible drug-cost savings were estimated as the difference between the potential and current use. The data on the patterns of consumption of ACE inhibitors in Finland and Norway was extracted from the databases of the representative national authorities. The actual prescription patterns were compared to the current Serbian National Guidelines, 2009 Guidelines of the European Society of Cardiology and European Society of Hypertension (ESC/ESH), 2003 American Society of Hypertension Guidelines and 2011 British National Institute for Clinical Excellence (NICE) Guidelines for Treatment of Hypertension.

Results

Over a 6-month period in the city of Novi Sad, Serbia, a total of 670,477 packages of medication were issued for treatment of hypertension – 85% of all cardiovascular drugs dispensed. Up to 2013, including our study period, prescription drugs were available for reimbursement only in state owned pharmacies. Total utilization of antihypertensives issued on prescription was 283.48 DID (Table 1). More medication

was prescribed to female outpatients, but no substantial differences in the structure of different groups of antihypertensives were observed between sexes. The use of antihypertensive medication increased with patients age, with over 70% being prescribed to patients over the age of 60 (Figure 1). ACE inhibitors were most commonly prescribed drugs in all age groups, followed by calcium channel blockers (CCBs), and beta-blocking agents (BBs). The consumption of diuretics and angiotensin II receptor blockers (ARBs) was low in all the groups of patients.

Sixteen drugs were within DU90%, accounting for 255.65 DID (Table 2). Amlodipine was most commonly used

Table 1

Consumption of the main groups of antihypertensive agents according to sex of patients

ATC group	Male		Female		Total	
	DID (n)	Share (%)	DID (n)	Share (%)	DID (n)	Share (%)
C03	3.62	3.19	6.28	3.70	9.90	3.49
C07	12.52	11.03	20.36	11.98	32.87	11.60
C08	26.83	23.64	37.72	22.18	64.55	22.77
C09A	58.37	51.42	84.98	50.00	143.35	50.57
C09B	9.42	8.30	15.77	9.28	25.19	8.88
C09C	2.52	2.22	4.41	2.59	6.93	2.44
C09D	0.24	0.21	0.45	0.27	0.69	0.24
Total	113.52	100.00	169.97	100.00	283.48	100.0

C03 – diuretics, C07 – beta blocking agents; C08 – calcium channel blockers; C09A – angiotensin converting enzyme inhibitors, C09B – angiotensin converting enzyme inhibitors, combinations; C09C – angiotensin II receptor antagonists; C09D – angiotensin II receptor antagonists, combinations; ATC – anatomical therapeutic clinical; DID – defined daily dose *per* 1,000 inhabitants *per* day.

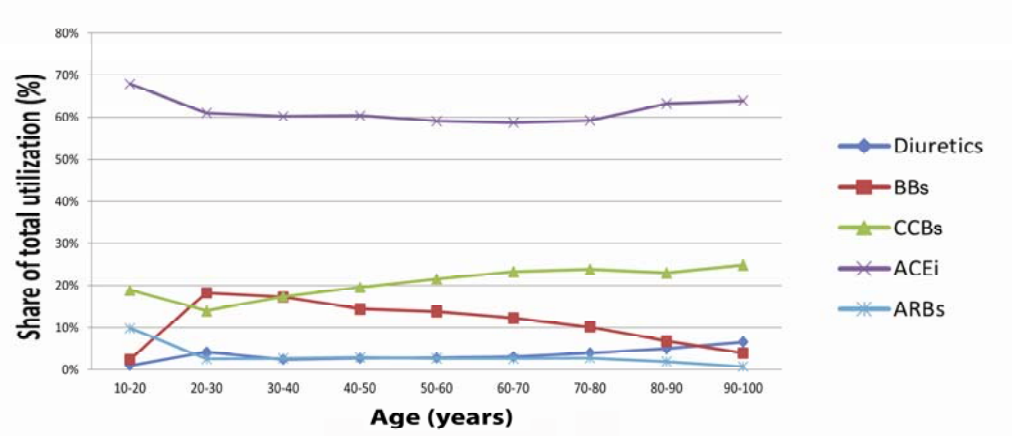

Fig. 1 – Trends in the use of the main groups of antihypertensives according to age of patients. BBs – beta blocking agents; CBBs – calcium channel blockers; ACEi – angiotensin-converting enzyme inhibitors; ARBs – angiotensin II receptors antagonists.

Table 2

Consumption of antihypertensives – DU90% profile expressed in the number of DDD/1,000 inh/day (DID), share, average price *per* DDD (in Euros), and patient co-payment

No	ATC	INN	DDD [n]	DDD [%]	Price *per* DDD [€]	Co-payment (%)
1	C08CA01	amlodipin	52.51	18.5	0.06	/
2	C09AA02	ramipril	39.42	13.9	0.06	/
3	C09AA05	enalapril	38.56	13.6	0.07	/
4	C09AA09	fosinopril	22.12	7.8	0.15	35
5	C07AB02	metoprolol	18.32	6.5	0.09	/
6	C09AA08	cilazapril	15.57	5.5	0.11	35
7	C09AA01	captopril	15.12	5.3	0.09	/
8	C09AA03	lisinopril	8.50	3.0	0.06	/
9	C09BA02	enalapril/HCTZ[1]	7.47	2.6	0.11	/[2]
10	C09BA05	ramipril/HCTZ	7.35	2.6	0.11	/[2]
11	C07AB07	bisoprolol	7.05	2.5	0.10	/
12	C08CA05	nifedipin	6.38	2.3	0.06	/[2]
13	C09DA01	losartan	5.61	2.0	0.11	25
14	C09BA09	fosinopril/HCTZ	4.82	1.7	0.18	30
15	C08DB01	diltiazem	3.49	1.2	0.17	/
16	C09BA03	lisinopril/HCTZ	3.36	1.2	0.10	/[2]
Within DU90% 1–16			255.65	90.2	0.10	8
Beyond DU90% 17–42			27.84	9.8	0.13	24
Total N = 42			283.48	100.00	0,12	19

[1]HCTZ – hydrochlorothiazide; [2]limited reimbursement (fixed combinations available for reimbursement after 3 months of treatment with single preparations); DDD – defined daily dose; ATC – anatomical therapeutic clinical; INN – international nonproprietary names; DU90% – drug utilization 90%.

and made up more than 18% of the total consumption and two more drugs of the same class, nifedipine and diltiazem were within DU90%. Metoprolol was the most commonly used beta-blocker, followed by bisoprolol. Out of 16 drugs in DU90% profile, 10 were ACEi or ACEi and combinations. Neither thiazide diuretics, nor ARB were within DU90%, as thiazide diuretics and combinations altogether comprised 2.4% and ARB comprised 2.6% (losartan 2.0%, other ARB 0.6%). The average price *per* DDD within the DU90% segment was 0.10 Euro/DDD, whereas for the antihypertensives beyond the DU90% segment the average price/DDD was 0.13 Euro/DDD. Direct drug cost of antihypertensive medication distributed was more than 1.5 million euro (Figure 2). Utilization and expenditure share were similar for the majority of drugs, and drugs within the DU90% segment had better utilization-expenditure ratio, especially amlodipine, enalapril and ramipril (Figure 3). A potential change in the use of ramipril and enalapril and corresponding decrease in the use of expensive ACE (i.e. fosinopril) provided a resulting net potential for direct drug-cost savings of 175,000 euro over a 6-month period in the city of Novi Sad (Table 3).

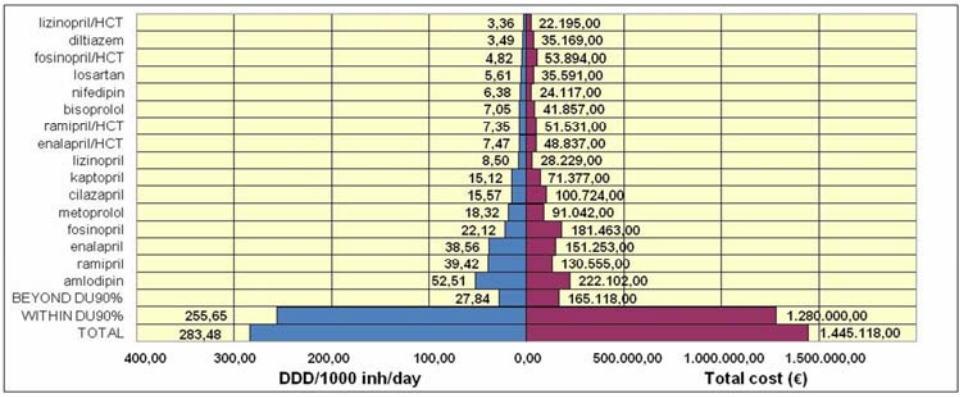

Fig. 2 – Drug utilization (DU90%) segment – utilization and expenditure comparison. DDD – defined daily dose.

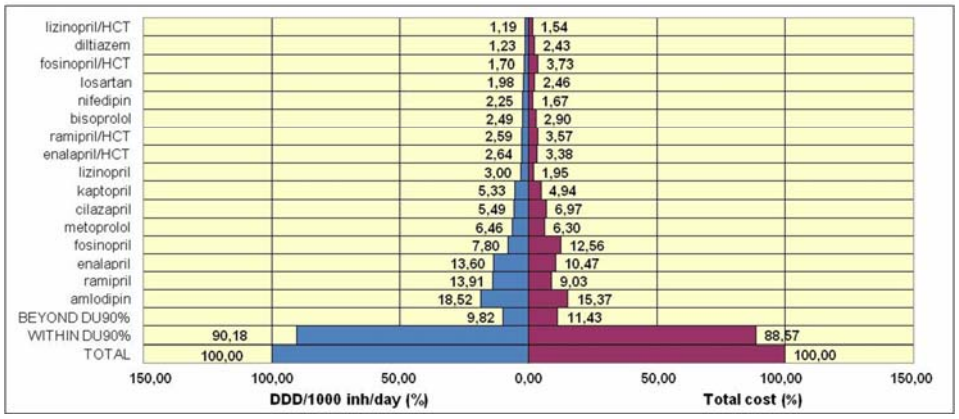

Fig. 3 – Drug utilization (DU90%) segment – comparison of utilization and expenditure share. DDD – defined daily dose.

Table 3

The impact of adjusting angiotensin converting enzyme inhibitors (ACEi) prescribing to that of Scandinavian countries - current total use in Novi Sad, Serbia (DID) and direct drug cost (in Euros), pattern ACEi prescribing adjusted to Scandinavian countries and potential savings over a 6-month period.

Drug (INN)	Price *per* DDD (€)	Current ACEi use in Novi Sad			Adjusted ACEi use*		
		DID	Share (%)	Cost (€)	Share (%)	Cost (€)	Savings (€)
Captopril	0.09	15.12	10.6	71,377	0.6	3,886	67,491
Enalapril	0.07	38.56	26.9	151,253	25.6	143,596	7,657
Lizinopril	0.06	8.50	5.9	28,229	4.7	22,467	5,762
Perindopril	0.11	1.60	1.1	9,534	4.5	38,346	-28,812
Ramipril	0.06	39.42	27.5	130,555	64.5	305,808	-175,254
Quinapril	0.11	2.30	1.6	15,692	0.2	1,588	14,104
Cilazapril	0.11	15.57	10.9	100,724	/	/	100,724
Fosinopril	0.15	22.12	15.5	181,463	/	/	181,463
Zofenopril	0.19	0.17	0.1	1,975	/	/	1,975
Total		143.19	100.0	688,827	100.0	515,691	175,111

***adjusted to the structure of use of ACE inhibitors in Scandinavian countries.**
ATC – anatomical therapeutic clinical; DDD – defined daily dose; DID – defined daily dose *per* 1,000 inhabitants *per* day; INN – international nonproprietary names.

Discussion

Both Serbian and ESC/ESH guidelines state that all the available antihypertensive classes are suitable for hypertension management [13, 14], so individual patient needs and comorbidities, as well as on economic aspects should be critical when determining preferential antihypertensive drugs. Despite neither of the main types of antihypertensives being favored in clinical guidelines, ACEi were the most commonly used drug group, used 3 times more than CCBs, 5 times more than BBs and 20 times more than diuretics. Vast usage of ACEi also reflected the DU90% profile – out of 16 drugs within DU90%, 9 were ACEi. Beneficial effects of ACEi in hypertensive patients have been well documented and in British NICE guidelines they are recommended as first line treatment of hypertensive patients under 55 years of age [15]. ACEi do not only lower blood pressure, but also have vasoprotective, antiaterogenic effect and improve prognosis of CVD, lower the incidence of myocardial infraction and stroke [16, 17]. No difference in efficacy has been documented between different drugs in the ACEi group, nor clinical guidelines favor specific ACEi, yet clinicians in Serbia prescribed extensive amounts of fosinopril and cilazapril, with two times higher price *per* DDD than, for example, enalapril. Fosinopril with less than 8% of total utilization accounted for nearly 13% of total spending. Fund reimburses 65% of costs for these drugs; the rest needs to be compensated by patients [18, 19]. Multiple studies [20–22] emphasized that higher prescription copayments were associated with poor compliance. The reasons for prescribing more expensive ACEi despite no proved clinical benefit are probably a combination of tendency of the physician to use a wide palette of drugs, inclination towards newer drugs and marketing pharmaceutical campaigns [23]. There are differences in the use of ACEi between Serbia and Scandinavian countries. In Norway and Finland, ACEi and combinations are also the primary drug class used, but they account for around 20–30% of all drug utilized, a share much smaller than in the city of Novi Sad [24, 25].

Most patients require two or more antihypertensive drugs to achieve blood pressure control [21]. Fixed combinations of antyhipertensives (ACEi or ARBs with diuretics or CCBs) allow combination therapy with a higher patient compliance and thus better control of hypertension compared to multiple drugs administration. These drugs, mainly ARBs and diuretics combinations are widely used in Scandinavian countries such as Norway and Finland, with well-developed pharmacotherapeutic practice [24, 25]. Utilization of these drug types is much lower in Serbia, especially for ARBs combinations. Many factors limit their use in Serbia, since a fixed combination of antihypertensives can be reimbursed only after a failure to achieve blood pressure control with a 3-month combination therapy with single drug preparation and copayement ranges from 25% [(most ACEi and hydrochlorothiazide (HCTZ)] to 70% (telmisartan and HCTZ) [18, 19]. CCBs were also widely utilized in hypertensive patients, amlodipine being the most commonly prescribed drug. CCBs utilization is probably associated with demographic characteristics of the city of Novi Sad, where 40% of inhabitants are older than 50, and guidelines recommend calcium channel blockers in treatment of hypertension in people over the age of 55. A higher usage of BBs was observed in people under 40, which is in accordance with the national guidelines [13], where BBs are the drugs of choice in case of hypertension associated with increased sympathetic tone, mostly observed in younger people. A lower usage of BBs in older population may be due to the fact that many of comorbidities in senior population are a contraindication for use of these drugs.

Both national and international guidelines state superiority and effectiveness of diuretics [13–15, 26] in treatment of hypertension in the elderly, but their consumption was unreasonable low despite the fact that over 70% of all antihypertensive drugs in the city of Novi Sad were dispensed to people > 60. The elderly are more likely to have developed organ damage related to hypertension or to have heart failure or diabetes as concomitant conditions, and thiazide diuretics, drugs with the highest cost-effect index, which are at least as effective as BBs and ACEi in reducing cardiovascular events (CVEs) in patients with hypertension, and that are more effective in reducing stroke [27] accounted for 2.4% of total consumption. A study conducted in the city of Niš region of Serbia [28] also demonstrated underutilization of diuretics which emphasizes the need to undertake efforts to change this practice in Serbia. In 1995, a total utilization of diuretics was similar to todays (10.221 DID in 1990 *vs* 9.9 in 2012), even though CVD drugs were used in much smaller extent [29]. In the last 25 years, the use of CVD drugs in the city of Novi Sad has doubled, yet the consumption of diuretics stayed the same. The use of thiazides in the city of Novi Sad, Serbia is low in comparison to the neighboring Croatia [30], as well as western countries. A share of thiazides in all drugs utilized in treatment of hypertension ranges from 7.8% in Sweden, 6.8% in Netherlands to 25.0% in Denmark [31]. The American Society of Hypertension guidelines [26] recommend thiazides as initial treatment of non-complicated hypertension and thiazide accounted for 14% of total utilization in the USA in 2012 [32].

Our analysis indicates that there is a substantial potential for savings if Serbian physicians prescribe low-cost ACEi more frequently, modeling the practice of Scandinavian countries. An increase in ramipril and enalapril consumption with an equivalent decrease in the use of more expensive ACEi, could result in the yearly savings around 350,000 euros in the city of Novi Sad alone. If we extrapolate these results to the national level, since in Serbia, in 2012, 44 million euro worth of ACEi were distributed [33], the savings worth 5.5 million euros could be provided. In Serbia, drugs for therapy are used much more than drugs for the prevention of CVDs, such as serum lipid-reducing drugs. The study that compared the use of lipid reducing drugs in Serbia and Scandinavian countries [34] has shown that the mortality rate for CVDs and the use of serum lipid-reducing drugs have an inverse relationship. Numerous obstacles to lipid-lowering treatment are present in Serbia, as the Republic Health Fund has a very stringent criteria for refunding the cost of statins. A saving provided with changes in ACEi patterns could be redistributed and widen the indications for statin reimburse-

ment, and make lipid-lowering drugs available for primary prevention of cardiovascular diseases.

The limitations of our study that should be mentioned are imposed by the type of data used which contained no information on compliance and actual consumption.

Conclusion

Despite the guidelines stating that all available antihypertensive classes are suitable for hypertension management this study demonstrates substantial differences in utilization of different groups of antihypertensive agents in the city of Novi Sad. Irrational prescribing and preference to more expensive drugs have been reported in the city of Novi Sad. Underutilization of valuable, efficacious, and cost-effective thiazide diuretics and over the use of expensive ACE inhibitors is unjustifiable. The modeling structure of the use of ACE inhibitors in Scandinavian countries could produce substantial savings and significantly reduce pressure on already limited health resources without negative effect on treatment outcomes.

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Modelling and assessment of the electric field strength caused by mobile phone to the human head

Modelovanje i procena jačine električnog polja mobilnog telefona u predelu glave

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Abstract

Background/Aim. Electromagnetic field exposure is the one of the most important physical agents that actively affects live organisms and environment. Active use of mobile phones influences the increase of electromagnetic field radiation. The aim of the study was to measure and assess the electric field strength caused by mobile phones to the human head. **Methods.** In this paper the software "COMSOL Multiphysics" was used to establish the electric field strength created by mobile phones around the head. **Results.** The second generation (2G) Global System for Mobile (GSM) phones that operate in the frequency band of 900 MHz and reach the power of 2 W have a stronger electric field than (2G) GSM mobile phones that operate in the higher frequency band of 1,800 MHz and reach the power up to 1 W during conversation. The third generation of (3G) UMTS smart phones that effectively use high (2,100 MHz) radio frequency band emit the smallest electric field strength values during conversation. The highest electric field strength created by mobile phones is around the ear, i.e. the mobile phone location. The strength of mobile phone electric field on the phantom head decreases exponentially while moving sidewards from the center of the effect zone (the ear), and constitutes 1–12% of the artificial head's surface. **Conclusion.** The highest electric field strength values of mobile phones are associated with their higher power, bigger specific energy absorption rate (SAR) and lower frequency of mobile phone. The stronger electric field emitted by the more powerful mobile phones takes a higher percentage of the head surface. The highest electric field strength created by mobile phones is distributed over the user's ear.

Key words:
cellular phone; electromagnetic fields; health; head.

Apstrakt

Uvod/Cilj. Izloženost elektromagnetnom polju spada u najvažnije fizičke agense koji aktivno utiču na žive organizme i na prirodu. Stalna upotreba mobilnih telefona povećava radijaciju elektromagnetnog polja. Cilj ove studije bio je da se izmeri i odredi jačina električnog polja koje stvara mobilni telefon u predelu ljudske glave. **Metode.** U ovoj studiji koristili smo softver COMSOL *Multiphysics* za utvrđivanje jačine električnog polja koje stvaraju mobilni telefoni oko glave. **Rezultati.** Druga generacija telefona 2G GSM (2nd *Generation Global System for Mobile*) frekvencije od 900 MHz i snage od 2 W ima snažnije električno polje od mobilnih telefona 2G GSM koji rade u višem opsegu frekvencije, tj. 1 800 MHz i snage do 1 W tokom razgovora. Treća generacija pametnih telefona 3G UMTS koji efektivno koriste visoki opseg radiofrekvencije, tj. 2 100 MHz, emituju najslabije električno polje tokom razgovora. Najjače električno polje mobilni telefoni zrače u predelu uha, tj. na mestu mobilnog telefona. Jačina električnog polja mobilnog telefona na model glave opada eksponencijalno kada se pomera na stranu od epicentra efektivne zone (uho) i iznosi 1–12% površine modela glave. **Zaključak.** Najviše vrednosti jačine električnog polja mobilnih telefona povezane su sa većom snagom, većom specifičnom brzinom apsorpcije energije (*specific energy absorption rate* – SAR) i nižom frekvencijom. Jače električno polje koje emituju mobilni telefoni veće snage zauzima veći procenat površine ljudske glave. Najjače električno polje mobilnog telefona stvara se u predelu uha korisnika.

Ključne reči:
mobilni telefon; elektromagnetna polja; zdravlje; glava.

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Introduction

The world we live in is constantly modernizing and the electromagnetic radiation emitted by electronic devices, electric power transmissions, television and radio stations, computers, radars, mobile phone antennas and, especially by mobile phones constantly affects people and can cause various health problems. Electromagnetic waves surround us, however, we do not smell, see, nor feel them [1]. A rapid increase in the level of electromagnetic radiation (many times exceeding original background) that can damage ecological balance of the environment has been observed; therefore it should be researched and evaluated by theoretical and experimental methods [2].

In the 21st century, with the spread of mobile phone networks and the introduction of GSM-900, GSM-1,800, UMTS-2,100 systems, the number of sources of electromagnetic radiation increased [3, 4]. A lot of people use mobile connection without being concerned about its operating principles: something that is not seen can cause harm [5]. Active use of mobile phones and their level of temporal dynamics allow for perceiving the component of electromagnetic background as one of the most important physical agents that actively affect live organisms and environment [6].

Due to the growing number and scope of sources of electromagnetic radiation from mobile phones, their effect is significant not only for specialists but for all the inhabitants of surrounding areas [7, 8]. In some countries, mobile-free zones are demanded. In such zones it is forbidden to install mobile communication base stations, it is also required to reduce the highest levels of allowed electromagnetic radiation or other restrictions may be imposed [9].

A mobile phone is one of the most actively used sources of electromagnetic radiation. It is a small compact transmitter and receiver in a single casing [10]. The owner always carries mobile phone nearby; therefore, it has effects on the owner [11, 12]. When the mobile phone is used, it is put to the ear, and when not – it usually stays in the pocket, near the body. In this way the electromagnetic radiation, emitted by (ringing) mobile phone directly interacts with the human body [13, 14]. While talking on the phone, the electromagnetic field is directly pointed towards the brain. During conversation, each mobile phone emits different strengths of electric field [15].

The World Health Organisation's International Agency for Research on Cancer on May 31, 2011 announced that when the mobile phone is used for a long time, 30 or more minutes per day, radiofrequency electromagnetic fields could increase risks of brain tumour. The World Health Organisation stresses that when used properly and all safety measures followed, the negative effects of a mobile phone on health caused by electromagnetic radiation can be minimised or avoided [16, 17]. The contemporary opinion on the negative effects of mobile phone antennas and electromagnetic radiation from mobile phones does not allow for making assumptions about future consequences [18].

Measurement and evaluation of electromagnetic radiation level, reduction of the highest allowed level of electromagnetic radiation and establishment of other regulations are the issues of worldwide importance. At the moment, the most important concern is the contradictory information about mobile phones and mobile communication base stations, and their declared effects on human health [19, 20].

Due to the lack of scientific research on electromagnetic field radiation from mobile phones and public concern, the electromagnetic field radiation strength parameters of various mobile phones and emission to the human body are discussed in this study. The aim of the study was to create and to assess the strength spread by the electric fields of mobile phones around the head.

Methods

We used the software "COMSOL Multiphysics" for modelling of the mobile phone electric field around the head during the call mode.

"COMSOL Multiphysics" is a powerful, interactive environment which we can apply for modelling of the mobile phone electric field. By using this software, we can simulate the propagation of electromagnetic fields induced in the human head while it is in active mode. For the propagation of electromagnetic fields, the program "COMSOL Multiphysics" uses the proven finite element method (FEM). Using a various number of solutions, the software performs finite element analysis, error control, and activates the adaptive grid (if selected). "COMSOL Multiphysics" is suitable for evaluating human exposure to radiofrequency of electric field strength.

"COMSOL Multiphysics" for simulation of the electric field strength uses the artificial head model (phantom) composed of different thicknesses layers: skin, fat, muscle, skull and brain. In addition to the head, it shows a mobile phone. The mobile phone simulates a half-wave length dipole, which is equal in frequencies 900 MHz – 166 mm, 1,800 MHz – 83 mm, and 2,100 MHz – 71 mm. The dipole antenna is divided into segments of length $\lambda/30$, and the brain, skin and other tissue segmentation selected $\lambda/15$ side, according to the creators of the program guidelines. The distance between the mobile phone and the head is equal to 1 cm (Figure 1). The program simulates electric field intensity distribution in the mobile phone active mode. In view of the use of mobile phones and antennas, the dipole model is used with a 2 W, 1 W, 0.8 W, 0.25 W and 0,125 W radiant power and dipole wave impedance of 50 Ω.

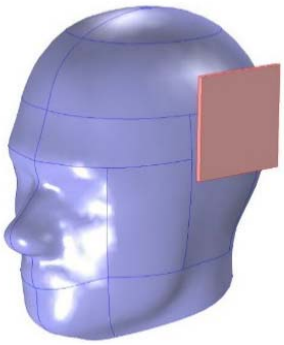

Fig. 1 – Electric field strength simulations using the artificial (phantom) head and a mobile phone.

Taking into account the use of mobile phones and their characteristics, the simulation program allows to change the basic settings and monitor the electric field distribution on the surface of the head. In order to simulate the electric field distribution in the artificial head, we also used the following data: the mobile phone of the specific energy absorption rate (SAR), the maximum transmission power, frequencies, and electromagnetic signal levels in the environment. After setting up the necessary parameters, the software "COMSOL Multiphysics" allows to model the spread of mobile phone electric fields in the head at the distance of 1 cm.

The research was conducted by using GSM second generation (2G) mobile phones that operated within frequency range from 900 MHz to 1,800 MHz and by UMTS third generation (3G) smart mobile phones that operated in 2,100 MHz frequency range. The main criteria in the choice of mobile phones were the differences in power, SAR, and frequency.

Results

SAR is a measure to estimate the absorbed energy by the human body when exposed to electromagnetic field. Reliable estimation of SAR values has become a very important concern. It is impossible to measure SAR *in vivo*, but reliable SAR values can be easily obtained from electric field data.

The experimental electric field distribution in the real human head is not a simple task. Thus, we used the artificial head with the following layers: skin, fat layer, skull and brain, and we accessed possible electric field strength values distribution around the head in active call mode.

Figure 2 shows smart mobile phone (power – 2 W, SAR 1.4 W/kg, frequency – 900 MHz) electric field intensity distribution in the active mode.

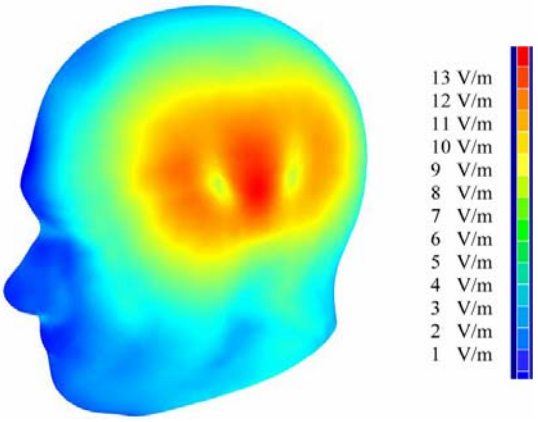

Fig. 2 – Mobile phone [power 2 W, specific energy absorption rate (SAR) 1.4 W/kg, frequency 900 MHz] and the distribution of electric field values during phone talk.

Figure 3 displays the distribution of mobile phone electric field strength during conversation (SAR 0.6 W/kg, maximum power – 0.8 W, used frequency – 900 MHz).

Figure 3 shows that the electric field strength created by the mobile phone (colour red) reaches 6–7 V/m around the ear (i.e. the place to which the mobile phone is put) in a 3 cm radius. In a 4–5 cm radius (colour yellow) the electric field strength is lower (4–5 V/m). At the distance of 5–10 cm radius (colour green) the electric field strength is 2–3 V/m. In the upper part of the head (light blue colour) the electric field strength is about 2 V/m. In the upper and lower parts of the head, at both sides of the head or on top of it the electric field strength decreases to 1 V/m. The electric field strength of the mobile phone takes about 8% of the whole artificial head.

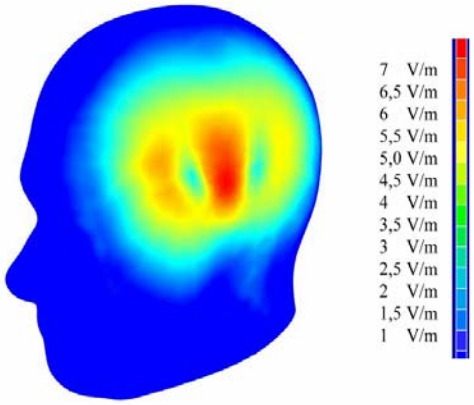

Fig. 3 – Mobile phone [power 0.8 W, energy absorption rate (SAR) 0.6 W/kg, frequency 900 MHz] and the distribution of electric field values during phone talk.

The study shows that the electric field strength of 0.8 W power mobile phones is weaker and smaller by size compared with a mobile phone 2 W power. As we see, the electric field strength of the mobile phone takes about half of the human head compared with the mobile phone of 0.8 W power. Here, the electric field strength takes only one-third of all the head.

Figure 4 displays the distribution of mobile phone electric field strength during conversation on the mobile phone of 0.99 W/kg SAR, 1 W maximum power, 1,800 MHz used frequency.

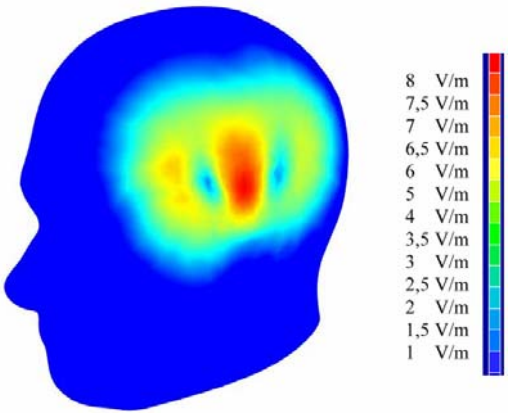

Fig. 4 – Mobile phone [power 1 W, energy absorption rate (SAR) 0.99 W/kg, frequency 1,800 MHz] and the distribution of electric field values during phone talk.

The study reveals (Figure 4) that the electric field strength created by the mobile phone (colour red) reaches 6–8 V/m around the ear (i.e. the place to which the mobile phone is put) in a 3 cm radius. In a 4–5 cm radius (colour yellow) the electric

field strength is lower (5 V/m). Furthermore, at the distance of 5–10 cm radius (colour green) the electric field strength is 2–4 V/m. In the upper part of the head (light blue colour) electric field strength is about 2–4 V/m. In the upper and lower parts of the head, at both sides of the head or on top of it the electric field strength is decreasing to 1 V/m. The electric field strength of the mobile phone takes about 7% of the whole artificial head.

Figure 5 displays the distribution of mobile phone electric field strength during conversation over the mobile phone of 0.6 W/kg SAR, 0.25 W maximum power, 1,800 MHz used frequency. The results show (Figure 5) that the electric field strength created by the mobile phone (colour red) reaches 4–5 V/m around the ear (i.e. the place to which the mobile phone is put) in the radius 1.5 cm. In radius 1.5–2 cm (colour yellow) the electric field strength is lower (3.8 V/m). At the distance of 2–3 cm radius (colour yellow green) the electric field strength is 2–4 V/m. In the upper part of the head (blue colour) electric field strength is about 1 V/m. In the upper and lower parts of the head, at both sides of the head or on top of it the electric field strength decreases to 1 V/m. The electric field strength of the mobile phone takes about 4% of the whole artificial head.

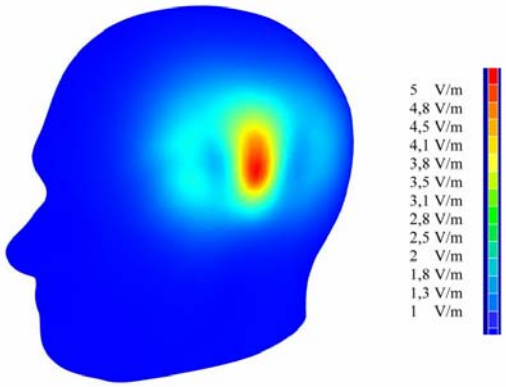

Fig. 5 – Mobile phone [power 0.25 W, energy absorption rate (SAR) 0.6 W/kg, frequency 1,800 MHz] and the distribution of electric field values during phone talk.

The experiments reveal that electric field strength of 0.25 W power mobile phones is 1.6 times weaker compared with 1 W (SAR 0.99 W/kg) power, 2.5 times weaker compared with 2 W (SAR 1.4 W/kg) power, and 1.4 times weaker compared with 0.8 W (SAR 0.99 W/kg) power mobile phones.

The third generation of smart mobile phones Universal Mobile Telecommunication Systems (UMTS) (3G) emits 2,100 MHz frequency range waves. The emitted wavelength of smart phones is quite short (about 7 cm). Figure 6 displays the distribution of smart mobile phone electric field strength during conversation over a mobile phone, 1.22 W/kg SAR, 2 W maximum power, 2,100 MHz used frequency. The study reveales (Figure 6) that the electric field strength created by the mobile phone (colour red) reaches 9–11 V/m around the ear (i.e. the place to which the mobile phone is put) in a 2 cm radius. In a 3–4 cm radius (colour yellow) the electric field strength is lower (3.8 V/m). At the distance of 2–3 cm radius (colour yellow green), the electric field strength is 4–7 V/m. At the distance of 4–6 cm radius (light blue colour) the electric field strength is 1–4 V/m. In the upper part of the head (blue colour) the electric field strength is about 1 V/m. In the upper and lower parts of the head at the distance of 6 cm, at both sides of the head or on top of it the electric field strength decreases to 1 V/m. The electric field strength of the mobile phone takes about 5% of the whole artificial head.

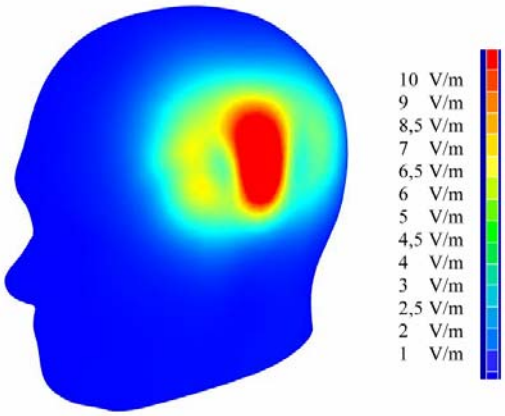

Fig. 6 – Smart mobile phone [power 2 W, energy absorption rate (SAR) 1.22 W/kg, frequency 2,100 MHz] and the distribution of electric field values during phone talk.

Figure 7 displays the distribution the electric field strength of the mobile phone during conversation over the mobile phone 0.57 W/kg SAR, 0.125 W, maximum power 2,100 MHz used frequency. The study reveals (Figure 7) that the electric field strength created by the mobile phone (colour red) reaches 1 V/m around the ear (i.e. the place to which the mobile phone is put) in a 1 cm radius. Because the mobile phone emits a very low electric field strength, its radiation area is very small, only 3 cm. In this area the prevailing electric field strength is 0.1–0.9 V/m. When the distance from the mobile phone is more than 3 cm, the electric field strength is not detected on the upper and lower parts of the head, at both sides of the head or on top of it. The electric field strength of the mobile phone takes about 1% of the whole artificial head.

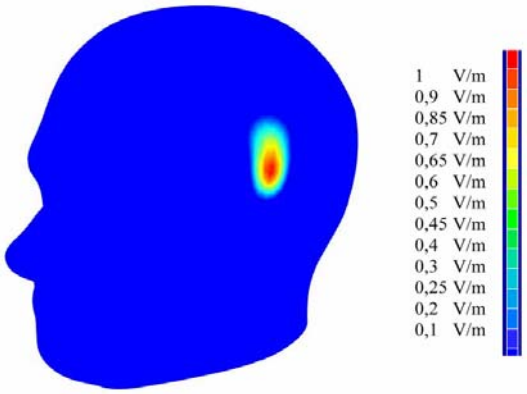

Fig. 7 – Smart mobile phone [power 0.125 W, energy absorption rate (SAR) 0.57 W/kg, frequency 2,100 MHz] and the distribution of electric field values during phone talk.

The experiments revealed that the electric field strength of 0.125 W power mobile phones was 10 times weaker compared with 2 W (SAR 1.22 W/kg) power mobile phone, 7 times weaker compared with 0.8 W (SAR 0.6 W/kg) power mobile phone, and 13 times weaker compared with 2 W (SAR 1.4 W/kg) power mobile phone.

The study shows that stronger power, higher SAR and lower frequency of the mobile phone is associated with the stronger electric field of the device. As we can see, the highest values of electric field strength are determined by the powerful mobile phones. The electric field strength of the powerful mobile phones is stronger and bigger. A stronger electric field emitted by more powerful mobile phones takes a higher percentage of the head surface. The third generation (3G) UMTS smart phones that operate in the frequency bond of 2,100 MHz, emit smallest electric field strength values during the conversation. This is determined by the effective usage (smart phones use communication types of extended spectrum) of radio frequency (2,100 MHz). In addition, the electric field emitted by smart phones constitutes the smallest percentage (only 1%) of the head's surface.

Discussion

The interaction between the human body and electromagnetic radiation can induce electric currents and electric fields inside human bodies, what can produce side effects to health. Adverse effects investigated by various clinical trials include the possible link to increased risk of leukaemia, sleep disturbances and brain tumours [1, 2, 4, 21]. Numerous studies stated that the intensive use of mobile phones can cause headache, fatigue, insomnia, muscle pains, hearing and eyesight defects, failures of memory, neck and facial skin redness, and increase stress [12, 13, 21]. The mentioned symptoms can short-termed arise either during or sometimes after phone conversation [18]. It means that adults, young people, and children spending a number of hours for talking with mobile phone are directly exposed to the harmful effects of electromagnetic fields. Considering all these points, the current studies are necessary to investigate the effects of electromagnetic fields on the humans [14]. Some uncertainties concerning possible carcinogenic effects should also be considered. According to epidemiological studies on mobile phones and cancer (children and teenagers leukaemia, adult leukaemia, lymphatic leukaemia, breast and brain tumour), it was concluded that the possibility of the enhanced cancer risk could not be excluded [2]. The use of mobile phones is associated with the increased risk for brain tumour after 10 years [1, 4, 12]. The International Agency for Research on Cancer stated overall evaluation that radiofrequency electromagnetic fields are possibly carcinogenic to humans [7, 21]. Exposure to electromagnetic radiation can cause nerve and muscle stimulation and changes in nerve cell excitability in the central nervous system [8].

Exposure to electromagnetic radiation normally results in negligible energy absorption and no measurable temperature rise in the human body. The effects of external exposure to electromagnetic radiation on the human body depend mainly on the time and strength of electromagnetic fields [8, 21].

In order to assess potential health risks, reliable exposure assessment and modelling are necessary. Radio frequency electromagnetic radiation from mobile phones can be reliably modelled using special programs like "COMSOL Multiphysics". In epidemiological studies on the adverse health effects of mobile phones, it is especially important to determine exposure size and strength considering that electromagnetic radiation has a potential hazard to the human head. Our results show that most of the electric fields are absorbed at the side of the head nearest to the mobile telephone.

The user of a mobile phone is exposed to radiofrequency radiation much higher than those found in the environment (mobile telecommunication, television or radio antennas). Mobile phones are in a very close contact with the human head. That is why the distribution of absorbed energy by the head of the user must be determined. From sophisticated computer modelling and measurements using models of heads, it appears that the energy absorbed from a mobile phone is not evaluated quite enough.

According to the results of the research, it is possible to renew the legal regulatory framework of electromagnetic expertise, that regulates the allowed intensity level of the electromagnetic fields, and to improve evaluation methods and methodologies for electromagnetic radiation. Considering the results of the analysis of electric field radiation of mobile phones, it is possible to provide recommendations for mobile phone safety. This could protect mobile phone users from biologic effects, health problems, and from the possible carcinogenic effects. According to other studies, using a safer device at the safe distance, and following safety measures, the harm caused by mobile phone could be minimised [16, 17, 21].

The most common document with recommendations concerning electromagnetic radiation is The Council Recommendation on electromagnetic field exposure limits (1999/519/EC), where standards of 41 V/m and 58 V/m are set as the limits (at 900 MHz and 1,800 MHz). However, many countries follow up the guidelines what are below this limit, because of public complaint and attitude. Lithuania does not have limitation for electric field strength at 900 MHz and 1,800 MHz (the only limitation for electromagnetic field power density is 10 $\mu W/cm^2$).

The strong side of the study was the assessment of the electric field strength exposure, to the phone user, by various models of mobile phones with different technical parameters. The results of the study could be used for public health risk and hazard prevention, measurement of electromagnetic radiation emitted by mobile phones, and regulatory documents, legislation, and limitation preparations.

Conclusion

The highest electric field strength values are emitted by more powerful mobile phones, with the bigger specific absorption rate and with lower frequency mobile phones. The second generation 2G Global System for Mobile phones that operate in the frequency band of 900 MHz and reach power of 2 W have the stronger electric field than the second gen-

eration 2G Global System for Mobile mobile phones that operate in the higher frequency band of 1,800 MHz and reach power up to 1 W (during conversation). The third generation 3G Universal Mobile Telecommunication System smart phones that effectively use high (2,100 MHz) radiofrequency band, emit the smallest electric field strength values during conversation.

The highest electric field strength created by mobile phones is absorbed by the user's ear (i.e. in the mobile phone location). The strength of mobile phone electric field to the phantom head decreases exponentially while moving sidewards from the center of the effect zone (the ear), and constitutes 1–12% of the artificial head's surface. A stronger electric field emitted by the more powerful mobile phones takes higher percentage of the head surface.

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Marketing communication in the area of breast and cervical cancer prevention

Marketinška komunikacija u oblasti prevencije karcinoma dojke i grlića materice

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Abstract

Background/Aim. Innovative marketing campaigns and promotional activities can successfully contribute to the improvement of public health by raising the level of general knowledge about health issues and benefits that the change of habits, eradication of undesirable behaviour and regular medical controls have. The focus should be on continuous marketing communication through various mass media or direct communication between medical staff and patients. The aim of this paper was to define the role that various communication channels have in the process of informing and educating the target group in case of breast and cervical cancer prevention. **Methods.** The survey based on polling a sample of 2,100 female patients of the Serbian Railways Medical Centre was conducted in the period October–December 2013. The questionnaire included questions about demographic characteristics, prevention habits of women, their level of information on that topic and communication channels they prefer. **Results.** There is a difference among respondents' awareness level about preventive measures depending on demographic and geographical criteria. The results indicate the existence of variations in frequency of performing gynaecological examinations and Pap tests depending on different age, educational and residential groups. Although the largest percentage of women stated familiarity with the way of performing breast self-examination (78%), the majority of them had never performed mammography or ultrasonography (67%). The greatest number of women were informed about the possibility of preventing breast and cervical cancer by posters or brochures in health institutions (71%) and mass media – television on the first place (74%), then specialized magazines about health (48%), radio (48%), web sites about health (42%), and daily newspapers (34%). The respondents consider the Ministry of Health and health institutions as the most responsible subjects for education of women about cancer prevention, while the self-initiative was given the least importance. **Conclusion.** Determined informing habits of the target group, their prevention habits and attitudes on the subject should be used as the basis for planning and implementation of prevention marketing campaigns that would be the most effective.

Key words:
breast neoplasms; uterine cervical neoplasms; health promotion; communications media.

Apstrakt

Uvod/Cilj. Inovativne marketinške kampanje i promotivne aktivnosti mogu uspešno uticati na poboljšanje zdravstvenog stanja stanovništva kroz podizanje nivoa njihove opšte informisanosti o zdravstvenim problemima i koristima koje menjanjem navika i iskorenjivanjem nepoželjnog ponašanja stiču. Fokus bi trebalo da bude na kontinuiranoj marketinškoj komunikaciji putem različitih masovnih medija ili direktnom komuniciranju medicinskog osoblja sa bolesnicima. Cilj rada bio je definisanje uloge koju različiti kanali komunikacije imaju u procesu infomisanja i obuke ciljne grupe u slučaju karcinoma dojke i grlića materice. **Metode.** Istraživanje je sprovedeno anketiranjem uzorka od 2 100 bolesnica Zavoda za zdravstvenu zaštitu „Železnice Srbije" u periodu oktobr/decembr 2013. godine. Upitnikom su obuhvaćena pitanja važna za ispitivanje demografskih karakteristika, preventivnih navika kod žena, stepena njihove informisanosti i kanala komunikacije koji im odgovaraju. **Rezultati.** Ustanovljeno je da postoji razlika u nivoima svesti ispitanica o preventivnim merama u zavisnosti od demografskih i geografskih kriterijuma. Rezultati su ukazali na postojanje varijacija u učestalosti obavljanja ginekoloških pregleda i Papanikolau testova u zavisnosti od godina života, obrazovne stukture i mesta stanovanja. Iako je najveći procenat žena upoznat sa načinom obavljanja samopregleda dojke (78%), većina njih nikada nije izvršila mamografski ili ultrasonografski pregled (67%). Najveći broj žena obavešten je o mogućnostima pre-

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vencije karcinoma dojke i grlića materice putem plakata i brošura u zdravstvenim ustanovama (71%), a zatim masovnih medija – televizije na prvom mestu (74%), specijalizovanih časopisa o zdravlju (48%), radija (48%), veb sajtova (42%) i dnevnih novina (34%). Ispitanice smatraju da su Ministarstvo zdravlja i zdravstvene ustanove najodgovorniji subjekti za obuku žena iz prevencije karcinoma, da je samoinicijativi žena dat najmanji značaj. **Zaključak.** Definisane navike ciljne grupe u informisanju, preventivne navike i stavovi o ovoj temi, trebalo bi da posluže kao osnova za planiranje i sprovođenje preventivnih marketinških kampanja koje bi imale najefikasnije rezultate.

Ključne reči:
dojka, neoplazme; grlić materice, neoplazme; zdravlje, promocija; komunikacijski mediji.

Introduction

Current statistics indicate that, worldwide, every two minutes one woman dies of cervical cancer. Globally, cervical cancer is the third most common cancer type in women – every year approximately 500,000 women are being diagnosed with it, and more than half of that number die from that disease [1]. Approximately 85% of the overall number of diagnoses occurs in less developed regions where there are no organized prevention programs [2]. According to the data pertaining to Serbia, cervical cancer represents the cause of 6% of all deaths in women. Serbia has the highest incidence of cervical cancer (24.3 *per* 100,000) in relation to all the other republics of the former Yugoslavia and one of the highest prevalence among the countries of South Eastern Europe. The incidence of cervical cancer in Serbia is two times higher than the incidence in Western Europe (10.43 *per* 100,000 women) and about three times higher than in European Union (EU) countries (where the figure is 8.1 *per* 100,000 women). Cervical cancer development shows a typical increase after the age of 30, with the most common detection in the age groups from 45 to 49 and from 70 to 74 years [3].

Public health data on the global level indicate that the burden of breast cancer in women, measured by incidence, mortality and economic costs is substantial and increasing [4, 5]. Worldwide, each year more than one million women are diagnosed with it and about 410,000 will die from the disease, which represents 14% of overall female cancer deaths [6, 7]. Statistics indicates that one in eight women either already has breast cancer or will become ill during lifetime [8]. In less developed countries, breast cancer incidences rates have been reported to be increasing by 5% annually [9]. Due to the poor infrastructure and lack of resources for routine screening mammography, breast cancers are commonly diagnosed at late stages when "women may receive inadequate treatment, pain relief, or palliative care" [10, 11]. In Europe, about 129,000 women die each year, and about 370,000 are diagnosed. Breast cancer is the most common malignant tumour in women in Serbia. There are about 4,000 new cases every year and about 1,600 of them die, which makes 18% of the overall number of deaths from all cancer types [8].

Besides underdeveloped public health infrastructure, that can limit access to preventive screenings, the reason for such prognoses, when it comes to cervical and breast cancer, lies in women's lack of basic health education [2]. Practice has shown that the problem of public health improvement is closely linked to the informing and education of the population in order to increase knowledge and awareness of the public [12–14], as the lack of information about prevention was reported to have been impeding women from getting tested [15]. At the international level, it has been proven that investments in marketing communication about prevention are more significantly associated with improved general health of the whole population than economic growth and investment in other social spheres [16]. The results suggest that the higher awareness level about prevention can lead to improved health outcomes in developing countries [17], such as Serbia. Although developed countries place greater attention to intensive and continual marketing campaigns dedicated to health promotion, in Serbia, conventional approaches still dominate, which are related to the rehabilitation and cancer treatment rather than disease prevention. By using mass media, which includes television, radio, print, a range of visual media, and the Internet, which can efficiently "disseminate accurate health information, promote changes in public attitudes toward health, and ensure the continuity of new health behaviours" [18], health communication nowadays has been significantly advanced and facilitated [19]. There are various empirical evidences in the existing literature that marketing communication campaigns have been effective in improving either cervical screening or mammography and achieved the diffusion of adequate knowledge, increase of awareness and understanding [15, 20–23]. So, if properly planned and implemented, marketing communication in the field of prevention has a tendency to influence the general public and lead to the change of social consciousness and timely recognition of the health problems [24], so the mortality rate of certain types of malignancies, including breast and cervical cancer, would be significantly reduced. Therefore, the aim of this paper is to define the importance that various communication channels have in the process of informing and educating women in Serbia in case of breast and cervical cancer prevention.

Methods

The survey based on polling a sample of 2,100 female patients of the Serbian Railways Medical Center was conducted in October, 2013. The female patients were given the questionnaire to fill in while waiting for the examination. The condition of eligibility was that women were older than 20. No other condition was required. The survey instrument was developed based on thorough literature review on this topic. The survey instrument was used to examine the following issues: demographic characteristics; knowledge of ways to prevent cervical and breast cancer; past history of gynaecological examinations and the Papanicolau (Pap)

tests; past history of mammogram screenings; past history of performing breast self-examination; main sources of information about prevention of cervical and breast cancer; significance of different mass media in preventive communication; the most responsible subjects for incitement of cancer prevention education programmes. Entire data analyses were performed by using the SPSS statistical software.

Results

The sample was structured by age into four groups: from 20 to 29 years (31% of the respondents), from 30 to 39 years (21%), from 40 to 49 years (16.05%) and 50 years or more years (31.95%). A greater percentage of the respondents (68.95%) came from urban areas, while the rest (31.05%) lived in rural areas in Serbia. In terms of education, the approximate percentages of the sample have completed their secondary school education (44.04%) and higher education (41.96%), while the remaining 14% finished only primary school.

First, we examined the association between demographic characteristics of the sample and habits of performing gynaecological examinations and the Pap test. The frequency of the respondents' answers is shown in Table 1.

Table 1

Preforming cervical cancer preventive activities

Characteristics of respondents	Gynecological examination, n (%)				Pap test, n (%)		
	More than twice	Twice	Once	None	Always	Not regularly	Never
Age (years)							
20–29	83 (12.74)	128 (19.65)	336 (51.61)	104 (16.00)	335 (51.46)	273 (41.93)	43 (6.61)
30–39	64 (14.53)	96 (21.75)	210 (47.62)	71 (16.10)	229 (51.93)	168 (38.09)	44 (9.98)
40–49	21 (6.24)	84 (24.92)	189 (56.08)	43 (12.76)	168 (49.85)	126 (37.39)	43 (12.76)
≥ 50	23 (3.43)	61 (9.09)	482 (71.83)	105 (15.65)	398 (59.31)	188 (28.02)	85 (12.67)
Area							
urban	152 (10.50)	272 (18.78)	813 (56.15)	211 (14.57)	901 (62.21)	440 (30.39)	107 (7.40)
rural	63 (9.66)	64 (9.81)	399 (61.21)	126 (19.32)	231 (35.43)	316 (48.47)	105 (16.10)
Education							
primary	8 (2.72)	14 (4.76)	136 (46.26)	136 (46.26)	23 (7.82)	203 (69.05)	68 (23.13)
secondary	49 (5.30)	140 (15.13)	609 (65.84)	127 (13.73)	504 (54.49)	315 (34.05)	106 (11.46)
high	56 (6.36)	108 (12.26)	635 (72.08)	82 (9.30)	594 (67.42)	246 (27.93)	41 (4.65)

The research results indicate that more than half of women who belonged to the age group of 20 to 29 years, in most cases, performed gynaecological examination annually. More than half of those who regularly went for check-ups always did Pap test, more than 40% sometimes and the least of them never. When it came to the age group of 30 to 39, the results are similar, as the majority of women in this group also went to gynaecological examination once a year. More than half of them did Pap test regularly, a great percentage sometimes and less than 10% never. Approximately 16% of the members of this age group did not go to the gynaecologist at all. Among women aged 40 to 49, the highest percentage went to annual examinations and the quarter twice a year. The Pap test was done regularly by half of them and sometimes by almost 40%. More than 70% of women who had 50 or more years went to gynaecological examination once a year, and this was definitely the dominant answer. The Pap test was always performed by almost 60%, which makes this age group the most responsible, regarding this issue ($\chi^2 = 131.388$, df = 9, $p < 0.01$). Considering the fact that they are at the higher risk, greater awareness of preventive behaviour is desirable and comprehensive. From the perspective of areas that the respondents come from, it can be concluded that there were some variations in responses, although not statistically significant. Contrary to our expectations, a greater number of women from rural areas stated that they went to regular gynaecological examination in comparison with women from urban areas. But, when it comes to regular performing of Pap test, women from urban areas lead (62.28% of them), in comparison to women in rural areas (35.48%). The results indicated the existence of statistically significant differences in the frequency of performing gynaecological examinations observed by education level ($\chi^2 = 237.633$, df = 6, $p < 0.01$). More than 70% of university educated women claimed to do annual gynaecological examination, almost the same percentage regularly did Pap test. When it came to women with secondary education, the survey results indicated that the greatest percentage did annual gynaecological examination. Pap test was a regular item for more than half of them, for more than one third occasional, while the rest did not consider that it should be done. Equal percentages of women (46.19%) who had only primary education performed gynaecological examinations once a year and never. Sixty nine percent of women within this age group were not examined for the Pap test regularly, while only 80% of them did it every year. The percentage of those who had never performed it was significant (23.00%). The results show a significant association between educational level and performing regular Pap testing, thus the higher the level of education was, women were more aware of the necessity of prevention and, therefore, preventive habits were more developed ($\chi^2 = 327.906$, df = 4, $p < 0.01$).

The results indicate that the largest percentage of respondents from all age categories were familiar with the way

of performing breast self-examination, which was especially obvious in case of the oldest group (96.90%). The percentage of women who performed self-examination was slightly higher in urban than in rural areas. The awareness of the necessity of performing self-examination increased with the level of education. Within the group of women with only primary education, the percentage of those who were not familiar with this type of examination was higher – 61.48%. A total of 8% of women with secondary education were familiar with the way of carrying out this examination, as well as 95% of those with higher education. Table 2 shows the results of breast cancer preventive measures.

Table 2

Performing breast cancer preventive activities

Characteristics of respondents	Breast self-examination, n (%)		Mammography / Ultrasonography, n (%)	
	Performed	Never performed	Done	Never done
Age (years)				
20–29	447(68.66)	204 (31.34)	85 (13.06)	566 (86.94)
30–39	315 (71.43)	126 (28.57)	106 (24.04)	335 (75.96)
40–49	261 (77.45)	76 (22.55)	125 (37.09)	212 (62.91)
≥ 50	650 (96.87)	21 (3.13)	377 (56.19)	294 (43.81)
Area				
urban	1218 (84.12)	230 (15.88)	669 (46.20)	779 (53.80)
rural	484 (74.23)	168 (25.77)	168 (25.77)	484 (74.23)
Education				
primary	181 (61.56)	113 (38.44)	67 (22.79)	227 (77.21)
secondary	740 (80.00)	185 (20.00)	371 (40.11)	554 (59.89)
high	839 (95.23)	42 (4.77)	264 (29.97)	617 (70.03)

A high percentage of women of all age groups had never performed mammography or ultrasonography, although it was significantly lower in case of women that had 50 or more years. There was a statistically significant relation between age categories and performing of mammography or ultrasonography ($\chi^2 = 298.817$, df = 3, $p < 0.01$). Although this habit is not highly established among respondents, it is obvious that older women are more likely to do mammographic checks than younger ones. The percentage of women who had done mammogram screening was higher in urban areas than in rural, but it remained evident that the overwhelming percentage of the women had never performed this type of examination. In the group of women with only primary education, more than one fifth of them had done this type of examination so far, as well as two fifths of women with secondary and one third of women with higher education.

The largest number of women of all ages had been informed about the possibility of preventing breast and cervical cancer by the media – more than 60% of those who belonged to the first three age groups and 40% of women older than 50. Medical providers were identified as the second important source of information in case of all four age groups. Among women aged 20 to 39, there were no uninformed about these issues, while some percent of women older than 40 identified themselves as uninformed. Family and friends were stated to be the third important source of information, while the least importance was given to lecturers at schools/universities or work places. For women who came from urban areas, the main sources of information were medical providers and then media and none of them were uninformed. In rural areas, the highest percent of respondents had received the information by the media, significantly less (close to 20%) by medical providers, while more than 6% stated not to be informed about this topic at all. From the perspective of the educational structure of the sample, women with primary education were mostly informed by the media, slightly fewer by the staff in medical institutions, then by family members and friends, while close to eight percent were not informed about prevention possibilities. Women with secondary education were also mostly informed about prevention by the media and by medical providers. A small percentage had an opportunity to listen to lectures about prevention at work or at school and get informed by family and friends, while 2% were not informed at all. Women with higher education preferred to get information by the media, but consider medical providers being also a significant source of information. Approximately 14% had listened to lectures on this topic at work/university. Family and friends were not noted as important source of information. There are not uninformed within this educational category (Table 3).

When it comes to information by television, a great number of respondents (74%) had the opportunity to watch TV programs dedicated to the prevention of cervical and breast cancer, while 17% saw such programmes only during special media campaigns dedicated to these topics. A small number of respondents were not interested in watching this type of content on television, while 2% believed that national television stations lacked this type of content. Radio programmes dedicated to the prevention of cervical and breast cancer had been listened so far by less than half of women and one fifth of them only during specific campaigns. A small percentage was not interested in listening to the radio content of this type, even though they had had a chance to do it, while a bit more than one fifth of women stated that radio stations generally lacked information of this type. When it comes to information by printed media, more than one third of respondents had the opportunity to read the articles dedicated to prevention of breast and cervical cancer in daily

Table 3

Sources of information on cancer prevention

Characteristics of respondents	Media n (%)	Medical providers n (%)	Lectures at the school/ university/ workplace n (%)	Family/ friend n (%)	Not informed n (%)
Age (years)					
20–29	400 (61.44)	143 (21.97)	37 (5.68)	71 (10.91)	0 (0.00)
30–39	287 (65.08)	106 (24.04)	32 (7.26)	16(3.62)	0 (0.00)
40–49	207 (61.42)	81 (24.03)	20 (5.93)	11 (3.26)	18 (5.36)
≥ 50	274 (40.84)	273 (40.68)	35 (5.22)	59 (8.79)	30 (4.47)
Area					
urban	504 (34.81)	776 (53.59)	79 (5.45)	89 (6.15)	0 (0.00)
rural	420 (64.42)	126 (19.32)	5 (0.77)	58 (8.89)	43 (6.60)
Education					
primary	126 (42.87)	110 (37.41)	10 (3.40)	27 (9.18)	21 (7.14)
secondary	525 (56.77)	315 (34.05)	41 (4.43)	25 (2.70)	19 (2.05)
high	526 (59.71)	198 (22.47)	116 (13.17)	41 (4.65)	0 (0.00)

newspapers and near half in specialized magazines about health. A small number of women were not interested to read this type of articles in the press, while the lowest percentage considered that not enough attention was paid to these issues in national newspapers and magazines. Forty two percent of women had informed themselves about preventive measures on sites dedicated to health and one fifth of them on the official sites of medical institutions. Health information on social networks, blogs and forums were followed mostly by younger women, while a quarter of respondents had never read about these topics on the Internet. The highest percentage of women (84%) had seen posters about prevention in health facilities or was given brochures with such content. Among them, more than seventy percent read the promotional material in detail, while the others were not interested in reading it. Near sixteen percent of respondents stated that they had never seen nor received promotional material of this type in medical institutions (Table 4).

In relation to the attitude of respondents regarding the most responsible subjects for the education of women about cancer prevention, in most cases these were health institutions and the Ministry of Health (Table 5).

Table 4

Informing about cancer prevention through different media

Response	n (%)
Informing about cancer prevention through television	
Yes, by watching TV documentaries about prevention	1,554 (74.00)
Yes, but only during special campaigns	357 (17.00)
Not interested in watching such content	146 (6.95)
There is lack of such content on television	43 (2.05)
Informing about cancer prevention through radio	
Yes, by listening program about prevention	1,007 (47.95)
Yes, but only during special campaigns	421 (20.05)
Not interested in watching such content	126 (6.00)
There is lack of such content on radio	546 (26.00)
Informing about cancer prevention through print media	
Yes, in daily newspapers	714 (34.00)
Yes, in health magazines	1,012 (48.28)
Yes, but only during special campaigns	122 (5.72)
Not interested in watching such content	168 (8.00)
There is lack of such content in printed media	84 (4.00)
Informing about cancer prevention on Internet	
Yes, on web sites dedicated to health	884 (42.09)
Yes, on web sites of health institutions	418 (19.91)
Yes, on social networks, blogs and forums	275 (13.09)
Not interested in searching such content	523 (24.91)
Informing about cancer prevention through posters and brochures	
Yes, and read it with interested	1,498 (71.33)
Yes, but without interest to read it	270 (12.86)
Never given to	332 (15.81)

Table 5

Role of different subjects in informing and education about cancer prevention

Characteristics of respondents	Ministry of Health n (%)	Health institutions n (%)	Media n (%)	Self -initiative of women, n (%)
Age (years)				
20–29	252 (38.71)	189 (29.03)	194 (29.80)	16 (2.46)
30–39	84 (19.04)	231 (52.39)	126 (28.57)	0 (0.00)
40–49	105 (31.16)	190 (56.38)	21 (6.23)	21 (6.23)
≥ 50	168 (25.04)	287 (42.77)	6 (0.89)	210 (31.30)
Area				
urban	440 (30.39)	566 (39.09)	170 (11.74)	272 (18.78)
rural	168 (25.77)	336 (51.53)	42 (6.44)	106 (16.26)
Education				
primary	113 (38.43)	113 (38.43)	45 (15.31)	23 (7.83)
secondary	85 (9.19)	546 (59.03)	105 (11.35)	189 (20.43)
high	409 (46.43)	246 (27.92)	164 (18.61)	62 (7.04)

There was a statistically significant relation between age categories and attitudes towards the most important subjects, as these two variables were highly associated (χ^2 = 648.545, df = 9, $p < 0.01$). A greatest percentage of women who belonged to the age group of 20 to 29 believed that the Ministry of Health played a major role, followed by the media and healthcare institutions. Less than 3% of younger respondents stated that every woman should educate herself on her own initiative. Women aged 30 to 39 thought that the main role belonged to health care institutions, more than to media and the Ministry of Health, while the initiative was not on women themselves. Women aged 40 to 49 also stated that health care institutions played the main role in education, then the Ministry of Health, media and women themselves. Women over 50, considered the role of health care institutions as the most important, but almost a third highlighted the importance of taking self-initiative. A quarter of women within this age category considered that the Ministry of Health had a fundamental role in encouraging the education of women in the area of cancer prevention.

The largest percentage of respondents from both, urban areas and rural areas, believed that health care institutions were the most responsible, followed by the Ministry of Health. Respondents from urban areas noted the role of the media and self-initiated education of women as less important. Women from rural areas valued more the role of women self-initiative than the role of the media. From the perspective of the level of education, the results were as follows: women with primary education equally valued the role of the Ministry of Health and health institutions, followed by the role of the media and self-initiative of women. Women with secondary education considered that institutions played the main role (59.09%), followed by self-education of women (20.49%). Around 11% thought that the media played a major role, while 9% thought that the Ministry of Health is more responsible. Higher educated women, at most, emphasized the responsibility of the Ministry of Health, then health care institutions, media, while the lowest percentage emphasized the role of self-initiative of women. Education level is, statistically observed, found to be associated with the respondents' attitudes towards the most important subjects in the process of prevention information and communication (χ^2 = 415.362, df = 6, $p < 0.01$).

Discussion

The results of the survey indicate that sociodemographic factors such as education and age affect individuals' choice of communication channels and preventive behaviour, as it was previously noted by some other authors [25]. The results show that a significant percentage of women of all age groups had the habit of going to gynaecological examinations at least once a year, and that women of 50 or more years have the highest developed awareness of that. More than half of respondents always do Pap test, but it can be seen that women in rural areas have less developed awareness of doing it on regular basis. A research carried out in the USA also suggested that rural residents are less likely to receive timely cancer screening test [26], as well as a research carried out in Italy [27]. A possible explanation of this finding can be in the presence of structural barriers and the larger distance from the health care provider that may limit the use of preventive care units. It is in accordance with Vernon's [28] statement that subjects who live close to the provider are often more likely to comply, while the others, who are rather distanced, may perceive the prevention activity, such as testing or screening, as a time consuming activity [29]. The results also indicate that awareness towards breast and cervical cancer prevention increases observed by education level and these two variables were found to be statistically significant. Like in some other studies, higher educational level is one of the main characteristics associated with a higher level of knowledge of cervical cancer aetiology and preventive behaviour [29]. Unlike some research results obtained in African countries, which found that women who had limited knowledge about cervical cancer, never had a Pap smear test and lacked access to screenings [2, 30–32], we obtained better, but not satisfactory results regarding preventive behavior. It is obvious that women with lower level of education are not well informed regarding necessity of the Pap test and, therefore, greater attention should be placed on targeting that group when conducting marketing communication program.

The results indicate that the largest percentage of respondents from all age categories noticed they were familiar with the way of performing breast self-examination, but the awareness of the necessity of performing self-examination again increased with the level of education. Nevertheless, the results are not positive at all when it come to mammography or ultrasonography, as the majority of women of all age groups had never performed them. The awareness level was higher in case of older women and there was a statistical significance of that. More women who lived in urban areas performed mammographic checks in comparison with those in rural areas, but the results are not particularly significant.

According to the results, the greatest number of women of all ages were informed about the possibility of preventing breast and cervical cancer by the media, which is in accordance with findings of some authors who came to the conclusion that mass media affect all sociodemographic groups of women [26]. That number is much higher in rural areas than in urban, where medical providers were marked as the main sources of information. The results of previous research also showed that the mass media campaign, generally, was more effective in rural resident women [29]. The reason for that, again, can be searched in the fact that visiting medical providers demands time, as many rural areas do not have their own health facilities. On the other hand, media-based information is easier to get without leaving home. For urban residents media-based information is playing a rather supporting role, as they find medical providers to be more reliable communication channel. Health providers were the most frequently cited information source in various research in the usa [25, 33, 34]. One more study showed that participants reported seeing or hearing information from clinicians on the first place, then media and their family [2]. Our results are similar to those found by authors in Vietnam, who found that older adults were more likely to use doctors as a source for cancer and cancer screening information, and that those sources had a greater influence on their screening decision than media [35]. Younger respondents are more likely to trust the media as a referent source. Family and friends were not considered as an important source; probably as such topics are not "appropriate enough" to talk with family members and friends. In Serbia, conservative attitude regarding various health topics still exists in everyday communication, so that the issue should be rather addressed through media to encourage behaviour changes and exchange of such information among women themselves. Considering responses given in our study, it is obvious that these topics are not given enough attention at work places or at schools or universities, which should be also integrated in prevention campaigns.

When it comes to information by television, the majority of respondents had the opportunity to watch programs dedicated to the prevention of cervical and breast cancer, which makes television a leading media in this area. Such findings are consistent with other earlier ones [15, 21, 22, 36]. While some authors considered that older generation may prefer to be informed by television programmes regarding health problems [25], our research show no significant differences among age groups. That could be explained with the fact that in Serbia television is still a dominant media at the national level. A significant percentage of women listen about cervical and breast cancer on the radio, but one quarter of women believed that radio stations actually lacked information of this type. Such findings clearly indicate that prevention information should be placed more often through radio, which is observed as the media for listening music and such "serious" topics are more often avoided to be discussed.

One third of respondents had the opportunity to read articles dedicated to prevention of breast and cervical cancer in daily newspapers and more than a half in specialized magazines about health, which makes printed media important source of information, as well. Although not considered as the most important, printed media is also one of the key channels through which the public is informed about important health issues such as cancer prevention, detection and treatment. Existing literature on news coverage of screening includes conflicting conclusions regarding the impact of news on understanding and engagement in cancer screening practices [37]. While some authors suggested that this topic is largely ignored by the printed media [38], others argued that screening is widely covered [39]. Some researches blame the printed media to cover cancer prevention stories only during awareness months [40], but our results did not reflect such situation. Nevertheless, health-promoting public bodies should work more closely with editors and journalists to ensure that the relevant messages reach wider audiences during whole year.

The significance of the internet as a valuable source of information is obvious, as 42% of women informed themselves about preventive measures on various sites dedicated to health and one fifth of them on the sites of medical institutions. There is a growing number of studies which have examined the influence of online activity in raising awareness of cancer risk and stimulating preventive behaviour [41–43]. Results of previous study suggest that the internet could be an important venue for women, particularly the younger generation, to find information about breast and cervical cancer screening [33]. Like we noticed, studies from other countries suggested that women receiving health information from the internet were more likely to belong to younger groups [25, 33, 44–46]. Although highlighted by many studies to reflect an opportunity for distribution of information targeting the youth [47–50], so-called 'new media' were not pointed out to be so popular among respondents in our study. Even though, among all mass media, the internet holds particular perspective as an effective platform for health communication and education, it may still be too early to use it for programmes targeting wider society groups [51]. Therefore, the internet may be a favourable source of information for those women who feel that they do not have time to go to the doctor's or feel that doctors do not pay enough attention just to talk with individuals. Also, web-based campaigns or targeted health messages conducted through new media could be innovative, supplementary ways to reach younger population.

The highest percentage of women (84%) in the sample noted to have the opportunity to see posters about prevention in health facilities or to be given brochures with the

same content. The majority of them read the promotional material in detail and received some new information. So, it is obviously a very significant communication channel for women in Serbia, who prefer to get informed on health issues while sitting in the waiting room in health institutions. The explanation may lie in the fact that, since they came to health care institution, they may have some problem and, therefore, become more sensitive to receive any kind of information regarding health and may take it more seriously than in other environments. Hence, future marketing campaigns should include distribution of similar promotional material as mandatory.

Surprisingly, in relation to the attitude of respondents regarding the most responsible subjects for the education about cancer prevention, the majority of women considered themselves as not responsible for taking self-initiative. In most cases these were health institutions and the ministry of health. The exception made women of 50 or older, who highlighted the importance of taking self-initiative in more than 30%, right after health institutions. Women of the first two age groups (20–39) emphasised the role of media more than older women. Such findings indicate that women who are 50 or older have the higher awareness level regarding prevention due to the fact that they may consider themselves as being at higher risk and understand the necessity of self-engagement and this appeared to be statistically significant. Nevertheless, it is obvious that marketing campaigns are absolutely necessary to be initiated by health care institutions on the national level in order to encourage health education. Media are considered to be a communication channel, not initiator of such campaigns. Also, women in Serbia, especially younger ones are not used to educate themselves in the prevention area on their own initiative, probably due to the lower risks of developing cancer in the younger age which results in the lack of the awareness.

These findings should be used as guides for future marketing campaigns based on culturally acceptable channels of communication and informing. As stated by earlier studies, programs that use culturally admissible information sources or that address key barriers to information seeking are more likely to be effective [52]. Our objective in this study was to conduct an initial exploration of main information sources and barriers that may be particularly relevant regarding breast and cervical cancer prevention. The results may also provide a direction for future research that could address some issues, which were found to be significant, in a more detailed way.

Conclusion

Our results indicate the lack of conducting some preventive measures (mammographic screening and Pap test). The results show the differences in awareness level according to education degree of respondents. Information through posters or brochures in health facilities are considered to be the most proper communication channel on these topics. Television is still the leading media in this area, but the emerging significance of internet is also noted. When it comes to the attitude of respondents regarding the subjects that are most responsible for education of women, health institutions and the Serbian Ministry of Health are considered as most important, while there is the absence of self-initiative, especially in younger women. Since women of 50 or more expressed the highest level of awareness, future marketing campaigns should target younger groups more and use channels more appropriate to them.

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Fever of unknown origin − diagnostic methods in a European developing country

Nejasno febrilno stanje – dijagnostičke metode u evropskoj zemlji u razvoju

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Abstract

Background/Aim. Fewer of unknown origin (FUO) remains amongst the most difficult diagnostic dilemmas in contemporary medicine. The aim of this study was to determine the causes of FUO and to identify the methods of diagnosis in patients with FUO in a tertiary care setting in the Republic of Macedonia. **Methods.** Retrospectively histories of 123 immunocompetent patients older than 14 years with classical FUO that had been examined at the University Hospital for Infectious Diseases and Febrile Conditions in the city of Skopje, during the period 2006−2012 were evaluated. FUO was defined as axillary fever of ≥ 37.5°C on several occasions, fever duration of more than 21 days and failure to reach the diagnosis after the initial diagnostic workup comprised of several defined basic investigations. **Results.** Infections were the cause of FUO in 51 (41.5%) of the patients, followed by non-infective inflammatory disorders (NIID) in 28 (22.8%), miscellaneous in 12 (9.7%) and neoplasm in 11 (8.9%) of the patients. Twenty one of the patients (17.1%) remained undiagnosed. The most common causes for FUO were visceral leishmaniasis, abscesses, urinary tract infections, subacute endocarditis, *polymyalgia rheumatica* and adult onset of Still disease. The final diagnosis was reached with histology in 24 (23.5%), imaging and endoscopic procedures in 21 (20.6%), clinical course and empiric therapy response in 20 (19.6%), serology in 18 (17.6%) and cultures in 16 (15.7%) of the cases. **Conclusion.** In the Republic of Macedonia infections are the leading cause of FUO, predominately visceral leishmaniasis. In the future in patients with prolonged fever, physicians should think more often of this disease, as well as of the possibility of atypical presentation of the common classical causes of FUO.

Key words:
fever; infection; diagnosis; diagnosis, differential; leishmaniasis, visceral; macedonia.

Apstrakt

Uvod/Cilj. Nejasno febrilno stanje (*fever of unknown origin* − FUO) ostaje među najvećim dilemama u dijagnostici savremene medicine. Cilj ovog rada bio je da se prikažu uzroci FUO i da se definišu metode kojima je postavljena dijagnoza kod ovih bolesnika u tercijernoj medicinskoj ustanovi u Republici Makedoniji. **Metode.** Retrospektivno su proučavane istorije bolesti 123 imunokompetentna bolesnika starija od 14 godina sa klasičnim FUO koji su bili ispitivani na Univerzitetskoj klinici za infektivne bolesti i febrilna stanja u Skoplju, u periodu 2006−2012. godine. FUO je bila definisana kao aksilarna temperatura ≥ 37,5°C u nekoliko navrata, trajanja dužeg od 21 dana i nepostavljanje dijagnoze posle inicijalnog dijagnostičkog pristupa sastavljenog od nekoliko definisanih ispitivanja. **Rezultati.** Infekcije su bile razlog za FUO kod 51 (41,5%) bolesnika, praćene neinfektivnim inflamatornim bolestima kod 28 (22,8%), raznim drugim stanjima kod 12 (9,7%) i neoplazmama kod 11 (8,9%). Kod 21 (17,1%) bolesnika razlog za FUO nije bio pronađen. Najćešći razlozi za FUO bili su visceralna lajšmanioza, apscesi, infekcije urinarnog sistema, subakutni endokarditis, reumatska polimijalgija kao i Stilova bolest. Krajnja dijagnoza bazirala se na histologiji kod 24 (23,5%), radiološkim i endoskopskim procedurama kod 21 (20,6%), kliničkom toku i odgovoru na empirijsku terapiju kod 20 (19,6%), serologiji kod 18 (17,6%) i kulturama kod 16 (15,7%) bolesnika. **Zaključak.** U Republici Makedoniji infekcije predstavljaju vodeći uzrok FUO, u prvom redu visceralna lajšmanioza. U budućnosti, kod bolesnika sa FUO lekari bi trebalo češće da misle na ovu bolest kao i na mogućnost za atipičnu prezentaciju uobičajenih klasičnih bolesti koje izazivaju FUO.

Ključne reči:
telesna temperatura; infekcija; dijagnoza; dijagnoza, diferencijalna; lajšmanioza, visceralna; makedonija.

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Introduction

Fewer of unknown origin (FUO) remains amongst the most difficult diagnostic dilemmas in contemporary medicine [1, 2]. Nowadays, there are more than 200 known causes of FUO [3–5], but their true incidence and prevalence are unknown [6]. The etiologic spectrum of diseases that cause FUO is determined by different factors like geographic conditions, economic characteristics of the country, the time period when the study was done, whether the study was prospective or retrospective one, inclusion and exclusion criteria, availability and quality of diagnostic methods, the increasing number of intravenous drug users, travelers, as well as by the development of new diagnostic tools, new vaccines and new antimicrobial and immunomodulating agents [7–10].

The physicians that manage this category of patients encounter a lot of difficulties: the possibility to conduct investigations in wrong direction due to the accentuation of some or disregarding other potential diagnostic clues (PDCs), unintentional omission of appropriate diagnostic techniques, or simply not recognizing the disease as a result of its atypical clinical presentation. The always present probability for worsening the patient's health due to delay of empirical treatment, as well as the possibility to harm the patient with certain investigations or the used drugs, make the management of this kind of patients even more complex [1, 11].

Concerning FUO in the Republic of Macedonia, there is a lack of epidemiological and clinical data, and this study aimed to present the causes of classic FUO and to determine the role of diagnostic methods performed in order to reach the diagnosis in this developing country.

Methods

This retrospective study evaluated medical records of 123 immunocompetent patients older than 14 years with non-hospital acquired FUO. The patients were investigated at the University Hospital for Infectious Diseases and Febrile Conditions in the city of Skopje, Republic of Macedonia, during the period January 2006−December 2012. The patients were assessed as inpatients or as outpatients and they were admitted directly, or were transferred from other hospitals. The study was approved by the Medical Faculty Review Board.

The inclusion criteria were: axillary fever of ≥ 37.5°C on several occasions; fever duration of more than 21 days, and failure to reach the diagnosis after the initial diagnostic workup comprising: of detailed medical history which included actual symptoms, their features and duration, previous illnesses, surgical procedures, comorbid conditions, medications, alcohol intake, occupation, social environment, sexual and travel history, hobbies, animal exposure, animal or insect bites, recent contact with persons with similar symptoms, familial disorders; thorough physical examination with special accent on the skin, nails, mucous membranes, lymph nodes, eyes, ears, nose, sinuses, oropharynx, heart, lung, abdomen, extremities, nervous system, temporal arteries, rectum and genital organs; initial laboratory tests − erythrocyte sedimentation rate, C-reactive protein, complete blood count with differential leukocyte formula, glycaemia, blood urea nitrogen, creatinine, sodium, potassium, bilirubin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine phosphokinase, alkaline phosphatase, gamma-glutamyl transpeptidase and urine analysis; other investigations − blood cultures (≥ 2), urine culture, serology for brucellosis, anti HIV test, chest x-ray, electrocardiography, abdominal ultrasonography and tuberculin skin test.

After enrollment, additional advanced evaluation consisted of the systematic approach which included repeated questioning of patients and their close relatives and frequent physical reexamination done by different physicians in order to evaluate changes in the presentation or appearance of new symptoms or signs. At the same time body temperature and heart rate were measured every few hours in the presence of medical personnel, and all unnecessary drugs were discontinued or replaced with more adequate ones. Depending on the actual PDCs the patients were submitted to repetition of some of the initial diagnostic tests, as well as to some of the following second line investigations: biochemical tests − serum protein electrophoresis, fibrinogen, complement, circulating immune complexes, thyroxin and thyroid stimulating hormone, angiotensin-converting enzyme, hemostasis, Bence Jones proteinuria, 24 hours proteinuria, occult blood in feces; anti-nuclear antibody, rheuma factor, anti-deoxy ribonucleic acid antibodies, anti-neutrophil cytoplasmic antibodies, tumor antigen assays (AFP, CEA, PSA, CA 125, CA 72-4, CA 19-9, NSE, CYFRA); microbiological analyses − sputum microscopy and sputum for acid fast bacilli, thick and thin blood smear for malaria; stool, throat, cerebrospinal fluid, pleural fluid, ascitic fluid cultures; serological tests − antistreptolysin-O test, Widal, WDRL; indirect immunofluorescent antibodies (IIF) for visceral leishmaniasis, *Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella, Coxiella, Rickettsia*; enzyme-linked immunosorbent assay for viral hepatitis, *Toxoplasma, Epstein-Barr* virus, *Cytomegalovirus, Leptospira, Borrelia, Clostridium difficile* toxin in stool; imaging studies − radiography of the paranasal sinuses, teeth, pyelography, craniogram, angiography; ultrasound examination of thyroid gland, heart, kidney, lung, pelvic region, doppler imaging; computed tomography or magnetic resonance imaging of brain, thorax, upper and lower abdomen; scintigraphy with marked leucocytes; invasive procedures and histological examination − bronchoscopy, cystoscopy, gastroscopy, colonoscopy with adequate biopsies, sternal aspiration, bone marrow, liver, lymph node, skin, muscle, and other solid organs biopsy.

Data on age, gender, fever duration prior to inclusion in the study, time from the inclusion in the study to establishing the final diagnosis, and diagnostic methods used for establishing the diagnosis were analyzed. To decide on the definitive diagnostic method we took into consideration biochemistry and hematological analyses, microbiological cultures and smears, serology (microbiological and immunological), imaging techniques, endoscopic procedures, histology, and clinical course and/or empiric therapy response. Interpretation of data and establishing the final diagnosis was made by one of the authors in concordance with at least one other specialist

in infectious diseases, rheumatology, hematology, oncology, or other related specialties.

The causes of FUO were classified into 5 diagnostic categories: infections, neoplasm, non-infective inflammatory disorders (NIID) including connective tissue illnesses, vasculitides and granulomatous disorders, miscellaneous, and non-diagnosed diseases.

Patient's age, fever duration before inclusion in the study and the time from inclusion in the study to establishing the final diagnosis are presented using median and range values. All other parameters are presented as frequencies and percentages.

Results

This retrospective study included 123 patients with the median age 49, range 15−82 years. Sixty four (52%) of the patients were males, and 59 (48%) females. Sixty six (53.6%) of the patients were previously investigated as inpatients in other hospitals. Before their inclusion in the study, all the patients had at least one course of antimicrobial therapy. Fever duration before the inclusion in the study was on the avarage 30 days, range 10−1,440 days. Twenty one (17.1%) of the patients during their first examination in our hospital had fever duration of less than 21 days and this criterion for FUO (fever duration) was fulfilled during investigations after their hospital admittance. As shown in Table 1, infections were the most common causes of FUO, followed by NIID. In the group of infections, visceral leishmaniasis and abscesses were the dominant conditions. There were 7 males and 3 females with visceral leishmaniasis, average age 47, range 23−60 years. In this group of patients fever duration prior to admission was median 30, range 21−90 days. The diagnosis was reached with detection of parasites in material obtained from sternal aspiration in 4 out of 6 examined patients and in the remaining patients with indirect immunofluorescence (IIF) test. IIF test was positive in 9 out of 10 examined patients. In all the patients with visceral leishmaniasis defervescence was reached up to 10 days after beginning of specific treatment with antimonial compounds. In the NIID group the commonest conditions were *polymyalgia rheumatica* and adult onset Still disease. As far as neoplasms are concerned, the metastatic carcinoma of the liver was dominant, and in the group of miscellaneous diseases deep vein phlebothrombosis was the leading cause. However, in 21 (17.1%) of the patients the cause of FUO remained obscure in spite of all investigations and follow-up. This category of the patients composed of 12 females and 9 males with median age of 45, range 18−67 years, prior to inclusion in the study had illness duration of 60 days, range 14 days to 4 years. Fourteen patients were previously investigated in other hospital settings. The duration of the hospital stay was median 21, range 14−60 days. One patient died during the hospital stay, in 9 fever continued to be present at hospital discharge, and in 11 fever resolved during the stay in the hospital (in 4 of them spontaneously, and in 7 with corticosteroid therapy). All of the patients on corticosteroids and those that were discharged with fever were advised to consult specialists of various internal medicine branches.

In cases with the diagnosis, the time from inclusion in the study to establishing the final diagnosis was median 12, range 8−60 days. As shown in Table 2 all of the cited methods had their own contribution to establishing the final diagnosis.

Table 1
Causes of fever of unknown origin (FUO) in 123 patients

Causes	Patient, n (%)
Infections	51 (41.5)
Visceral leishmaniasis	10
Abscess*	10
Urinary tract infection†	6
Subacute endocarditis‡	6
Tuberculosis§	5
Pansinusitis	3
Cytomegalovirus infection	2
Sepsis‖	2
Lyme borreliosis	2
Other¶	5
Non-infective inflammatory disorders	28 (22.8)
Polymyalgia rheumatica	6
Adult onset Still disease	6
Vasculitis	4
Systemic lupus erythematosus	3
Crohn disease	3
Reactive arthritis	3
Other**	3
Neoplasm	11 (8.9)
Haematological disorders††	4
Metastatic adenocarcinoma in the liver	3
Colonic adenocarcinoma	2
Prostatic cancer	1
Leiomyosarcoma	1
Miscellaneous	12 (9.7)
Deep vein phlebothrombosis	5
Subacute thyroiditis	2
Ulcerative colitis	2
Other‡‡	3
Undiagnosed	21 (17.1)

***6 cases with abdominal/pelvic, 2 with dental, 1 with breast and 1 with cervical lymph gland abscess; †Urine culture: *Escherichia* (*E.*) *coli* in 3, *Enterococcus* in 2 and *Proteus mirabilis* in 1 patient; ‡Blood culture: *Staphylococcus* (*S.*) *aureus* in 1, *Enterococcus* in 1, negative in 4 patients; §1 case each with miliaria, tuberculous spondylitis, epididymitis, lymphadenitis, pericarditis; ‖Blood culture: *S. aureus* in 1, *E. coli* in 1 patient; ¶1 case each with leptospirosis, rickettsiosis, *Clostridium difficile*, parvoviral infection and cholecystitits; **1 case each with sarcoidosis, granulomatous hepatitis and erythema nodosum; ††1 case each with Hodgkin lymphoma, non Hodgkin lymphoma, chronic leukemia and myelofibrosis; ‡‡1 case each with lung embolia, cardiac myxoma, and drug fever.**

Table 2

Final diagnostic method in 102 patients with fever of unknown origin (FUO) in whom diagnosis was established

Diagnostic method	Patients n (%)
Biochemistry and hematological analyses	3 (2.9)
Cultures and smears	16 (15.7)
Serology (microbiological and immunological)	18 (17.6)
Imaging, endoscopic and other invasive procedures	21 (20.6)
Histology	24 (23.5)
Clinical course and/or empiric therapy response	20 (19.6)

Discussion

Although there were several attempts to define FUO prior to 1961 [12], this condition has had its true placement with the establishment of criteria by Petersdorf and Beeson [13], which include illness duration of more than 3 weeks, documented temperature higher than 38.3°C on several occasions, and uncertain diagnosis after one week of the hospital diagnostic workup. In 1991 Durack and Street [14] modified the previous definition by replacing the last criterion with the following modification "uncertain diagnosis after 3 days of hospital stay or more than 2 outpatient visits". For some authors this modified definition of FUO is also not satisfactory, considering that it is based on quantitative parameters. Today more current is the tendency to define FUO with the help of qualitative criteria where the time period during which no diagnosis or reasonable diagnostic hypothesis has been made is replaced with a standard initial diagnostic intelligent investigational protocol conducted in or out of a hospital setting [15, 16]. It is recommended that the standard diagnostic protocol should be adapted to the regional epidemiological factors. This was nicely demonstrated in our study by the number of cases with visceral leishmaniasis.

The definition of FUO in this study differs from the classical definition in two criteria. Firstly, in this study the temperature was measured axillary, the method that has deep roots and tradition in this region due to hygiene habits and as a result of having prejudices, especially in male population where rectal measurement of temperature is generally not accepted. Axillary measurement of the temperature is reported in several Japanese studies, although their definition for elevated body temperature varies from ours [17, 18]. The decision we chose for temperature cut off of $\geq 37.5^{\circ}$C was arbitrary – we intended to exclude conditions with habitual hyperthermia and cases with more expressed circadian temperature daily rhythm. Secondly, instead of criterion from 7 or 3 day hospital stay, or more than 2 outpatient visits we used qualitative criteria, considering it to be more objective especially in developing regions, where results from samples taken for examination were received with delay. In addition, it takes more time than usual to obtain the results partly due to objective reasons (some analyses are processed in continuity only in certain days, there is a periodical shortage of reagents, some analyses are done in facilities out of the state etc.), but also due to subjective reasons (indolence in preparation, issuing and collecting the results, absence of priority etc.).

According to numerous literature data, the occurrence of infections, NIID, neoplasms and miscellaneous conditions is 11% [19] to 59% [20, 21], 2% [22] to 38% [23], 6% [24, 25] to 31% [11, 26] and 2% [20, 23] to 22% [27], respectively. The proportion of undiagnosed cases ranges from 5% [26, 28] to 53% [3, 29]. This study similar to others conducted in university clinical centers in developing countries, as well as in secondary hospital care centers in developed countries shows the predominance of infections compared to other causes of FUO [30]. In our series the highest frequency was found for visceral leishmaniasis, an autochthonic disease in our region, but yet rarely thought of, and until recently with sparse diagnostic possibilities. Visceral leishmaniasis as a cause for FUO has been reported in other series as well [7, 20, 31, 32], but not as a predominant cause. From the abundance of other infective causes, dominant were abscesses (intra-abdominal but extra-abdominal as well), urinary tract infections (UTI), subacute endocarditis and extrapulmonary tuberculosis, similarly seen in another studies [9, 20, 33, 34]. A rather high frequency of bacterial infections such as abscesses, UTI and endocarditis can be ascribed to the all too common practice of repeatedly prescribing antibiotics to patients with prolonged fever. Interestingly, in our study there were no cases with human brucellosis as a cause of FUO, something that has been mentioned by other authors [7, 35, 36]. Possibly this is due to the fact that brucellosis, predominantly an endemic disease in this region [37], is often thought of by doctors and patients, as well. From non-infective diseases there was the marked occurrence of *polymyalgia rheumatica*, adult onset Still disease, haematological malignancies and deep vein phlebothrombosis, which were similarly reported in other studies [9, 17, 19, 29, 32, 34, 38].

Having in mind that we did not perform some of the sophisticated diagnostic tests (positron emission tomography – PET scan, genetic investigations, polymerase choin reaction – PCR, fungal diagnostic, temporal artery biopsy), and that all of the patients had prior empirical antimicrobial treatment, the percentage of cases without diagnosis, is comparable to the findings in other studies [24, 39–42]. This may be due to the spectrum of diseases that cause FUO in this region, the fact that half of the patients sought medical help for the first time, and that in some cases we accepted the probable diagnosis as a definite one, with special accent towards diagnostic meaning of clinical course and /or empiric therapy response.

In this study during advanced investigation no algorithms were used [5, 43–45] and to all the patients we had individualized approach. Also, the choice for investigations undertaken was based on PDCs, personal physicians' intuition and experience, but also on the availability and cost of the diagnostic investigations, a fact that is economically justified in areas with limited material resources and technologic potentials. At the same time invasive diagnostic tests were left as an ultimate option when no result was obtained with any of the other previously done investigations which would help solve the case, or when deterioration of the condition was expected. Histology had a leading role in reaching a diagnosis in cases with neoplasms, but also in some other diseases (inflammatory bowel diseases, vasculitis, and tuberculosis). Clinical course and empiric therapy were of special significance, especially in adult onset Still disease, *polymialgia rheumatica*, reactive arthritis and some of the miscellaneous disorders, while in cases of infections microbiological cultures, serological tests and imaging techniques were of special aid. Also, imaging and endoscopic techniques had helped in localization of some of the lesions and in subsequent histological examination. There is a great diversity in the literature considering the definitive diagnostic steps regarding the causes for FUO, available resources in the hospital in question, the interpretation of the results, used definitions and some regional specifics [19, 20, 23, 34, 46–48].

Conclusion

In conclusion, our study showed that in tertiary care hospitals in our country infections are the leading cause of FUO. In the forthcoming period in patients with FUO special concern should be paid to visceral leishmaniasis, abscesses, UTI, subacute endocarditis and tuberculosis, generating the clinical suspicion more often and aiming to improve the available techniques in order to achieve the final diagnosis more quickly. Our findings stress the importance of leishmaniasis, too often considered a tropical disease, as a cause of FUO in travelers visiting these parts of Europe. The great importance that clinical approach has in making the diagnosis of FUO should not marginalize the tendency towards new medical achievements and attempts for introducing new modern diagnostic procedures in order to help solve the diagnosis in some of the cases.

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Quantitative morphometric analysis of the myenteric nervous plexus ganglion structures along the human digestive tract

Kvantitativno-morfometrijska analiza ganglijskih struktura mijenteričkog nervnog spleta duž digestivnog trakta čoveka

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Abstract

Background/Aim. All the functions of the digestive system are controlled, guided and initiated by the autonomic nervous system. A special part of this system placed in the wall of the gastrointestinal tract is known as the enteric or metasympathetic nervous system. The aim of this study was to analyse myenteric nervous plexus in different parts of the digestive tract. **Methods.** We examined the myenteric nervous plexus of the esophagus, stomach, duodenum, jejunum, ileum, transverse colon and rectum in tissue samples taken from 30 cadavers of persons aged 20–84 years. After standard histological processing sections were stained with hematoxylin-eosin, cresyl violet (CV) and $AgNO_3$ method. Multipurpose test system M42 was used in morphometric analysis. The results were analyzed by *t*-test and analysis of variance. **Results.** The number of neurons *per* cm^2 surface was the lowest in the esophagus (2.045 ± 310.30) and the largest in the duodenum (65,511 ± 5,639). The statistical processing showed significant differences ($p < 0.001$) in the number of neurons between the esophagus and all other parts of the digestive tract. The maximal value of the average surface of the myenteric nervous plexus neurons was observed in the esophagus (588.93 ± 30.45 μm^2) and the lowest in the stomach (296.46 ± 22.53 μm^2). **Conclusion.** There are differences in the number of ganglion cells among different parts of the human digestive tract. The differences range from a few to several tens of thousands of neuron/cm^2. The myenteric nervous plexus of the esophagus was characterized by a significantly smaller number of neurons but their bodies and nuclei are significantly larger compared to other parts of the digestive tract.

Key words:
digestive system; myenteric plexus; neurons; cell count; histological techniques; cadaver.

Apstrakt

Uvod/Cilj. Sve funkcije digestivnog sistema su kontrolisane, usmeravane i inicirane od strane autonomnog nervnog sistema. Poseban deo ovog sistema, smešten u samom zidu delova digestivnog trakta, poznat je pod nazivom enterički ili metasimpatički nervni sistem. Cilj ovog istraživanja bio je da se prouči mijenterički nervni splet u različitim delovima digestivnog trakta. **Metode.** Ispitivan je mijenterički nervni splet jednjaka, želuca, duodenuma, jejunuma, ileuma, poprečnog kolona i rektuma u uzorcima tkiva uzetim od 30 kadavera, osoba starih od 20 do 84 godine. Nakon standardne histološke obrade preparati su bojeni hematoksilin-eozin, crezil violet (CV) i $AgNO_3$ metodom. Gotovi preparati su podvrgnuti morfometrijskoj analizi korišćenjem višenamenskog test sistema M42. Određivan je broj ganglijskih ćelija mijenteričkog nervnog spleta navedenih delova digestivnog trakta po jedinici površine (cm^2). Dobijeni rezultati obrađivani su *t*-testom i analizom varijanse. **Rezultati.** Broj neurona po cm^2 površine bio je najmanji u oblasti jednjaka (2 045 ± 310,30), a najveći u duodenumu (65 511 ± 5 639). Utvrđena je statistički značajna razlika ($p < 0,001$) u broju neurona između jednjaka i svih ostalih delova digestivnog trakta. Najveća vrednost prosečne površine neurona mijenteričkog nervnog spleta zabeležena je u jednjaku (588,93 ± 30,45 μm^2), a najmanja u želucu (296,46 ± 22,53 μm^2). **Zaključak.** Postoje razlike u broju ganglijskih ćelija između pojedinih delova digestivnog trakta čoveka. Razlike se kreću u rasponu od nekoliko, pa do više desetina hiljada neurona/cm^2. Mijenterički nervni splet jednjaka odlikuje se znatno manjim brojem neurona, ali su njihova tela i jedra znatno krupnija u odnosu na ostale delove digestivnog trakta.

Ključne reči:
digestivni sistem; plexus myentericus; neuroni; ćelije, broj; histološke tehnike; leš.

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Introduction

One of the basic features of the digestive tract is that it has its own nervous system, which extends from the pharynx to the rectum. All functions of the digestive tract are initiated and controlled by this system. The enteric nervous system contains a large number of nerve cells and nerve fibers, organized in the form of three large ganglionic (the myenteric – Auerbach's, the submucous – Meissner's, and the mucous) and more non-ganglionic nervous plexuses [1–3]. Enteric neurons form the ganglion plexus inside the wall of the gastrointestinal tract which are much more complex than any other component of the peripheral nervous system [4] and use a wide range of transmitters in very organized neural junctions [5]. Most regions of the gastrointestinal tract contain two main ganglion plexuses, the myenteric plexus between the longitudinal and circular smooth muscle layers, and the submucosal plexus, located in the connective tissue between the membrane and the circular muscle layer. *Plexus myentericus* is characterized by a dense network of nerve fibers that are interposed into many ganglions, usually grouped into nodes. Ganglions are diverse in terms of size, shape, number of neurons and structure. The shape, size and number of ganglia mainly depend on the characteristics intermuscular spaces that occupies. Many of myenteric ganglia are flat, irregular or lens- shaped and by observing to their longitudinal section surfaces they are usually outlined angular depending on the number and arrangement of the networks around them. In the myenteric nervous plexus are differentiated three interlaced network at the same plane (primary, secondary and tertiary plexus). Primary plexus represents quite a robust network of large nerve bundles that connect the ganglia by different sizes. This network of primary plexus shows only a longitudinal arrangement. The proximal border of distribution of myenteric plexus in humans is at 3–4 cm below the lower end of the larynx. Above this border, in the wall of the esophagus, can be seen only horizontally oriented, small bundles of nerve fibers without presence of ganglia, so is impossible to name these myenteric plexus. Some of studies [6, 7] on guinea pigs suggest that these nerve fibers participate in the innervation of striated muscle of the upper part of the esophagus. The highest density of ganglia and neurons in the human esophagus has been found at 4–6 cm above the posterior cardiac incisure [6]. In the distal direction the myenteric nervous plexus extends to the anus. Neurons of myenteric ganglia were larger in comparison to the other autonomic ganglions. Most of the enteric ganglion cells are multipolar, but unipolar, bipolar, ovoid, polygonal, or stellar neurons were also described. The number of neurons in the intestinal intramural ganglia is large, but varies from case to case [4], with different densities, different sizes of neurons in different segments of the alimentary canal. The smallest neurons are located in parts of the intestine closer to the attachement of the mesentery. In the myenteric system of guinea pig small intestine there are 14 types of neurons, each of which has a characteristic combination of morphological, neurochemical and biophysical features [8].

The main aim of our research was to establish neuronal density *per* cm² surface of the myenteric plexus of all main parts of the digestive tract, as well as, some of the basic morphological characteristics of myenteric neurons. Surely, also important is comparative analysis of the myenteric plexus different parts of the digestive system. Our research shows a very high neuronal density in all the parts of the alimentary tract.

We also tried to determine possible quantitative differences in the structure of the myenteric nervous plexus among these parts of the alimentary tract.

Methods

Tissue samples of the esophagus, stomach, duodenum, jejunum, ileum, transverse colon and rectum, with the consent of the Ethics Committee, were taken from autopsy material of the Institute of Forensic Medicine from 30 cadavers of both sexes, age range from 20 to 84 years. Samples were always taken from the same topographic places of the mentioned parts of the digestive tract (the middle part of the esophagus, central part of the anterior gastric wall, the middle of the upper part of the duodenum, anterior wall of the jejunum at 80 cm from duodenojejunal flexure, the anterior wall of the ileum 80 cm from the ileocecal junction, middle part of the free taenia of transverse colon and middle of the anterior wall ampullar part of the rectum). Thereafter, parts of samples (1 × 1 cm) were fixed in 10% buffered paraformaldehyde for 48 hours. After the routine processing through the series of alcohol, samples were embedded in paraffin blocks, and then cut into 6 μm thick section by two ways: sections perpendicular to the long axis of the anterior wall of the alimentary tract (classic), and longitudinal sections parallel to its long axis, serially from the serosa to the myenteric plexus, and through it. Sections were stained with hematoxylin-eosin method, but for identification of the ganglion structure and cells neighboring sections were stained with silver-nitrate (Mason-Fontana) and with cresyl-violet.

Silver-nitrate staining by the method of Mason Fontana included: hydrated sections were dipped in a previously prepared solution of silver-nitrate for 2 hours at 56°C, rinsed with distilled water and toning with a 0.2% solution of gold-chloride for 2–3 minutes, again rinsed with distilled water and 1 minute put down to the 5% sodium thiosulphate solution; again, rinsed with distilled water and dipped for 5 minutes in the nuclear-fast red color, then mounted on glass slides and cover with covering glasses. The result of staining was: argentaphilic granules in nerve cells were black, nuclei are pink-reddish and cytoplasm pale-pink.

Cresyl-violet staining for nerve cells included: hydrated sections were left in a previously prepared 0.5% solution of cresyl-violet for 30 minutes. After that, they were discolored in 96% ethyl-alcohol to which 1 drop of HCl was added, and discoloration controlled under a microscope. When the desired staining of sections was achieved they were dehydrated and mounted on glass slides. The result of staining were: dark blue nucleus, cytoplasm slightly lighter, while nerve fibers were not colored.

Quantification

Analysis was performed by the test system M42 calibrated to the proper magnification of light microscope (Carl Zeiss Jena). For measurements of the diameters of the cells and their nuclei we used an ocular micrometer calibrated to the appropriate magnification. At each section we analyzed 10 visual fields, and the obtained data were entered into spread sheets. The number of points of the test-system which fall on areas of ganglion structure was counted, as well as the number of points that fall on the profile of the myenteric plexus ganglion cells body. Also, the total number of neurons located within the area of ganglion structure was counted. Then we calculated the number of neurons *per* cm^2 surface of the nervous plexus, as well as the surface of individual profiles of the nerve cells bodies and their nuclei. Within morphometric analysis the surface of individual ganglion cells and the surface of their nuclei in different parts of the human digestive tract (relationship of the total surface profile of neurons to the surface area of the ganglionic structures in which they are located) were determined.

The results presented in the text and tables are expressed as mean ± standard deviation. The statistical significance (at the level of $p < 0.05$ or $p < 0.001$) between the mean values was estimated using Students *t*-test for independent samples and the analysis of variance (Med Calc statistical software 12.5.0.0.).

Results

The myenteric nervous plexus (ganglions interconnected by bundles of nerve fibers) forms a polygonal network interposed between the longitudinal and circular smooth muscle layers of the digestive wall. On cross sections the ganglionic structures of the myenteric plexus are of relatively small dimensions and within each of them we noticed a lower or higher number of interconnected neurons (Figure 1), some of them also connected to the muscle cells by nerve fibers. However, longitudinal sections through the longitudinal axis of the plexus allowed ganglion structures of different shapes and sizes to be clearly visible (Figure 2). Their obtained shape and size depended on the extent of specific section

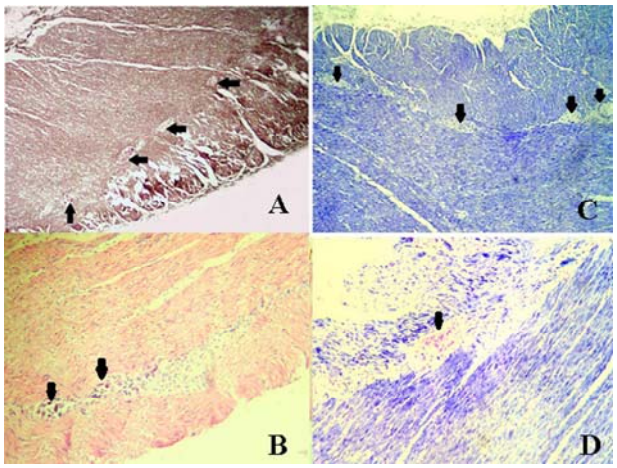

Fig. 1 – (A) Cross-section of the rectum wall, the arrow points to the myenteric ganglion (AgNO3, × 100); (B) Cross-section of the rectum wall, the arrow points to the myenteric ganglion [hematoxilin-eosin (HE), × 100)]; (C) Cross-section of the rectum wall, the arrow points to the myenteric ganglion [cresyl violet (CV), × 100)]; (D) Cross-section of the transverse colon wall, the arrow points to the myenteric ganglion (CV, × 200).

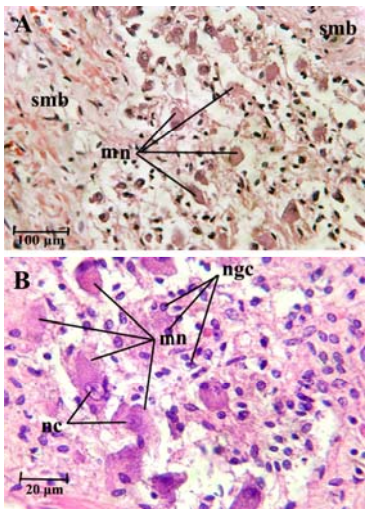

Fig. 2 – (A) Longitudinal section of the duodenum wall [hematoxilin-eosin (HE, × 400)]; (B) Longitudinal section of the stomach wall (HE, ×1000).
smb – smooth muscle bundles; mn – myenteric neurons; nc – nuclei of neurons; ngc – nuclei of glial cells.

affecting the plexus. Identification of neuronal bodies was clear on cresyl-violet stained sections (Figure 3). The result of this method of staining was neurons bodies coloring with unstained surrounding structures. Within the ganglion structures, which were generally arranged in groups of various size, the neurons of different shapes with vesicular nuclei which contain a very small quantity of chromatin were located. Around the neurons were located irregularly scattered oval nuclei of supporting or glial cells whose cytoplasm was not stained.

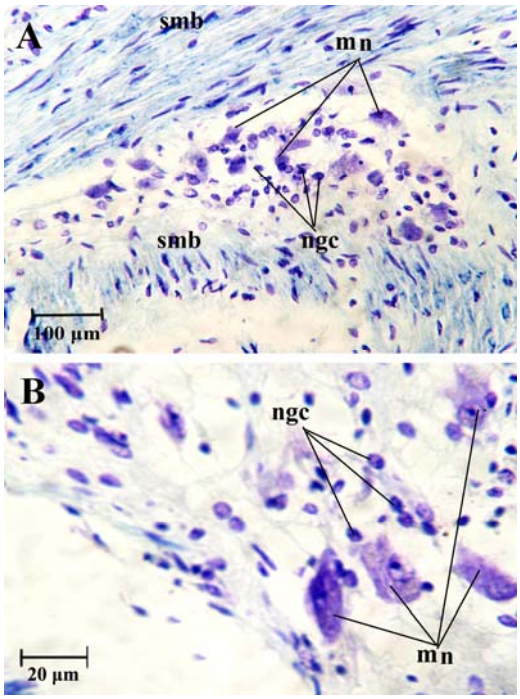

Fig. 3 – (A) Longitudinal section of the rectum wall [cresyl violet (CV), × 400]; (B) Longitudinal section of the stomach wall (CV, ×1000).
smb – smooth muscle bundles; mn – myenteric neurons; nc – nuclei of neurons; ngc – nuclei of glial cells.

Table 1 shows our results for the average number of myenteric plexus neurons *per* unit surface (cm^2). The lowest values were found in the esophagus (2,045 ± 310.30) and the largest ones in the duodenum (65,511 ± 5,639). There were noticeable very large differences in the number of neurons between the esophagus and of the other parts of the digestive tract. The results of measurements of the surface area (mm^2) of the myenteric plexus ganglionic structures along the alimentary tract are also presented in Table 1. The values found for surface area of ganglionic structures of different parts of the human digestive tract were different. The largest surface area was found in the stomach (0.01787 ± 0.0030 mm^2) and the smallest one in the duodenum (0.01185 ± 0.0016 mm^2). With the exception of the esophagus, statistical analysis (ANOVA/analysis of variance) did not show statistically significant differences at the level of $p < 0.05$ among investigated ganglion structures. However, we did not obtain data about surface of the esophagus ganglion structures, because during the study a significantly smaller number of ganglion cells was observed in the esophageal myenteric plexus, scattered in the intramuscular layer of the *tunica muscularis*. In addition, esophageal myenteric plexus ganglions were present in large parts of the sections only as a single (isolated). They were closely related to the circular and longitudinal layers of smooth muscle and only in some areas were in the groups and with a very smaller number of neurons. Therefore there was no possibility to include esophageal ganglionic structures into analysis by the same methodology which was used for the other parts of the digestive tract.

For complete analysis we also determined the surface which occupied the profiles of neurons within the ganglion structure, i.e. that phase of the surface of myenteric plexus ganglion structures which belonged to the profiles of nerve cells. The obtained values, presented in Table 1 (mm²), showed that the total surface neurons of the myenteric plexus ganglionic structure among the different parts of the digestive tract differed significantly. The largest difference (with the above explained exclusion of the esophagus) was between the stomach and transverse colon ($p < 0.001$).

The results of calculation of total surface percentages of the ganglion structures occupied by neuronal profiles are shown in Table 1. These data inform us about the density of plexus neurons in different parts of the digestive tube. The highest density of neurons we found in the area of duodenum (22.02%) and lowest one in the ileum (15.26%).

The results of the study of individual ganglion surface cells and of their nuclei (µm²) are shown in Table 2. The lar-

Table 1

Morphological characteristics of the myenteric plexus in the human digestive tract

Parts of Alimentary tract	Number of ganglion (cells/cm^2)	Surface area of ganglion structure (mm^2)	Surface phase of ganglion belonging to the profiles of nerve cells (mm^2)	
	x̄ ± SD	x̄ ± SD	x̄ ± SD	%
Esophagus	2,045 ± 310.30*	0	0	0
Stomach	46,260 ± 3,829	0.01787 ± 0.0030	0.00301 ± 0.00060	16.80
Duodenum	65,511 ± 5,639	0.01185 ± 0.0016	0.00261 ± 0.00035	22.02
Jejunum	53,794 ± 4,659	0.01344 ± 0.0016	0.00229 ± 0.00042	17.03
Ileum	44,850 ± 4,006	0.01395 ± 0.0021	0.00213 ± 0.00039	15.26
Transverse colon	37,836 ± 4,126	0.01227 ± 0.0017	0.00196 ± 0.00041*	15.97
Rectum	50,106 ± 3,004	0.01560 ± 0.0021	0.00260 ± 0.00041	16.66

x̄ – mean value; SD – standard deviation; *$p < 0.001$ compared to other groups (*t*-test);
****$p < 0.05$ compared to other groups (*t*-test).**

Table 2
Morphological characteristics of the myenteric plexus neurons in the human digestive tract

Parts of alimentary tract	Surface of neurons (μm²) x̄ ± SD	Surface of nuclei (μm²) x̄ ± SD
Oesophagus	588.93 ± 30.45*	82.88 ± 4.64*
Stomach	296.46 ± 22.53	37.19 ± 3.96
Duodenum	320.23 ± 29.39	38.97 ± 3.55
Jejunum	309.24 ± 27.76	38.14 ± 1.55
Ileum	318.05 ± 22.05	39.16 ± 2.60
Transverse colon	315.10 ± 19.97	37.72 ± 1.86
Rectum	303.04 ± 27.42	38.17 ± 1.86

x̄ – mean value; SD – standard deviation;
***p < 0.001 compared to other groups (*t*-test);**
****p < 0.05 compared to other groups (*t*-test).**

gest average surface of myenteric plexus neurons was found in the esophagus (588.93 ± 30.45 μm²) and the lowest one in the stomach (296.46 ± 22.53 μm²). The only statistically significant difference ($p < 0.001$) we found between the surfaces of the esophagus ganglion cells and those of the myenteric neurons in other parts of the digestive tract. A similar distribution we obtained for nuclei, so that the maximal average value of neuronal nuclei surface (82.88 ± 4.64 μm²) was observed in the esophagus and the lowest one (37.19 ± 3.96 μm²) in the myenteric plexus ganglion cells of the stomach. The only statistically significant difference ($p < 0.001$) we found between the surfaces of nuclei of the esophageal neurons and those of the myenteric neuronal nuclei of all the other investigated parts of the digestive tract.

Discussion

In the available literature we found only several research reports on human myenteric nervous plexus. Our study shows that the myenteric plexus, on the cross sections of the wall of the digestive tract appears as a thin, wrinkled, interrupted lamellar structure inserted between longitudinal and circular layers of smooth muscle. In these sections the plexus is a relatively thin with uneven diameter, with thinner and thicker parts. Bodies of the nerve cells are grouped on the places of thickening.

For an appropriate and completed morphometric research and evaluation of the myenteric nervous plexus the longitudinal sections have proven useful. However, this requires a greater effort, with unavoidable continuous control of native section by light microscopy, which is necessary because a large number of serial sections going from serosa to mucosa. On the sections, along its longitudinal axis, the myenteric plexus appears in the form of smaller or larger groups of ganglion cells around which are scattered the bundles of longitudinal and circular smooth muscle.

The total number of neuron/cm² in the middle part of the esophagus was on the average 2,045 ± 310.30 indicating that the myenteric nervous plexus of the esophagus has much lower neuronal density/cm² than the other parts of the human digestive tract. Similar results for the lowest neurons in the esophagus reported Maifrino et al. [9] in the study on myenteric plexus of the rodent digestive tract. De Souza et al. [10] in quantitative research of the myenteric nervous plexus in the distal part of the human esophagus (11 cm from cardial orifice) found the highest density of ganglion cells in the segment of the esophagus, at 4 to 6 cm above the cardiac incisure. They reported variability of the neuronal density in human esophagus between 659 and 3,316 neuron/cm² that is similar to our results. Our analysis included the middle third or more proximal part of the esophagus. The neurons in the esophagus are quite dispersed, especially in the upper part, so the borders of ganglia should not be defined clearly. Generally, we found that moving across from cervical to thoracic segment of the esophagus the density and the size of myenteric ganglia increased.

In the available literature we did not find data about the number of neurons in the myenteric nervous plexus ganglion structures of the human stomach. Our results show the presence of enormously larger number of neurons in the myenteric plexus of the stomach as compared to the esophagus (average 46,260 ± 3,829 neurons/cm²).

In our research the myenteric plexus of the duodenum showed a very high neuronal density (average 65,511 ± 5,639 neurons/cm²). In the available literature we found no data for humans, but high neuronal density (52,000 – 55,300 neurons/cm²) [11] was reported for the myenteric plexus of horse duodenum. In the duodenum of rats, 13,047 to 89,335 neurons/cm² depending on the age of animals were found from the oldest to the youngest animals, respectively [12].

It is important that we found a decreasing trend in the number of myenteric nervous plexus neurons in the parts of the small intestine going distally to the ileocaecal junction. The jejunum has a smaller number of neuron/cm² (53,794 ± 4,659) than the duodenum, and the ileum has smaller number of neuron/cm² (44,850 ± 4,006) in relation to the jejunum. In the absence of relevant data about the number of neurons in the human small intestine in the available literature we can only compare our results to those of Young et al. [13] of spatial density of myenteric neurons in the guinea pig ileum (17,300/cm²), and of Miranda-Neto et al. [14] in the rat ileum in the area of 8.96 mm² where they found 1,647 ± 76.67 to 2,144.40 ± 161.03 neurons. In a rat, on the surface of 25.2 mm² Moreira et al. [15] found 6,648.6 ± 790.2 neurons. In guinea-pig small intestine the number of the myenteric plexus neurons follows decreasing sequence: duodenum > jejunum > ileum [16] what corresponds to our findings on humans.

In the available literature there was no study on the rating of the number of neurons in the human colon. In the myenteric plexus of the human transverse colon we found 37,836 ± 4,126 neurons/cm^2, but in rat colon de Araújo et al. [17] revealed the distribution 30,968 neuron/cm². High variations in the number of neuron/cm^2 depending of the colon part was shown to existe, even these differences were found in the single segment of the colon [18] depending on which region of the same level section was explored.

The rectum, similarly to the stomach shows a slightly higher average number of neuron/cm^2 (50,106 ± 3,004 neurons/cm^2) in relation to the ileum and transverse colon. This is probably because both these organs, unlike of other parts of the digestive tract, are characterized by the presence of very strong smooth muscle able for large amplitudes of alternating relaxation and a very strong propulsion.

Except the number of ganglion cell/cm^2 of myenteric plexus, our research included the areal surface of nerve structures in the parts of the digestive tract. Table 1 shows that the areal surfaces of the ganglion structures of the myenteric plexus ranged from 0.01185 ± 0.0016 mm^2 in the duodenum to 0.01787 ± 0.0030 mm^2 in the stomach. Neuronal section surfaces have a certain percent of ganglion structure surfaces (Table 1) and, the highest percent (22.02%) of the ganglion surface has the neurons of the duodenal myenteric plexus, corresponding to their large number.

There are no data showing that the phase surface belongs to myenteric neurons, as well as their possible differences within the digestive tract. In the effort to find out more about the morphological characteristics of neurons we focused our research on determining the nerve cells body surface and their nuclei (Table 2). The largest areas of neurons were observed in the esophagus (588.93 ± 30.45 μm^2). Liberti et al. [19] also found the highest value of pericaryonic fields (profiles of neurons) in the esophagus (489.97 ± 212.35 μm^2). Neuron body surfaces in other parts of the digestive tract in our research range from 296.46 ± 22.53 μm^2 (stomach) to 320.23 ± 29.39 μm^2 (duodenum), while similar to our results, Liberti et al. [19] have found smallest neuronal surfaces in the sigmoid colon (241.64 ± 122.62 μm^2), without significant differences between the mean values in the stomach (284.77 ± 134.70 μm^2) and duodenum (291.39 ± 157.86 μm^2). Smaller average size of neurons in the rat duodenum was found (229.7 ± 3.75 μm^2) [20] than in our study, maybe due to the variety of tissue processing methodologies and the ways of treatment and staining, as well as because of different sampling sites from the digestive tract. Future studies should use the most objective method of analysis for the enteric nervous plexus, as well as the standardization of staining for all nervous structures.

Conclusion

Longitudinal sections of the myenteric nervous plexus are necessary for its morphometric studies, to make them completely visible as a wide branching structure containing ganglion cells, surrounding glial cells and nerve fibers, clearly delineated from the surrounding smooth muscle bundles. Ganglion cells are large, oval, round or polygonal, eosinophilic cytoplasm with round eccentric vesicular nucleus and clearly visible nucleolus. Generally they are found in smaller or larger groups, and in rare cases (typically in the esophagus) as single neurons. The esophageal myenteric nervous plexus shows a less dense structure than the other parts of the digestive tract and is characterized by much larger neuronal surfaces and neuron nuclei compared to other parts of the digestive tract.

There are differences in the number of ganglion cells between the different parts of human digestive tract, ranging from a few to several tens of thousands of neuron/cm^2. Our results strongly indicate a specific decreasing gradation in density and size neurons within human digestive tract: duodenum > jejunum > rectum > stomach > ileum > colon transverse > esophagus.

Our results show specific morphometric characteristics of the myenteric plexus for each of the investigated parts of the human tract.

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Efficacy of external warming in attenuation of hypothermia in surgical patients

Efikasnost spoljašnjeg zagrevanja u ublažavanju hipotermije kod hirurških bolesnika

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Abstract

Background/Aim. Hypothermia in surgical patients can be the consequence of long duration of surgical intervention, general anaesthesia and low temperature in operating room. Postoperative hypothermia contributes to a number of postoperative complications such as arrhythmia, myocardial ischemia, hypertension, bleeding, wound infection, coagulopathy, and prolonged effect of muscle relaxants. External heating procedures are used to prevent this condition. The aim of this study was to evaluate the efficiency of external warming system in alleviation of cold stress and hypothermia in patients who underwent major surgical procedures. **Methods.** The study was conducted in the Military Medical Academy in Belgrade. A total of 30 patients of both genders underwent abdominal surgical procedures, randomly divided into two equal groups: the one was externally warmed using warm air mattress (W), while in the control group (C) surgical procedure was performed in regular conditions, without additional warming. Oesophageal temperature (Te) was used as indicator of changes in core temperature, during surgery and awakening postoperative period, and temperature of control sites on the right hand (Th) and the right foot (Tf) reflected the changes in skin temperatures during surgery. Te and skin temperatures were monitored during the intraoperative period, with continuous measurement of Te during the following 90 minutes of the postoperative period. Heart rates and blood pressures were monitored continuously during the intraoperative and awakening period. **Results.** In the W group, the average Te, Tf and Th did not change significantly during the intraoperative as well as the postoperative period. In the controls, the average Te significantly decreased during the intraoperative period (from 35.61 ± 0.35ºC at 0 minute to 33.86 ± 0.51ºC at 120th minute). Compared to the W group, Te in the C group was significantly lower in all the observed periods. Average values of Tf and Th significantly decreased in the C group (from 30.83 ± 1.85 at 20th minute to 29.0 ± 1.39ºC at 120th minute, and from 32.75 ± 0.96 to 31.05 ± 1.09ºC, respectively). **Conclusion.** The obtained results confirm that the external warming using warm air mattress was able to attenuate hypothermia, i.e. substantial decrease in core temperature, compared with the similar exposure to cold stress in the control group.

Key words:
anesthesia, general; digestive system surgical procedures; hypothermia; intraoperative period; postoperative complications; monitoring, physiologic; heating.

Apstrakt

Uvod/Cilj. Hipotermija kod hirurških bolesnika može biti posledica dugotrajne hirurške intervencije, opšte anestezije i niske temperature vazduha u operacionoj sali. Postoperativna hipotermija može dovesti do brojnih postoperativnih komplikacija kao što su aritmija, ishemija miokarda, hipertenzija, krvarenje, infekcija rane, koagulopatija i produženo dejstvo mišićnih relaksanata. Za prevenciju ovog stanja koristi se spoljašnje zagrevanje. Cilj ove studije bio je procena efikasnosti sistema za spoljašnje zagrevanje radi ublažavanja hipotermije kod bolesnika podvrgnutih dugotrajnim hirurškim procedurama. **Metode.** Ovo istraživanje sprovedeno je u Vojnomedicinskoj akademiji u Beogradu na 30 bolesnika oba pola koji su nasumice bili podeljeni u dve jednake grupe: u jednoj je primenjeno spoljašnje zagrevanje madracem sa toplim vazduhom, dok je druga bila bez dodatnog zagrevanja (kontrolna grupa). U toku hirurške intervencije, kao i tokom postoperativnog perioda buđenja, promene unutrašnje temperature praćene su preko ezofagusne sonde (Te), a promene temperature kože intraoperativno preko kontrolnih tačaka na desnoj šaci (Th) i desnom stopalu (Tf). Srčana frekvencija i vrednosti krvnog pritiska praćeni su kontinuirano tokom hirurške intervencije, kao i u toku perioda buđenja. **Rezultati.** U zagrevanoj grupi, prosečne vednosti Te, Tf i Th nisu se značajno menjale

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tokom intraoperativnog i postoperativnog perioda. U kontrolnoj grupi, prosečna vrednost Te značajno se smanjila tokom intraoperativnog perioda (sa 35,61 ± 0,35ºC u nultom minutu na 33,86 ± 0,51ºC u 120. minutu). U odnosu na zagrevanu grupu, Te je u kontrolnoj grupi bila značajno niža u svim posmatranim periodima. Prosečne vrednosti Tf i Th značajno su se smanjile u kontrolnoj grupi (sa 30,83 ± 1,85 u 20. minutu na 29,0 ± 1,39ºC u 120. minutu, odnosno sa 32,75 ± 0,96 na 31,05 ± 1,09ºC). **Zaključak.** Rezultati ove studije podvrđuju da spoljašnje zagrevanje madracem s toplim vazduhom može da ublaži hipotermiju, odnosno da spreči značajno sniženje unutrašnje temperature do kojeg dolazi tokom izlaganja stresu usled hladnoće tokom hirurške intervencije.

Ključne reči:
anestezija, opšta; hirurgija digestivnog sistema, procedure; hipotermija; intraoperativni period; postoperativne komplikacije; fiziološke funkcije, praćenje; grejanje.

Introduction

Unintended periopertive hypothermia is common in surgical patients and is related to significant morbidity and mortality. Monitoring of body temperature and avoiding the unintended perioperative hypothermia using active and passive warming techniques are key factors of prevention of complications including surgical infection, delayed wound healing, adverse myocardial events, and increased bleeding [1]. Surgical patients are prone to hypothermia as a combination of thermoregulation impairment induced by anaesthetics, long duration of surgical procedures, widely open abdominal or thoracic cavity, and relatively low air temperature in operating room [2]. Induction of general anaesthesia also leads to redistribution of heat from the warm core thermal compartment to peripheral tissues [3]. Additionally, the characteristics of volatile anaesthetics to inhibit thermogenesis and muscle relaxants to inhibit perioperative shivering, also contribute to hypothermia [4, 5].

Perioperative hypothermia enables the onset of numerous postoperative complications such as arrhythmia, myocardial ischemia, blood pressure increase, bleeding, coagulopathy, wound infection, and prolonged activity of muscle relaxants [6]. Another common consequence of perioperative hypothermia is postoperative shivering, which is experienced by patients as extremely unpleasant. Shivering itself may lead to wound distension, hence may increase postoperative pain. Monitoring of vital functions is also more difficult when shivering is present. Both oxygen consumption and carbon dioxide production are increased, together with increase in metabolic rate, which can worsen the preexisting illnesses [6].

Up to 90% of body heat is lost from the skin [7]; hence, the prevention of heat loss *via* skin is necessity in surgical interventions. Elimination of heat loss from skin enables the increase of core temperature up to 1ºC every hour. This may be achieved by passive insulation of body surface with cover sheets. It may reduce the heat loss by 30%, whether the sheets are warmed or not [8], and the same results are achieved with infrared lamps. The most effective prevention of heat loss so far has been achieved by usage of blankets with circulating warm water, while forced-air warming mattresses may even conduct a substantial amount of heat into the body [7].

Perioperative temperature monitoring, as well as active warming of patients are still non-standard procedures in Europe [9]. In the previous study, Zeba et al. [10] investigated efficiency of perioperative internal warming. The intravenous application of amino acids resulted in alleviation of hypothermia, due to their thermogenic properties. Considering the characteristics of heat exchange during general anesthesia, in this study we wanted to investigate the other aspect of maintaining the constant temperature – reduction of heat loss from skin, using perioperative warming with forced-air mattress.

Methods

The participants enrolled in this investigation were 30 patients who underwent the extensive abdominal surgical procedure, duration of which was 2 hours and longer. In all the patients the same method of general balanced anaesthesia (GBA) was used. For premedication, 10 mg of diazepam [(intramuscular injection) was administered one hour before anaesthesia induction. Midazolam [0.05–0.15 mg/kg of body weight (BW)], fentanyl (2–6 mg/kg BW), propofol (1–2.5 mg/kg BW) and rocuronium (0.6–1mg/kg BW) were used for induction of GBA. Anaesthesia and analgesia were maintained with 2–4 vol% of volatile aesthetic sevoflurane (respiratory volume of 6–8 mL/kg BW) with intermittent bolus of 25–50 mg of fentanyl. Neuromuscular blockade was maintained with intermittent bolus of 0.15 mg/kg BW of rocuronium. The participants were randomized into two groups: 15 patients were externally warmed during operation with warming mattress (W group), while other 15 were non-warmed controls (C group). The patients in both groups were similar by age, gender distribution, body weight, diagnosis and surgical procedure, including duration of anaesthesia (Table 1).

Table 1

Characteristics of the patients and environments in both groups

Characteristics of the patients	W	C
Age (years), $\bar{x}$ ± SD	63.25 ± 5.8	65.19 ± 4.89
Males (%)	53.33	60
Females (%)	46.66	40
Body weight (kg), $\bar{x}$ ± SD	72.9 ± 6.12	74.28 ± 5.16
Duration of anaesthesia (min), $\bar{x}$ ± SD	133.46 ± 12.02	141.28 ± 16.13
Temperature in operation theatre (ºC), $\bar{x}$ ± SD	21.14 ± 1.64	21.25 ± 1.52
Relative humidity in operation theatre (%), $\bar{x}$ ±SD	55.6 ± 5.55	57.13 ± 6.28
Wind speed in operation theatre (m/s), $\bar{x}$ ± SD	0.22 ± 0.04	0.21 ± 0.04

W – warming mattress (W group); C – control group.

The investigation was approved by the Ethical Committee of the Military Medical Academy and corresponded to the standards of thermal strain evaluation by physiological measurements [11]. Signed informed consents were obtained from each participant, in accordance with the standards of medical safety during examination in cold environment [12]. The investigation was conducted in the Military Medical Academy, Belgrade, during 2013.

The external warming system tested in this study was KANMED WarmCloud OT-600-001 (Kanmed AB, Bromma, Sweden). This electric medical device consists of warm air mattress placed under the patient where it produces warm air cushion. Temperature and pressure in the warm air mattress are generated by a main unit, which is placed under the operating table, and controlled by a hand controller. The main unit's air hoses are connected to the mattress. Once the mattress is pressurized, warm air circulates under the patient in a closed system.

The patients were introduced into operation theatre nude, covered with a cotton sheet. Climatic conditions were continuously measured by the automatic monitoring system AMI300 (Kimo Instruments, France). Values of temperature, relative humidity, and air velocity were recorded every 10 minutes. The patients were placed onto the operating table, either with warming mattress (W group), or without it (C group). At 0th minute, anaesthesia was induced, and oesophageal and skin temperature probes were placed. In the following 20 minutes, preparations before surgery were done: isolation of operative site, covering the rest of body surface with sheets, disinfection of the skin. Approximately at the 20th minute, the surgeon started with the surgical procedure, which lasted at least 2 hours. When surgery was done, the patients were awakened, the mattress was removed and they were transported to awakening room. Oesophageal temperatures were monitored for further 90 minutes.

Skin temperatures were automatically monitored and recorded in real-time by physiological data monitoring system MP150 (Biopac Systems, Inc. USA), with interface module UIM100C and skin temperature amplifier modules SKT100C and skin thermistor tranducers TSD202E. Core temperature was measured as oesophageal, [11] using temperature probes 400 Series (Datex-Ohmeda Instrumentarium Corp. Finland). A probe was introduced in the anesthetised patient, into the lower part of the oesophagus and placed up against the left wall of the oesophagus. This position enables registration of temperature changes in arterial blood in descendent aorta, which leans with its back surface onto that side of the oesophagus. Oesophageal temperature (Te) was measured continually, with data recording every 10 minutes.

In extreme ambient conditions skin temperature tends to vary over the body surface. In surgical patients, the measurement of local skin temperatures, i.e. the choice of measurement sites is dictated by the given position of the body during surgery. Considering the prerequisite demands of the major abdominal surgical procedures, we selected two measuring points: right foot (Tf) and right hand (Th). Local skin temperatures were continually measured.

During intraoperative and awakening period, heart rates and blood pressure were continuously monitored using Datex-Ohmeda General Electric Anaesthesia Monitor S/5 (Beaverton, USA), and recorded every 10 minutes. Blood pressure was monitored *via* the intra-arterial catheter placed into radial artery after the induction of anaesthesia.

Data are presented as mean values and standard deviations (±SD). Normal distribution was tested by Shapiro-Wilk's test. The differences between the warmed and non-warmed group, as well as the differences between two terms of measurement in the same group were tested by Student's t-test for independent samples, Man-Whitney and Kruskall-Wallis test. The statistical significance was accepted at $p < 0.05$.

Results

Comparable reviews of core i.e. a oesophageal temperature values in the groups with a warming system and without it are shown in Figure 1. At the beginning of the observation period, core temperatures did not differ between the groups: 35.83 ± 0.35 in the W *vs* 35.61 ± 0.35ºC in the C group ($p = 0.1922$). In the W group, average core temperature did not change significantly from 0th minute towards the 120th minute: 35.83 ± 0.35 *vs* 35.33 ± 0.50ºC, respectively ($p = 0.2719$). In the group C temperature steadily decreased from the beginning towards the end of the observation period, with a statistically high significance: 35.61 ± 0.35 *vs* 33.86 ± 0.51ºC, respectively ($p < 0.000$). Also, except at the very beginning (0th minute), patients in the group C had significantly lower core temperatures. The average decrease in core temperature from 0 to 120th minute in the non-warmed patients was 1.75 ± 0.47ºC.

In the postoperative period we recorded the increase in oesophageal temperatures in both groups (Figure 2). However,

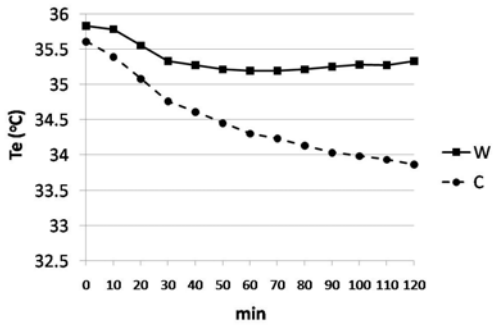

Fig. 1 – Average oesophageal temperatures (Te) during surgery in both groups.
W – warming mattress (W group); C – control group.

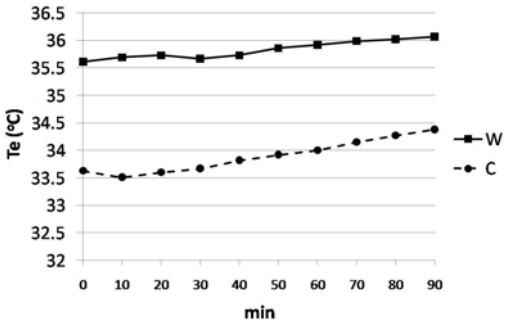

Fig. 2 – Average oesophageal temperatures (Te) during the awakening period in both groups.
W – warming mattress (W group); C – control group.

temperatures in the warmed patients were significantly higher throughout the entire 90 minutes of awakening, with the temperatures at the end (90th minute) as follows: 36.07ºC ± 0.69 in the W, and 34.38 ± 0.79ºC in the group C ($p < 0.000$).

Peripheral skin temperatures were measured on the right foot and right hand (Figures 3 and 4). In the first 20 minutes of the observed period (before surgical incision), temperatures were rising, due to the effects of premedication, as well as isolation of operative site for surgical procedure itself. In the warmed patients, the foot temperatures remained at the similar level from the 20th towards the 120th minute: 33.41 ± 0.89 *vs* 32.97 ± 1.09ºC, respectively ($p = 0.4621$), while in the control group the decrease was statistically highly significant: 30.83 ± 1.85 *vs* 29.0 ± 1.39ºC, respectively ($p < 0.000$). The average decrease in foot temperature in the control group was 1.83 ± 1.13 ºC, and the mean temperatures during the entire period were significantly lower compared to the warmed group ($p < 0.000$).

The mean temperatures of skin surface at the other measurement site (right hand) followed the same pattern: in the warmed patients the temperature was maintained throughout the observed period (from 33.78 ± 0.66 at the 20th minute to 33.55 ± 0.74ºC at the 120th minute, $p = 0.2447$), while in the control group hand temperatures decreased significantly (from 32.75 ± 0.96 at 20th minute towards 31.05 ± 1.09ºC at the 120th minute, $p = 0.0076$). The temperatures were significantly higher in warmed group compared to the controls in all the observed periods ($p < 0.05$). The average decrease in the mean hand temperature in the control group was 1.69 ± 1.65ºC.

Average values of heart rates at the beginning of surgery did not differ between the groups: 76.60 ± 11.04 in the W group *vs* 72.73 ± 16.04 bpm in the C group. Average values of both systolic and diastolic blood pressure did not differ as well, and were 126.93 ± 21.24 *vs* 131.53 ± 15.26 mmHg and 76.60 ± 11.65 *vs* 72.80 ± 9.39 mmHg, respectively. Throughout the intraoperative period, average values of heart rates were higher in the warmed group, with a statistical significance recorded from 80–100th minute (78.47 ± 13.95 *vs* 65.40 ± 13.33 bpm; $p = 0.046$ in 80th minute; 76.53 ± 12.02 *vs* 64.47 ± 12.90; $p = 0.043$ in 90th minute; 77.40 ± 10,11 *vs* 64.87 ± 14.18; $p = 0.030$ in 100th minute), but at the 120th minute, there was no statistical difference between the groups.

Both systolic and diastolic pressures were similar in both groups from the beginning toward almost the end of the intraoperative period. Statistically significant differences were recorded only at 100th minute (for systolic pressure 126.93 ± 21.24 in the group W *vs* 131.53 ± 15.26 mmHg in the group C, $p = 0{,}000$, and for diastolic pressure 80.40 ± 9.50 *vs* 68.13 ± 7.59 mmHg; $p = 0.000$).

During awakening, average values of heart rates were significantly higher in the warmed group in almost every period of monitoring, and ranging from 74.73 ± 14.43 at 0th minute to 83,40 ± 10,41 bpm at 90th minute, comparing to the controls (from 66.00 ± 16.32 at 0th minute to 76.87 ± 17.95 bpm at 90th minute). At the same time, in both groups the similar average values of systolic and diastolic pressure were measured.

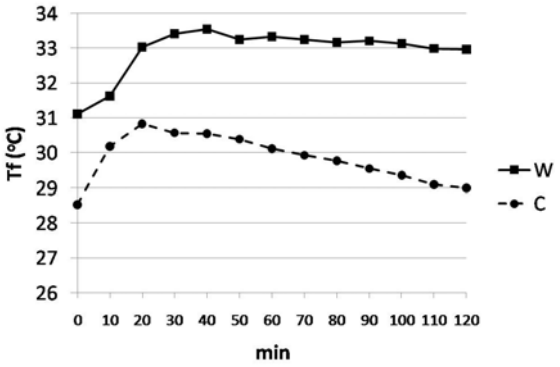

Fig. 3 – Average foot temperatures (Tf) during surgery in both groups.
W – warming mattress (W group); C – control group.

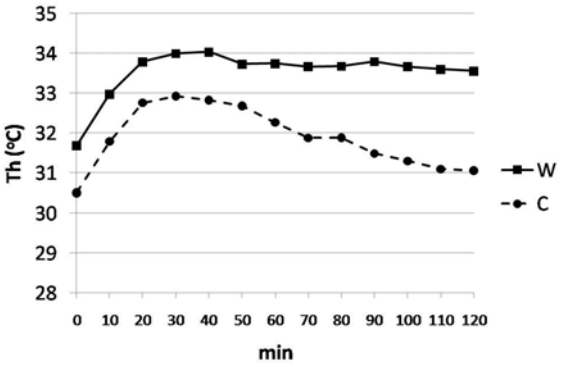

Fig. 4 – Average hand temperatures (Th) during surgery in both groups.
W – warming mattress (W group); C – control group.

Discussion

Data collected by measurements of physiological parameters of cold stress in the warmed and non-warmed patients during surgical interventions point to several key conclusions. First, our results confirm the influence of general anesthetics on thermoregulation impairment, which is in surgical patients enhanced with relatively low environmental temperature. In our investigation temperatures in operation room ranged from 19.8 to 22.7ºC, in combination with relative humidity between 47% and 78% and still air, which resulted in lower equivalent temperatures by approximately 2ºC. In surgical patients hypothermia is pronounced by inhibition of efficient mechanisms of thermogenesis such as shivering, which can be explained by properties of muscle relaxants and volatile anaesthetics. Another contributing factor to hypothermia in our patients was widely open abdominal cavity [13]. Evaporation and convection from wet tissues may eliminate up to 2 kJ *per* mL evaporated water [14].

Operating room does not represent extreme environment, except for surgical patients. With air temperature below 23ºC, it does not provide enough warmth for provide the maintenance of normal core temperature in patients. When temperature in operating room drops below 21ºC, every patient experiences hypothermia [5]. However, colder environment is more approp-

riate for surgeons and other stuff, considering the heat stress induced by sterile uniforms, rubber gloves, rubber aprons, and high level of stress resulting in increased sweating.

Body temperature is usually unequally distributed, with core values limited to head and trunk (internal organs in skull, chest and abdomen), while peripheral parts of the body are typically colder by 2–4ºC. Normally, there is temperature gradient from core to peripheral areas, which is maintained by constriction of blood vessels in skin. This distribution is substantially impaired during general anaesthesia. First phase of decrease in core temperature is the result of redistribution of heat from central compartment towards colder peripheral tissue (particularly in distal parts such as hands and feet) [15]. This is the result of dilatation of blood vessels in skin which is one of the effects of general anaesthesia. Redistribution also impose the enlargement of central compartment of the human body during general anaesthesia, from the volume limited to the head and trunk (internal organs in the skull, chest and abdomen) to expanded volume including arms and legs, as well. This redistribution of warm blood towards the peripheral areas leads to warming of hands and feet, but also contributes to decrease of core temperature by 0.5–1.5ºC [16].

When heat loss from the skin is eliminated, metabolic heat would increase the mean body temperature by approximately 1ºC every hour. Although we found a number of papers reported heat exchange between human body and environment, monitoring of heat flux by infrared (IR) thermography in surgical patients, as an example of objective measurement was not used so far. In our study, we also continued with the approach of collecting data by physiological measurements. Giesbrecht et al. [7] showed that active skin warming using forced-air warming systems may completely eliminate heat loss from skin surface. In 6 volunteers the authors investigated thermal exchange under conditions of operating room (ambient temperature 24.5 ± 0.8ºC; subjects minimally clothed, reclined on hospital bed), and recorded a significant increase in core temperatures when warming systems were applied.

Our results showed that in the warmed group core temperature after induction of anaesthesia decreased, particularly in the first 30 minutes, but not significantly. After that period, we observed a *plateau*, i.e. the temperature levels remained in the normal range (above 35ºC). In the non-warmed group, however, decrease is more pronounced in the first 30 minutes, with the constant drop throughout the entire observed period. Mean values from the 30th minute towards the 120th minute were below 35ºC, with end-point temperature 33.86 ± 0.51ºC, which means that non-warmed patients were hypothermic.

This is in accordance with the results of randomized, prospective clinical trial conducted by Hynson and Sessler [17]. The authors investigated the effect of forced-air warmer on tympanic temperature in 10 patients who underwent prolonged surgery and found that in warmed patients there was significantly less decrease in core temperature compared to controls: the temperature in warmed patients dropped from preinduction moment towards the third hour of anaesthesia by 0.5ºC, while in the control group, at the same time, the decrease was 2.0 ± 0.7ºC.

In our investigation, at the end of surgery core temperatures in the control group were between 32.7ºC and 35.4ºC, meaning that all the patients had temperature below 36ºC. In the warmed group, however, the values varied from 33.5ºC and 36.3ºC, and in 5 patients of 15 the temperature was above 36ºC. During awakening period in the group W we measured normal core temperature in additional 5 patients, while at the same time only one control became normothermic.

Vanni et al. [18] in 2007 evaluated the effect of preoperative (in 10 patients) and intraoperative (in other 10 patients) skin-surface warming using forced-air warming system during lower abdominal surgery under spinal anaesthesia and concluded that the applied warming procedures did not avoid, but did minimize hypothermia. Ambient temperature was similar as in our investigation (20ºC to 23ºC). In this investigation, despite the significantly higher temperatures in warmed patients compared to controls, at the end of surgery only 50% of warmed patients were normothermic (tympanic temperature above 36ºC). This can be explained by the differences between spinal and general anaesthesia regarding thermoregulation. Spinal anaesthesia obliterated any effective peripheral and central thermoregulatory control and hence might worsen cold stress.

Hemodynamic and respiratory stability in anaesthesia is crucial in response to all the perioperative influences, including hypothermia [19]. Even mild hypothermia may lead to cardiovascular impairments such as postoperative increase in heart rate and blood pressure, increase in vascular resistance, ventricular dysrrhytmia and irritability, and myocardial depression [20]. In our investigation, average values of heart rates recorded in the control group were lower than in the warmed group both during the intraoperative and postoperative period. The results obtained in a study of Bahar et al. [21] on the possibilities of attenuating the perioperative hypothermia indicate that hypothermic patients have higher heart rates, but other authors, like Chi et al. [22] report lower heart rates, which is in accordance with our results.

The same authors report that mild intraoperative hypothermia does not influence arterial blood pressure [22]. Our results may confirm their findings, because we did not observe any significant differences between the warmed patients and the controls regarding systolic and diastolic blood pressure. The average values in the warmed group were slightly higher compared to the controls, but remained in the normal range. However, there are some limitations in results interpretations in our study, which are derived from the fact that our patients were sedated in the monitored postoperative period in order to facilitate the presence of oesophageal temperature probes.

The duration of warming is also important. In a larger prospective randomized trial conducted in 2003, Wong et al. [23] investigated the effect of perioperative systemic warming on nasopharyngeal temperature in 47 warmed and 56 control patients during major elective abdominal surgery. They found that preoperative warming in duration of 2 hours, and continual warming throughout the surgical procedure and 2 hours after can completely prevent the intraoperative hypothermia. The difference between this study and our re-

sults may be explained by the duration of warming: in our study we did not used preoperative warming, for that reason, our patients had lower core temperatures on anaesthesia induction (35.83ºC compared to 36.5ºC).

However, there is another major difference in the results obtained in our investigation and in the given study. Our patients were not warmed during the awakening period, contrary to patients in Wong's study, but core temperatures at the end of the observation period were similar (36.7 *vs* 36.3ºC). These results point that pre- and intraoperative warming are more important in minimizing general anaesthesia-induced hypothermia than postoperative warming.

Conclusion

The results of temperature monitoring confirm that body core temperature (measured as oesophageal temperature) as well as local skin temperatures are significantly higher in the patients who underwent major abdominal surgery when warmed with a forced-air mattress, compared to the control conditions. The external warming proved to be efficient in alleviation of peri- and postoperative hypothermia. Considering the above, our results may be useful for further research activities directed to elimination or minimizing this important problem in surgical units.

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ORIGINAL ARTICLE

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The assessment of the stability of the corneal structure after LASIK correction of myopia by different optical zone diameters

Procena stabilnosti strukture rožnjače nakon korekcije kratkovidosti metodom LASIK sa različitim prečnicima optičke zone

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Abstract

Background/Aim. Enlargement of optical zone (OZ) diameter during laser *in situ* keratomileusis (LASIK) correction of myopia postoperatively improves the optical outcome, however, it also leads to the increased stroma tissue consumption – progressive corneal thinning. The aim of this investigation was to present the possibility of safe OZ enlargement without impairing the structural stability of the cornea, while obtaining an improved optical outcome with LASIK treatment of shortsightedness. **Methods.** Preoperative assessment of the cornea structure and prediction of the ablated stroma tissue consumption was conducted in 37 patients (74 eyes) treated for shortsightedness by means of the LASIK method. With the eyes that, according to their cornea structure, had the capacity for OZ diameter enlargement of 0.5 mm, LASIK treatment was performed within the wider OZ diameter of 7.0 mm compared to the standard 6.5 mm. The following two groups were formed, depending on the diameter of the utilized OZ: the group I (the eyes treated with the OZ 6.5 mm, n = 37) and the group II (the eyes treated with the OZ 7.0 mm, n = 37). **Results.** No significant difference in the observed structural parameters of the cornea was detected between the groups of patients treated with different OZ diameters. The values of all the parameters were significantly bellow the threshold values for the development of postoperative ectasia. **Conclusion.** Diameter enlargement of the treated OZ, if there is a preoperative cornea capacity for such enlargement, will not impair the postoperative stability of the cornea structure, and will significantly improve the optical outcome.

Key words:
myopia; keratomileusis, laser in situ; ophthalmologic surgical procedures; cornea; tretmant outcome.

Apstrakt

Uvod/Cilj. Proširenjem optičke zone (OZ) tokom korekcije kratkovidosti metodom *laser in situ keratomileusis* (LASIK) poboljšava se postoperativni ishod tretmana laserom, ali se istovremeno povećava potrošnja tkiva strome rožnjače – dolazi do progresije njenog istanjenja. Cilj rada bio je da se pokaže mogućnost bezbednog proširenja OZ bez narušavanja strukturne stabilnosti rožnjače, uz poboljšani ishod tretmana laserom nakon korekcije kratkovidosti. **Metode.** Preoperativno je sagledana struktura rožnjače i procenjen ablativni profil korekcije, na osnovu čega je predviđena postoperativna struktura rožnjače na očima 37 kratkovidih pacijenata (74 oka). Na osnovu sagledavanja strukturnih parametara procenjen je kapacitet strome rožnjače za proširenje tretirane OZ sa standardnih 6,5 mm na 7,0 mm OZ. U zavisnosti od prečnika OZ korišćene tokom lečenja formirane su dve grupe: grupa I (oči tretirane korišćenjem OZ 6,5 mm, n = 37 i grupa II (oči tretirane korišćenjem OZ 7,0 mm, n = 37). **Rezultati.** Analizom posmatranih strukturnih parametara uočeno je da su u obe posmatrane grupe njihove vrednosti bile značajno veće od graničnih koje su označene kao kritične za pojavu postoperativne ektazije nakon korekcije kratkovidosti metodom LASIK. **Zaključak.** Proširenjem prečnika tretirane OZ za 0,5 mm, ukoliko postoji odgovarajući kapacitet tkiva strome rožnjače, postoperativno se nakon korekcije kratkovidosti metodom LASIK ne narušava strukturna stabilnost rožnjače, a poboljšava se ishod laserske korekcije miopije.

Ključne reči:
miopija; keratomileusis, laser in situ; hirurgija, oftalmološka, procedure; rožnjača; lečenje, ishod.

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Introduction

Excimer laser pulses are used for remodeling of the corneal stroma with the aim of refractive anomaly correction, with the tissue being removed by means of evaporation and therefore leading to corneal stroma remodeling and thinning. The quantity of the removed tissue is proportional to the size of the corrected diopter ametropia and to the radius of the treated optical zone (OZ). According to Munnerlyn et al. [1], for corrections up to – 7.0 diopter sphere (Dsph) the following formula is used: the depth of the ablation (μm) is equal to the number of diopters multiplied by the square of the OZ diameter (mm) divided by three: Depth ab (μm) = Dsph x OZ^2 (mm)/3.

Impairing the cornea structure beyond the critical values of the structural parameters (corneal pachymetry, corneal thickness progression and corneal volume reduction), will lead to the most severe complication of laser *in situ* keratomileusis (LASIK) treatment – corneal ectasia [2]. Enlargement of the OZ diameter during LASIK correction of myopia will postoperatively improve the optical outcome, however it will also lead to the increased stroma tissue consumption – progressive corneal thinning.

The aim of this work was to present the possibility of safe OZ diameter enlargement without impairing the structural stability of the cornea, while obtaining an improved optical outcome with LASIK treatment of short-sightedness.

Methods

A prospective clinical study, conducted at the Military Medical Academy, Clinic for Ophthalmology (January 2010 – June 2012), included 37 patients treated for the existing ametropia by means of the LASIK method with a Wavelight Allegreto 400 Hz excimer laser. The study was approved by the Military Medical Academy Ethics Committee. The study included patients with myopia and myopic astigmatism. The total number of the eyes treated was 74.

The following two groups were formed, depending on the diameter of the utilized OZ during the treatment with the excimer laser: the group I (the eyes treated with the OZ 6.5 mm, n = 37); the group II (the eyes treated with the OZ 7.0 mm, n = 37).

The decision criteria for the selection of OZ diameter was based on evaluation and application of the excimer laser software for the expected postoperative thickness of the residual stromal bed (RSB) for each eye, and simulation of OZ treatment for 6.5 mm or 7.0 mm, so that the eye with greater postoperative thickness of the RSB was treated with the OZ 7.0 mm, and the eye with lower RSB value, in the same patient, was treated with the OZ 6.5 mm which was larger than the scotopic pupile diameter.

Camera recording, using the Scheimpflug system, and measuring of the postoperative structural parameters: corneal pachymetry, corneal thickness progression and corneal volume reduction, were performed after six months.

The obtained data were analyzed and showed in the text and figures. The results were presented as means with standard deviations (SD). Throughout the study, Wilcoxon and Mann-Whitney *U*-test with a statistically significant cutoff of $p < 0.05$ were used to assess the statistical significance of parameters observed.

Results

The average patient's age was 32.34 years and the youngest treated patient was 21 year old. The highest value for myopia was 8.75 diopter sphere. The average value of corneal pachymetry in our patients was 545.86 ± 33.94 μm, ranging from 503 μm to 599 μm, while the average value of the postoperative corneal pachymetry from the two observed groups was 492 μm.

Postoperatively, the average value of corneal pachymetry for the group I, treated with the OZ 6.5 mm, was 491.55 ± 48.24 μm (Figure 1) while in the group II, treated with the OZ 7.0 mm, 493.04 ± 49.23 μm of the thinnest corneal part was registered.

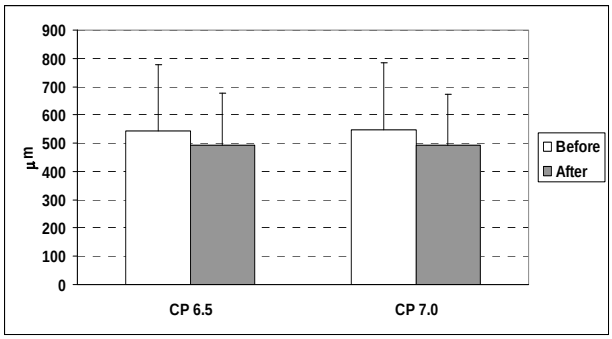

Fig. 1 – Corneal pachymetry (CP) changes in the patients after the laser *in situ* keratomileusis (LASIK) treatment in different optical zones – 6.5 mm : 7.0 mm.

When comparing the preoperative values of corneal pachymetry between the group I and the group II (Table 1), no significant differences were observed. So, an equivalence of corneal pachymetry was observed between the groups before the treatment (Mann-Whitney; Z = 0.63; $p = 0.52$).

Moreover, the comparison of the total postoperative values of the thinnest part of corneal pachymetry between the

Table1

Statistical analysis of corneal pachymetry (CP) values before and after the laser *in situ* keratomileusis (LASIK) treatment in different optical zones – 6.5 mm : 7.0 mm

Groups (type of comparison)	Z value	*p*
CP 6.5 (before : after)	0.34	0.73
CP 7.0 (before : after)	0.45	0.65
CP 6.5: CP 7.0 (before)	0.63	0.52
CP 6.5: CP 7.0 (after)	0.32	0.75
% of before-after change (CP 6.5: CP 7.0)	0.09	0.92

groups showed no significant differences (Mann-Whitney; Z = 0.32; $p = 0.74$).

The comparison of the decreased corneal pachymetry values by OZ ablation in the diameter of 6.5 mm and the wider diameter of 7.0 mm, showed no significant difference in the change of corneal pachymetry at different ablation parameters (Mann-Whitney; Z = 0.09; $p = 0.92$); (Table 1).

The values (Figure 2) and comparison of corneal volume consumption at the OZ ablation diameter of 6.5 mm, when compared to the OZ ablation diameter of 7.0 mm (Table 2), showed no significant differences in stroma consumption (Mann-Whitney; Z = 0.60; $p = 0.54$).

The values of both groups (Figure 3) and comparison of the corneal thickness progression after the LASIK procedure between the group I and the group II showed no significant difference as well (Table 3). It can be concluded that the enlargement of the OZ radius of 0.5 mm does not make a significant difference to the postoperative increase of the corneal thickness progression in central 6.5 mm of measured diameter of cornea.

Discussion

The diameter enlargement of 0.5 mm for the treated OZ, starting with the standard 6.5 up to 7.0 mm, increases the ablation depth and the volume consumption of the stromal tissue – progressive corneal thinning occurs during the LASIK procedure. The criteria for the selection of patients – eyes for the wider diameter of the treated cornea were based on the references from Binder and Trattler [3] and Randleman et al. [4], as well as the predictions on the postoperative structure of cornea based on the software program for Wawe Light Allegreto Q 400 laser, and our own previous research results [5,6]. We monitored and analyzed the cornea structural stability parameters, such as the value of corneal pachymetry, corneal volume and corneal thickness progression. Other parameters such as age as well as the highest myopic value for myopia we consider as parameters of lower level of importance. We believe that these parameters have no relevant importance for the development of ectasia as much as those structural parameters that we followed in this study.

Corneal pachymetry is perceived as the basic numerical parameter for the assessment of the postoperative stability of the cornea after the LASIK treatment and for the comparison between the observed groups. Postoperatively, the average value of corneal pachymetry in our study was 491.55 μm for the group treated with the OZ 6.5 mm, while for the group treated with the OZ 7.0 mm, the average value of corneal pachymetry was 493.04 μm. According to the empiric conclusions of Ambrósio et al. [7], the treshold value of corneal pachymetry for the development of the ectatic process is 424 ± 50.93 μm which is significantly lower than the values measured in both groups after the LASIK treatment.

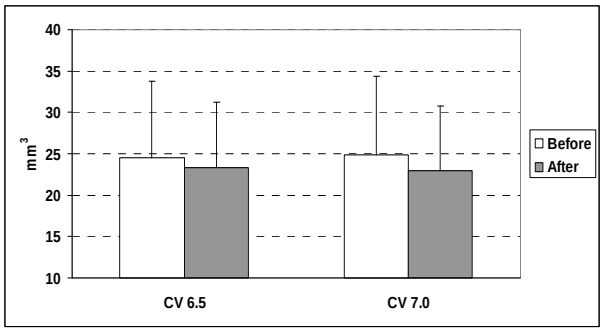

Fig. 2 – Corneal Volume (CV) changes in patients after the laser *in situ* keratomileusis (LASIK) treatment in different optical zones – 6.5 mm : 7.0 mm.

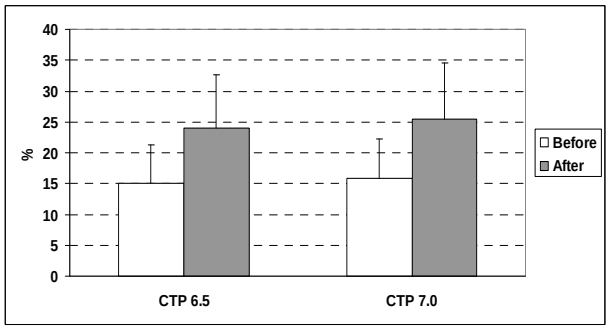

Fig. 3 – Corneal thickness progression (CTP) changes in the patients after the laser *in situ* keratomileusis (LASIK) treatment in different optical zones – 6.5 mm : 7.0 mm.

Table 2

Statistical analysis of corneal volume (CV) values before and after the laser *in situ* keratomileusis (LASIK) treatment

Groups (type of comparison)	Z value	*p*
CV 6.5 (before : after)	0.28	0.79
CV 7.0 (before : after)	0.38	0.69
CV 6.5: CV 7.0 (before)	0.59	0.55
CV 6.5: CV 7.0 (after)	0.71	0.48
% of before-after change (CV 6.5: CV 7.0)	0.60	0.54

Table 3

Statistical analysis of corneal thickness progression (CTP) values before and after the laser *in situ* keratomileusis (LASIK) treatment

Groups (type of comparison)	Z value	*p*
CTP 6.5 (before : after)	4.03	< 0.001*
CTP 7.0 (before : after)	3.98	< 0.001*
CTP 6.5: CTP 7.0 (before)	0.27	0.79
CTP 6.5: CTP 7.0 (after)	0.31	0.75
% of before-after change (CTP 6.5: CTP 7.0)	0.10	0.88

***statistically significant difference ($p < 0.05$).**

The lower limit of the residual corneal volume of 22.4 ± 2.0 mm^3, within the measurement radius of the central 7.0 mm corneal area, was based on the statement of the same author – Ambrósio et al. [7], who defined the parameter (Corneal Thickness Spatial Profile–CTSP) as the critical value for the development of the ectatic process. The average value of the postoperative corneal volume in our study, determined in the group treated with the OZ 6.5 mm was 23.33 ± 1.30 mm^3, and in the group treated with the OZ 7.0 mm 22.98 ± 3.37 mm^3. This is significantly beyond the above mentioned limit of 22.4 mm^3, which is significant for the development of the ectatic process.

Corneal thickness progression after the LASIK treatment, with the value of 31.4% for the optical zone radius of 6.0 mm, was marked as the critical value for the development of the postoperative ectasia, based on the statement of Ambrósio et al. [7]. Throughout the research, it was determined that the average postoperative value of corneal thickness progression was 24.02 ± 7.04%, for the group treated with the OZ radius of 6.5 mm, and for the group treated with the OZ 7.0 mm, the corresponding value was 25.37 ± 8.70% (which is, in case of both observed groups, significantly below the above mentioned critical value of 31.4 %).

In some of their earlier studies, our Clinic's authors [5, 6] determined the average consumption of 0.43 ± 0.18 mm^3 in the group treated with the OZ 6.5 mm *per* one diopter correction, and in the group treated with the OZ 7.0 mm, the ablated volume was 0.61 ± 0.31 mm^3 *per* one diopter correction of myopia.

Throughout our research, we were guided by the statements of Ambrósio et al. [7], regarding the critical value of the postoperative volume of 22.41 mm^3 within the measurement diameter of 7.0 mm, as the critical value for the changes in the corneal structure. In no case was it determined that the measurement of the residual corneal volume with Oculyzer device, six months after the LASIK procedure, exceeded this treshold value.

The comparison of the corneal thickness progression after the LASIK correction between the groups treated with the OZ radius of 6.5 mm and the group treated with the OZ radius of 7.0 mm, showed no significant difference as well.

Having in mind a relatively short period of follow-up (only 2 years) the possibility for the appearance of postoperative ectasia cannot be absolutely excluded but it can be concluded that the enlargement of the OZ radius of 0.5 mm does not make a significant difference to the postoperative increase of the corneal thickness progression.

As recommended by the American Academy of Ophthalmology, and based on the available literature data, throughout our clinical work we were guided by the reference to the minimal value of the preoperative corneal pachymetry of 500 μm [8, 9], while the average value of corneal pachymetry in our patients was 545.86 ± 33.94 μm, ranging from 503 μm to 599 μm. Twa et al. [2] in the abovementioned research on the characteristics of corneal ectasia after myopic LASIK treatment states that the average corneal pachymetry within the group of patients who developed ectasia was 512 μm (in the range of 498–530 μm), and that the limit value of ≤ 400 μm is considered to be a significant value of the postoperative corneal pachymetry for the development of ectasia. In our patients the average value of postoperative corneal pachymetry observed groups was 492 μm, which indicates that the preoperative corneal pachymetry values in our study were significantly higher than this value.

Preoperative assessment of the cornea structure, based on Scheimpflug camera recording and processing by means of the Oculyzer device software system, and the assessment of the ablation profile with the Allegreto Wave Light Q 400 laser software system, adhering to the corneal topography criteria [5, 10], provides a reliable estimate of the postoperative cornea structure. When structural characteristics, together with the ablation profile assessment and the stromal tissue consumption, during LASIK correction of myopia, indicate the stable postoperative cornea structure, diameter enlargement of the treated optical zone improves the optical outcome of LASIK treatment.

Conclusion

Our experience indicates that, in patients with the adequate capacity of corneal stroma, the diameter enlargement of the optical zone from the standard 6.5 mm to 7.0 mm does not impair the cornea structural stability parameters after the LASIK treatment and after six months of monitoring, while the optical outcome is improved, and that, therefore, it is advisable to enlarge the diameter of the optical zone when there are potential opportunities for such a procedure.

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New metaphysics in Serbian rehabilitation medicine

Nova metafizika u srpskoj rehabilitacionoj medicini

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Key words:
philosophy; physical and rehabilitation medicine; religion; humanities.

Ključne reči:
filozofija; medicina fizikalna i rehabilitacija; religija; altruizam.

The worst beings for Allah are those who are deaf and mute, and who will not understand [1].

Introduction

Metaphysics is philosophy. This is a story of reality. If we say it in Serbian language, its metaphorical meaning is that something does not exist, that it is completely unrealistic. We should be careful with the word metaphysic. This word could mean: "the finding of bad reasons for what we believe upon instinct" [2]. Metaphysical opinion is hard and work in accordance with this opinion is even harder. Metaphysical societies, which were founded by some philosophers, priests, biologists, critics and politicians quickly collapsed [3]. To all appearances, philosophy is the sky and medical science is the Earth. If this is correct, then metaphysic is another galaxy for the medical science. Is this true? Do we know that "every truth needs a truthmaker [4]?" Are we considering "the thrutbearers [4]?" Some philosophers claim that metaphysic introduces us into the world liberated from all rules [5]. This is important both for philosophy and medicine because: "Where physics ends, the problem does not end [6]." One philosopher of science regards that all science is pervaded by metaphysics. Especially if we take into consideration mathematics and natural sciences [7]. This claim is sustained by some experts in biometaphysics: for example, the gene ontology is a part of many biological and biomedical studies [8]. We can talk, for example, about cognitive psychology, the changing of the neurological code into the cognitive code, but the questions of consciousness, understanding and learning are scientific as well as philosophical and metaphysical questions [9].

Which philosophical questions are essential for biomedicine? It is understandable that medicine is possible without questions such as: does God exist and what about life after death, where does the cosmos originate from and where does it expand; or, does a human being have the gene predestination or is free will possible? However, it would be hard to imagine medicine without the following philosophical and metaphysical questions: what does one ideal society look like; what is the truth, righteousness, good, evil, causality or should one be an honest man [10, 11]? Rehabilitation medicine is a skill of teamwork rehabilitation of disabled people [12]. It has its own philosophy [13]. Physiatry or rehabilitation medicine is the most sociable specialization among all medical specializations. Namely, a great number of medical and nonmedical workers are engaged around a patient and their family. Physiatrists, physiotherapists, nurses, occupational therapists, psychologists and speech therapists are included in the medical core of the rehabilitation team [14–17]. But the nonmedical core, the so-called "sociable part" of the rehabilitation team, is much larger. It consists of social workers, art therapists and connselors [15]. There are residential adaptations engineers and assistive technology engineers [17]. There are music therapists, dance therapists, horticultural therapists, and experts for homeopathy, osteopathy, healing touch as well as Western herbalism [16, 18, 19]. Finally, there are teachers and chaplains as experts on religiosity and spirituality [15, 16]. How are these experts incorporated into the harmonious whole in terms of reaching rehabilitation aims – it is a philosophical, sometimes also metaphysical, question. In that respect, numerous problems exist in the world rehabilitation practice and the European rehabilitation practice, too. These are most commonly organizational-teamwork problems having to do with a small number of rehabilitation leaders and their unclear role [20]. There are also prob-

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lems connected with the rehabilitation philosophy. Specifically, fragmentizing the rehabilitation medicine and fragmentizing its ethic [21]. The roles of members of the rehabilitation team are being carefully reconsidered. The domain of the rehabilitation work and the funding of rehabilitation are being discussed. Robotics and new technologies are in the focus of researchers in the rehabilitation medicine today. The telerehabilitation and the community based rehabilitation are being developed. Experts in the rehabilitation medicine are trying to narrow the gap between the rehabilitation science and practice [17]. The rehabilitation philosophy requires the members of the rehabilitation team to pay attention to disabled people's personal factors and the environmental factors as well (ICF Classification). Besides others, religiosity, spirituality and art are these contextual factors [22].

Language is culture. It is the symbol and the sign of culture [23]. Symbols, money and signs float around us [24]. Medicine is culture, too. The word metaphysics has a literal and metaphorical meaning. Let us use this word in its metaphorical meaning. And let us say: religiosity, spirituality and philosophy are metaphysics for Serbian rehabilitation medicine. These notions do not exist in Serbian rehabilitation medicine. This is our old and sad metaphysics. What is happiness? The notion of happiness and the suppositions of happiness are well defined by philosophers. These are health, food, place of residence, love and the respect of one's own nation [25]. These matters are metaphysics for the average Serbian citizen today.

Rehabilitation medicine here has a long tradition [26]. Today the circumstances in our rehabilitation are worrying. These circumstances are: poverty, bad organization of rehabilitation work, great inflow of patients, educational problems, lack of rehabilitation leaders, political influence in the choice of the top rehabilitation management, corruption and the dampening of the rehabilitation spirit on the whole [13]. Education in the rehabilitation field is an enormous problem, especially education of all members of the rehabilitation team. It is hard to say which of these problems is bigger: the rehabilitation programs themselves or the teachers who should carry out these programs. Having in mind the philosophical question – who trains educators [27] – the problem of education in the Serbian rehabilitation is both a medical and a philosophical problem. We need new metaphysics for the Serbian rehabilitation medicine. This means, alongside everything else, the introduction of religiosity, spirituality and philosophy in the rehabilitation theory and practice. We are struggling for our rehabilitation identity. In this struggle and aspiration to symmetry there are elements of metaphysic. The identity in the being itself, the struggle and the symmetry are the three main metaphysical principles [28]. This work has the aim to explain the necessity for humanities in Serbian rehabilitation medicine according to the famous Buddhist question: "Do you put your being at disposal to the enormous human suffering, oh, candidate of light [29]?"

Crippled world

Our world is a crippled world. This is the world which "suffocates all authentic forms of human mind such as religiosity, philosophy and art [24]." This world is characterized by alienation and instrumentalisation of human beings. The business and the technical models dominate. The crippled world suffers from maximal individualism, egoism and neglect of interest for future generations [30]. This is the state of cultural and ethical relativism. This is one distorted materialistic civilization "without an ear for all which is not measurable [31, 32]." It is known, even from Plato's time: this rampant egoism leads us to misery [33]. Similarly to the man in Pascal's era, the modern man is unstable and upset [34]. Europe today is a space of the civic culture and the lay culture which have replaced the dominant Christian culture [35]. Even though the European Christian culture has been replaced by the lay culture, religiosity in Europe will not be extinguished. Religiosity is alive even in this era of globalization, whose main question is: "Who gets what, how and when [36]?" Religiosity is an important carrier of culture [37]. It did not die in the communism, nor will it die in this civic, lay and global Europe [38, 39].

Invalids in Serbia live in this crippled world. They are strangers in their own skin. Their world is the world of fear. Fear is the most important goods which is being produced by the mass media [40]. The dominant form of this fear is the fear of uncertain future. This is rational fear. Poverty is our present. Debt, banks and bankers are our future as well as our children's future [41]. Invalids here live from day to day, from case to case. Talking in the language of Karl Jaspers, in terms of the philosophical notion of the word "case", we could say that life in Serbia has been one long borderline situation for the last decades [24]. This metaphysical life is not reality only for our invalids, of course, but for all citizens in Serbia, consequently for members of the rehabilitation team, too. Serbian philosophers call this life the life in the black but accepted banality [42]. The crisis of identity has had an epidemic proportion in this crippled world [43, 44]. This is the crisis of individual and collective identity. In this world there is a yearning for freedom and there are rare few persons who dare to be extraordinary [45]. Postmodernism, bureaucracy and globalization are taking their toll [46–49].

What about identity of invalids in this world of virtually created false impressions? This is the world of calculated persons [50] and the world of confirmed simulacrum where identity theft is not metaphysical, but bitter reality [51]. Today, the Bacon's idols of the marketplace and the idols of the theatre [24] are joined into the idols of the "steal cobweb". The "steal cobweb" is a metaphor for the misconceptions into which we have been drawn by the virtual world and the Internet. Researchers in the medical anthropology are dealing with a cultural and ontological aspect of invalidity. It is recognized that physical suffering is a threat to the ontological security of invalids who, in turn, become suspicious toward their own culture, being and identity [52]. The Movement of Invalids emphasizes the importance of notions such as subjectivity, knowledge and spirituality for disabled people [53]. In our country, there are a lot of disabled people in the old population. Similarly to other parts of the world, they are a socially discriminated category [54]. Nevertheless, this is a category of patients with the pronounced religious needs [55].

Is there anybody in the average Serbian rehabilitation team who could satisfy these needs. Unfortunately, no!

The idols of the "steal cobweb" are a current issue especially in this era of liberal capitalism. The scientific world today discusses the nonsense called medicine completely turned to the market [56]. This nonsense is still one of the "mantras'' of the crippled world. We can merely imagine what this completely trade medicine would look like in Serbia. But, everything is possible! Nietzsche wrote about the culture of lie which easily becomes the law [57]. In this completely trade medicine here, the invalid would be a by-product of the industry of money which would, unfortunately, have the stethoscopes but which would not listen to anybody. A biopsy of patient's wallet would probably be the first care of a physican [56]. This is a completely foreseeable metaphysics.

Why and how religiosity and spirituality in our rehabilitation medicine?

Religion is fear of God, love for man and a conscience. It is: "a system of beliefs, practices and symbols designed to facilitate closeness to higher power [58]". "Spirituality is the quest for understanding life's ultimate questions and the meaning and purpose of living [58]". Religiosity in Serbia today is an "old wine in a new bottle [59]". Science has proved a significant relationship between religiosity/spirituality and health [60–80]. These are mainly qualitative researches with the correlation designs. The value of pastoral care has also been confirmed by the randomized controlled trials [76]. Researches direct their attention to the medical prevention, diagnosis and therapy. Samples are varied, from several participants [75], to even 37000 participants in the famous Baetz and Bowen [81] study on religiosity and the chronic pain [62]. In the statistical analysis, as a rule, compound statistical methods, mostly the regression analysis, are used [58, 61, 68].

Much more has been written and published on spirituality, and religiosity and spirituality, than only on religiosity [82]. Religious students and young people misuse alcohol less and have a lower level of risky behavior [65, 67, 68]. Physicians, nurses and students of medicine with a high level of education are less religious [83]. Religiosity and spirituality help nurses in their daily work [84, 85]. Chaplains, as the carriers of pastoral support, mostly have contact with nurses [86]. The physicians-residents in family medicine and the occupational therapists are in need of special education in religiosity and spirituality [87, 88]. Some medical leaders have inner conflict between religiosity and capitalistic ethos [88]. Religiosity affects daily physicians' practice [89]. Patients want to share their religious experiences and habits with physicians [61]. A full cooperation with persons engaged in the pastoral care is recommended to medical workers [63]. Chaplains consider that there is an antagonism between physicians and themselves [90].

Professor Harold G. Koenig, the most authoritative in the field of religious medicine, defined religion-health problem, condensed its causes and gave us suggestions for its solution. Religiosity of patients is being neglected in the daily clinical practice [91]. The main problem are physicians who: a) do not believe that they should waste their time and energy on religious questions; b) have no time and c) are afraid of crossing the professional borders [86]. Professor considers that the solution is in the religious and spiritual education of physicians and in the real multi-, inter- and transdisciplinary model of team work [91]. Religiosity and spirituality can be measured (Table 1). New metaphysics of Serbian rehabilitation medicine is based on these recommendations. The expression "new metaphysics" in medicine was created by Mr. Bruce Eperly who has written about process-relational metaphysics [92]. It is the physician-patient relationship which implies a full religious and spiritual interaction. The Serbian rehabilitation medicine should base its work on these postulates.

Table 1

Clinical tools for measuring religiosity and spirituality

The first author (Ref)	The title of the article and a journal	Clinical tool
Koenig HG. (58)	• Religion, spirituality and health service use by older hospitalized patients. J Relig Health 2003; 42(4):301–14	• Daily spiritual experiences scale (DSE)
Hall DE. (120)	• Measuring religiousness in health research: review and critique. J Relig Health 2008;47	• Duke Religion Index (DUREL) •Spiritual beliefs inventory (SB1-15R)
Hyman C. (59)	• Definitions and evaluation of religion and spirituality items by religious professionals: a pilot study. J Relig Health 2006; 45(2): 264–82.	• Personal religiosity inventory • Religious maturity scale
Makros J. (77)	• The relationship between religion, spirituality, psychological adjustment, and quality of life among people with multiple sclerosis. J Relig. Health 2003;42(2):143–59.	• Spiritual experience index • The brief measure of religious coping (B-RCOPE)
Kretchy I. (80)	• Spiritual and religious beliefs: do they matter in the medication adherence behavior of hypertensive patients? BioPsychoSocial Med 2013; 7(1):15.	• Spiritual perspective scale (SPS)

From the anthropologic point of view physicians and patients are moving inside the hermeneutical cultural circle [93]. Considering that physiatry is the most sociable medical specialization, our rehabilitation programs are too narrowly designed. We have to introduce, in the first place, education in religiosity and spirituality to our resident (doctor) curricula. This would not be advocacy of "introducing the Christian-conservative revolution" [94] to the Serbian medical rehabilitation, this would be an attempt to correct a long-lasting injustice. If there is only one believer among rehabilitation patients, then a denial of the pastoral care to them is injustice. What should we do? Priests, sociologists and philosophers should be engaged in teaching religious and spiritual systems of the Eastern and Western world [95–97]. Priests or imams, who work in our hospitals and rehabilitation centers, would have to pass courses in clinical work and staff communication. These courses would be held by physicians, psychologists, and social workers who have experience and knowledge of religious and spiritual medical issues. The success of this new metaphysic in our rehabilitation medicine depends on rehabilitation leaders. They must work on the three strategic levels. Firstly, the doors of our clinical and rehabilitation centers should be open for a truly pastoral work. Secondly, a religious-spiritual anamnesis should become an obligatory part of the patient's history. Thirdly, imams, priests and clinicians together, should carry out clinical trials and publish their results.

Why and how philosophy of medicine in our rehabilitation medicine?

"Philosophy of medicine is not a school of philosophical thought but the end and purpose for which philosophy is applied to medical topics [98]." These are the words of Edmund D. Pellegrino, one of the pioneers in philosophy of medicine. Medicine is not merely ethics. There is also logic, philosophy of science, epistemology, aesthetic and metaphysic. The philosophy of medicine has an equal importance both for medical workers and patients. By means of studying philosophy of medicine, medical workers acquire an essential breadth of perception. Without this, it is hard to imagine medicine as a truly humanistic science. Besides medicine and philosophy of medicine, the experience of human suffering is covered by literature, history, philology and theology [97]. It has been proved that people, who participate in religious societies, recover more quickly after diseases and injuries and live longer [99]. Philosophy and religiosity rise our hope and contribute to our understanding of life [100]. It is well known that most people have spiritual life and want to discuss it [101]. Philosophy of medicine helps medical workers to pay attention to contextual factors in treatment and rehabilitation of patients [102]. Philosophy reminds us that medicine is love with all love's modalities [103–106]. Medical workers are fighters for human goodness. Philosophy and religion help us reach this goodness and keep peace with ourselves [107, 108]. From the philosophical point of view one thing is clear: philosophy should be introduced into medicine because the trouble with the meaning of life never ends [109]. Toynbee and Ikeda [110], the famous historian and the famous Buddhist, claim that the only way to avoid the quandaries of modern medicine is humanism based on religiosity. Seneka [111] said a long time ago: to be healthy means to be engaged in philosophy.

A need for humanities as a part of medical education has been noticed. Pellegrino [98] states that out of 125 medical schools in the USA, some kind of humanistic programs are offered in 118. It is considered that humanities stimulate free thinking, develop imagination and create an experience of closeness to being [98]. Humanities in the classical sense – *Studia humanitarum* – are the study of literature, philology, history and philosophy [98]. This kind of education is important for all medical workers, especially physicians. Especially in this "Post-Evangelical Period", [98] the Medical Faculty of the Military Medical Academy in Belgrade offers this kind of education. Medical workers do not have a broad enough approach to patients. Chaplains, priests, imams – as the religious-spiritual persons in medical staff - are not completely ready for the clinical work [112]. Because of that, elements of humanities have been introduced in the general practice and the rehabilitation medicine of developed countries. These are rehabilitation programs ennobled by music, songs, prayers and reading of artistic and religious literature [113]. Humanistic sciences help us develop love, firstly towards ourselves, secondly towards other persons and other opinions and attitudes [114, 115]. Philosophical education for members of the rehabilitation team is their elixir of youth. Philosophical spirit does not allow a medical worker to one day become an impassive cynic and a vain expert [116].

The essentials of humanities should be introduced into the specialist curricula of the Serbian rehabilitation studies – the philosophy of medicine in the first place. These courses should be held by philosophers, sociologist and physicians who have additional humanistic education. Not only would medical ethic be studied in these courses. Medical logic, epistemology, aesthetic, philosophy of science and – why not! – metaphysics could have importance for members of the rehabilitation team. The first task of these humanistic studies would be the study of life. Participants in these courses should study how to use a philosophical way of thinking in resolving certain medical problems. All humanistic issues, theoretically covered by these courses, would practically be checked throughout the case studies, a contextual attitude and a phronetic scientific frame [117]. "Damned be the one who teaches his son the Greek wisdom" [118]. We respect the Jewish religion but we cannot accept this quotation from the Talmud. The questions asked by old Greeks are also current today for members of the Serbian rehabilitation teams – alongside with the medical knowledge and skills. These questions are: what is good and what is evil; what is the truth and how can we recognize the truth; what is righteousness; what is causality; what is the cosmos and where does it expand; what is the role of language and finally, and should one be an honest man [11].

Conclusion

New metaphysics of Serbian rehabilitation medicine is the struggle against alienation, inaccessibility, superficiality

and dehumanization of this splendid medical specialization. We need education in humanities for all the members of the rehabilitation team. Introduction of humanities in Serbian rehabilitation medicine is the introduction of religiosity, spirituality and philosophy of medicine in daily rehabilitation. "Oh faithless generation, how long shall I be with you [119]?" Because of that, believe in God, believe in man and let us be on our way.

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CASE REPORTS

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Pheochromocytoma of the urinary bladder – A case report

Feohromocitom mokraćne bešike

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Abstract

Introduction. Pheochromocytoma of the urinary bladder is a rare tumor and presents less than 0.06% of all urinary bladder tumors. **Case report.** We presented a 49-year-old female patient with a history of daily paroxysmal hypertension accompanied with flushing of the face and upper chest, palpitations and excessive sweating prior to micturition. Ultrasonography reported a 3 cm bladder wall tumor. The ^{131}I-metaiodobenzylguanidine (^{131}I-MIBG) scan showed a pathological isotope accumulation in the projection of the bladder. The patient underwent a partial cystectomy. One year following the operation the patient was normotensive and without recurrence. **Conclusion.** The most efficient treatment option for bladder pheochromocytoma is surgical resection. The most important fact in the diagnostics is suspicion on this rare condition.

Key words:
pneochromocytoma; urinary bladder neoplasms; ultrasonography; radionuclide imaging; urologic surgical procedures.

Apstrakt

Uvod. Feohromocitom mokraćne bešike je veoma redak i javlja se kod manje od 0,06% svih tumora mokraćne bešike. **Prikaz bolesnika.** Prikazali smo bolesnicu, staru 49 godina, sa svakodnevnom paroksizmalnom hipertenzijom praćenom crvenilom lica i gornjeg dela grudnog koša, lupanjem srca i preznojavanjem koji su prethodili aktu mokrenja. Ultrasonografski, viđen je tumor mokraćne bešike, promera 3 cm. Metajod-benzil-guanidin (MIBG) sken pokazao je patološku akumulaciju izotopa u projekciji mokraćne bešike. Bolesnici je učinjena parcijalna cistektomija. Godinu dana nakon operacije, bolesnica je bila normotenzivna, bez recidiva tumora u mokraćnoj bešici. **Zaključak.** Najefikasniji vid lečenja feohromocitoma mokraćne bešike je njegovo hirurško uklanjanje. Najvažnije u postavljanju dijagnoze je to da lekar posumnja na ovo retko oboljenje.

Ključne reči:
feohromocitom; mokraćna bešika, neoplazme; ultrasonografija; scintigrafija; hirurgija, urološka, procedure.

Introduction

Pheochromocytoma of the urinary bladder is a rare tumor which presents less than 0.06% of all urinary bladder tumors and less than 1% of all pheochromocytomas [1, 2]. They are tumors of the chromaffin tissue of the sympathetic nervous system of the urinary bladder wall [3]. They are most frequently derived from the adrenal medulla and in approximately 10% of cases are extraadrenal [4]. In the genitourinary tract the most frequent localization is the bladder (79.2%), urethra (12.7%), renal pelvis (4.9%) and the ureter (3.2%) [2]. Aside from the localization features the diagnosis and treatment of these tumors is not much different than pheochromocytomas of other localizations.

Case report

We presented a 49-year-old female patient with a history of daily paroxysmal hypertension (from 220/130 mmHg), accompanied with flushing of the face and upper chest, palpitations and excessive sweating. The paroxysmal hypertension was preceded by morning urination. Ten minutes following the attack there was a spontaneous decrease of the blood pressure. Similar lower intensity episodes which the patient subjectively better tolerated were reported occasionally during the day after voiding. The concentration of vanillylmandelic acid (VMA) in the 24 hour urine was in two occasions in normal range. Also, normal levels of epinephrine and norepinephrine were recorded.

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Ultrasonography of the lower pelvis reported a 3.2 × 2.8 × 1.8 cm well vascularized, clearly defined tumor localized on the edge of the left lateral and anterior wall of the bladder (Figure 1). Computed tomography (CT) of the chest, abdomen and lower pelvis showed a round mass approximately 1 cm behind and under the main bronchi that can be by differential diagnosis a ganglion or lymph node. Also there was an oval well vascularized mass, 2.3 × 3.4 cm in diameter on the margin of the anterior and left lateral wall (Figure 2). The ^{131}I-metaiodobenzylguanidine (MIBG) scan showed a pathological isotope accumulation in the projection of the bladder (Figure 3). The patient underwent partial cystectomy. The tumor was identified in the bladder wall covered by intact mucosa (Figure 4). During the operation a few hypertensive episodes occurred that were revolved. No intraoperative or postoperative complications were registered.

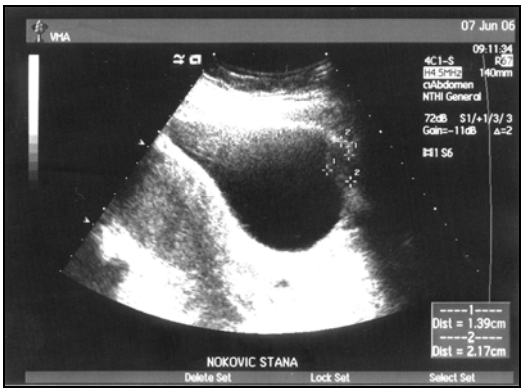


Fig. 1 – Ultrasonography shows a tumor mass at the edge of the bladder wall.

Fig. 2 – Computed tomography scan shows a tumor mass at the edge of the anterior and the left lateral bladder wall 2.5 × 3.5 cm in diameter.

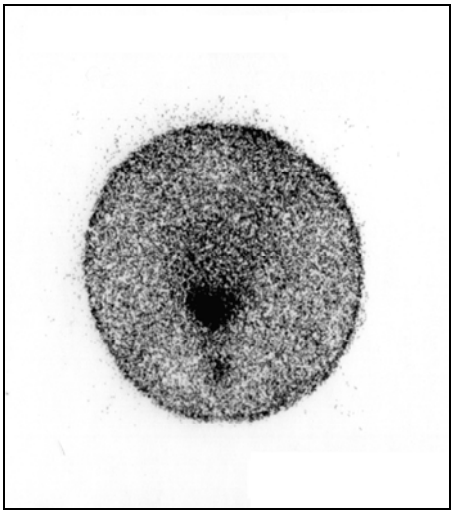
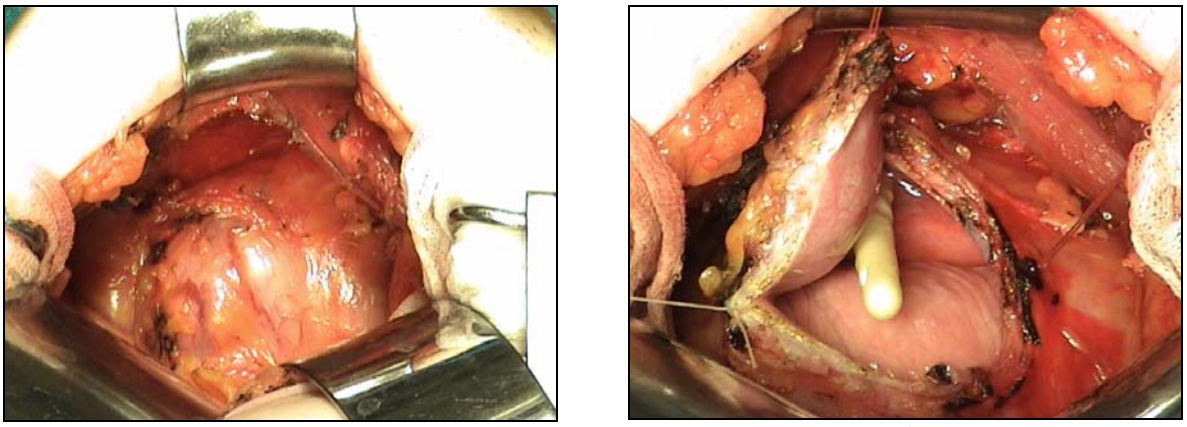


Fig. 3 – The ^{131}I-metaiodobenzylguanidine (MIBG) scan shows pathological isotope acumulation in bladder.



A) **B)**

Fig. 4 – A) Urinary bladder; B) Resection of the urinary paraganglioma.

The patient was discharged on the day 9 after surgery. Pheochromocytoma of the urinary bladder was confirmed by pathological and immunochemical examination. One year following the operation the patient was normotensive and without recurrence. The MIBG scan showed no pathological accumulation of the isotope.

Discussion

The first case of pheochromocytoma of the bladder was published by Zimmerman et al. [5] in 1953. and from that time about 200 cases have been published. Pheochromocytoma of the bladder can be diagnosed in children and the elderly, at the age of 10 to 80 years [6, 7]. It is more common in the female population [8–10]. The localization of these tumors differs in various studies. By some authors they are solitary tumors that most frequently arise on the dome or trigone of the bladder, while by other authors they are more often on the lateral bladder walls [9, 11]. Approximately 10% of urinary bladder pheochromocytomas are malignant [2]. Some authors consider invasion into surrounding organs and tissues or lymph node involvement as a criteria for malignancy [12, 13]. The presence of necrosis, angiolymphatic invasion, increase of mitosis and DNA ploidia indicate the malignant potential of the pheochromocytoma [1, 5, 14–19]. The current literature reported clinical signs range from the micturition attacks of headaches, palpitations, visual disturbances, tachycardia, sustained or paroxysmal hypertension, hematuria, dysuria, suprapubic pain or sweating after voiding [20, 21]. In around 17% of cases tumors are hormone inactive with practically no symptoms or can be presented only by pain or hematuria [13, 19, 20]. Only 40% of cases are preoperatively diagnosed, and the majority got adequate diagnosis after bladder surgery [21]. The suspicion on bladder pheochromocytoma with the presence of the characteristic symptoms should be investigated using imaging techniques (ultrasonography, CT) measurement of hormonal metabolites, and visualization *via* cystoscopy [21–23]. MIBG scan is highly recommended in determining the localization and diagnosis of pheochromocytoma. Its specificity is very high (nearly 100%) [24]. Biochemical measurements of urine/plasma catecholamine's and their metabolites are mandatory, such as measurement of norepinephrine 3 hours prior and after voiding [15, 21]. The most efficient treatment option for pheochromocytomas is surgical resection. The preoperatively confirmed diagnosis and adequate preparation facilitate the safety of surgical treatment [25]. Partial cystectomy is the first choice option [8, 9, 21, 24]. There are studies where tumors were treated by transurethral resection with or without hypertension attacks and recurrence, especially tumors that were not preoperatively diagnosed as pheochromocytomas [26, 27]. Nowadays, these tumors are treated by laparascopy or by robot assisted partial cystectomy with pelvic lymph node resection in the centers of excellences [28–30].

In the follow-up for benign tumors it is recommended to do the biochemical measurements of urine/plasma catecholamine's and their metabolites every 3–6 months and ^{131}I-MIBG and CT scan annually [15]. Patients with malignant tumors should have a monthly check of catheholamine levels and imaging studies twice a year [31]. Long term follow-ups are necessary because there are published cases with local recurrence and development of metastasis decades following surgical treatment [32].

Conclusion

Paraganglioma of the urinary bladder is a very rare tumor. When suspected the diagnosis can be reliably confirmed or excluded almost in all of the cases. Surgical resection is the standard treatment modality and requires the same preparation as in pheochromocytomas of different localizations. Almost all of the patients with benign tumors can be cured by surgery. The surgical mortality for all of the pheochromocytomas is around 2–3% with an experienced anesthesiologist and the surgeon. The histological confirmation of the tumor must be completed with immunohistochemical examination. Because of frequent recurrence, especially in malignant pheochromocytomas there must be a long term follow-up of these patients. The optimal treatment of these patients must be reserved for highly specialized medical centers.

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CASE REPORT

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A series of drawings of a patient with schizophrenia-like psychosis associated with epilepsy: captured illustration of multifaced self-expression

Serija crteža bolesnice sa psihozom sličnom shizofreniji udruženom sa epilepsijom: zabeležena ilustracija različitih oblika samoizražavanja

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Abstract

Introduction. Drawings may give an insight into the complex mental process, however they have been underutilized in patients with psychosis associated with epilepsy. **Case report.** A 33-year-old, right handed female, diagnosed with schizophrenia-like psychosis associated with epilepsy, was treated in the Day Unit due to psychosocial rehabilitation. Besides other treatments, the patient underwent group analysis of drawings once a week. Qualitative analysis of the form and content of drawings made by free associations were performed. The varying of size and motives with vivid colours and the curve of perspective were documentated in three figures, followed by the last drawing which was without such elements. **Conclusion.** A series of drawings of patients with psychosis associated with epilepsy is needed to record a possible variations and disturbance of the immediate mental process. Group sessions may additionally contribute to diminish stigma and enhance psychosocial reintegration.

Key words:
psychotic disorders; epilepsy; comorbidity; ambulatory care; art therapy; psychology.

Apstrakt

Uvod. Crteži mogu pružiti uvid u složeni mentalni proces, ali su nedovoljno primenjeni kod bolesnika sa psihozom udruženom sa epilepsijom. **Prikaz bolesnika.** Žena, stara 33 godine, dešnjak, sa postavljenom dijagnozom psihoze slične shizofreniji udružene sa epilepsijom, lečena je u dnevnoj bolnici radi psihosocijalne rehabilitacije. Pored ostalih oblika integrativnog lečenja bolesnika, bila je uključena u grupnu analizu crteža koja se odvijala jednom nedeljno. Izvršena je kvalitativna analiza oblika i sadržaja crteža koji su nastali prema slobodnim asocijacijama. Variranje veličine i motiva crteža sa živim bojama i zakrivljenom perspektivom zabeleženo je kod tri crteža, dok kod poslednjeg nisu bili prisutni ti elementi. **Zaključak.** Neophodna je serija crteža kod osoba sa psihozom sličnom shizofreniji udruženom sa epilepsijom da se registruju moguće varijacije i poremećaji trenutnog mentalnog procesa. Grupne sesije dodatno mogu doprineti smanjenju stigme i olakšati psihosocijalnu reintegraciju ovih bolesnika sa komorbidnim poremećajima.

Ključne reči:
psihotički poremećaji; epilepsija; komorbiditet; lečenje, ambulantno; lečenje umetnošću; psihosocijalni faktori.

Introduction

Epilepsy is related to numerous psychological challenges and may impair the overall emotional, cognitive, behavioral and social functioning among affected persons with many underlying causes [1]. In accordance with the International Classification of Mental and Behavioral Disorders (ICD-10) schizophrenia-like psychosis associated with epilepsy appears in clear consciousness, is not directly linked to the ictus and persistent or recurrent delusions, hallucinations, thought disorder and behavioral abnormalities may be present [2]. The psychiatric symptomatology in epileptic patients was remarkably frequently similar to schizophrenia noted in non-epileptic patients [3]. After epilepsy was diagnosed, chronic interictal psychoses *de novo* developed with prevalence and average annual incidence of 6.0% *vs* 0.38%, respectively [4].

Postictal and chronic interictal psychoses require psychopharmacological and multidisciplinary management [5]. Psychological interventions may be used combined in treatment of epilepsy to reduce the seizure frequency and impro-

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ve the quality of life [6]. To explore the artwork produced by patients with epilepsy rare controlled studies were performed [7]. Drawing is a complex neurological task and may give an insight into mental processes and neuropsychological deficit, but it have been underutilized in patients with epilepsy [8]. There have been a few reports showing that art therapy focus groups may enhance the self-expression of young persons and children with epilepsy [9].

A qualitative analysis of a series of drawings of a patient with psychosis associated with epilepsy as an illustration of self-expression is presented.

Case report

A 33-year-old, right handed female of elementary school educational background, was diagnosed with schizophrenia-like psychosis associated with epilepsy. She was referred to the Day Unit of our Clinic of Psychiatry due to psychosocial rehabilitation. The diagnose of psychosis was established according the ICD 10. The data were obtained from medical records, the history of the patient and her mother, as well as clinical examination. Epilepsy was diagnosed when the patient was 15 years old. The patient started an antiepileptic therapy, but in the beginning epilepsy was not well controlled, thus the patient experienced amnesic episodes and felt sleepy. So, her school performance achievement was poor and she was labeled as epileptic person by her school peers who avoided her. After epilepsy occurrence, parents controlled her day and night. They worried that she could fall and get hurt. She had no siblings, and her mother, a housewife, was devoted to her. Two years later she was hospitalized due to the first episode of psychosis and the antipsychotic was added to antiepileptic medication. She continued out-patient treatment by the neurologists and the psychiatrist. There was the history of tongue bite. However, a better control of epilepsy was achieved for the last three years and seizures with *grand mal* manifestations were not observed, but psychotic symptoms became more prominent. Despite it, she continued to be afraid of going out without her mother. She spent most time at home and rarely went out solely. She experienced paranoid delusions and hallucinations. She had no head injuries. There was no history of focal weakness and no family history of seizures recorded. The patient has never used the psychoactive substances except caffeine, that has consumed for 15 years.

Pre-treatment assessments were done by the use of Mini Mental State Examination (MMSE, Folstein) with the score 24 of 30 that revealed a mild dysfunction of concentration and attention, visual and verbal memory and of perceptuomotor functioning were presented. The assessment was performed by using the Positive and Negative Syndrome Scale (PANSS). The PANSS total score was 57 (negative subscale score 16, positive subscale score 19 and general symptoms score 22). The patient gave informed consent on admission and Ethic approval was also obtained.

Integrative day treatment consisting of group psychotherapy three times a week and everyday occupational and recreative activities were applied. The patient participated group analysis of drawings once a week. The heterogenous group consisted of patients with various mental disorders. The patients were asked to draw by free associations and free choice of colours. The same size of paper was offered. The drawings were exibited in the group setting and, after voting, there was group discussion about them. Exploring the form and content of drawings series and group protocol analyses were done. For the first two drawings the presented case prepared by herself on a half of standard format of paper. She made the two similar motives consisting of the mosaic partially filled by vivid colours (Figures 1 and 2).

Fig. 1 – Mosaic partially filled by vivid colours (1/2 paper sheet).

Fig. 2 – Similar picture to the first one (1/2 paper sheet).

She avoided to discuss about them. On the next session she chose double size of paper and titled it "Town at night" (Figure 3). She used only two colours (black and yellow) with great contrast and with numerous squaers for windows. Other patients liked her work and asked her about the perspective in the left corner of the drawing, but she told that there was not enough place for the tree. She named the last drawing "A boy with the guitare" (Figure 4) and explained that she had the guitare at home and played sometimes it. Following completion of day treatment the repeated PANSS score was 48. However, the patient was positive about her participation in the group analysis of drawings and in other group activities, suggesting a qualitative benefit from the treatment. The team members observed that she slightly diminished avoidance with less fear and spoke with other patients not only during therapies, but also between structured activities.

Discussion

Considering the drawings of the presented patient with epilepsy and psychosis, some artistic features different from the drawings of other patients with psychosis were noticed [10]. The motives with intensive lines and mosaics of geometric details are repeated, but only partly filled by colours despite the double diminished size of the paper. It might illustrated the great anxiety and "horror vacui" which were described at art works of psychotics patients. However, these characteristics of the drawings of the presented case are similar to visual illusions described by some patients with epilepsy: increase or reduction in the size of objects of the real world, a dis-

Fig. 3 – "Town at night" with two contrast colours – black and yellow (2 x paper sheet).

Fig. 4 – "The boy with the guitare" (1 paper sheet).

tortion of proportions or contours, i.e. metamorphopsia, intensified appearance of colours (dyschromatopsia). These phenomena could be elicited by electrical stimulation of the occipital or the left temporolateral cortex affecting either a part or the whole visual field [11].

The presented patients spoke rarely in the group during the first two weeks. She showed dysfunction in personal, social, and cognitive areas, mild dysfunction assessed by clinical rating scales. After that, she partly adjusted to group activities and the setting in the day hospital. The day treatment program was the first structured psychosocial therapeutic and rehabilitation process that she was involved after many years. Besides her paranoid delusions, her social skills were poor, but the confidence in the treatment setting developed. This advantage was illustrated by the large size of paper she chosen to finish all the parts of the third drawing. The curving of perspective in one part of the drawing (Figure 3) could suggest that in the period of drawing she experienced some visual illusion. Some studies have shown that in the temporal lobe epilepsy seizures affect all the aspects of cognitive functioning [12]. The psychosis associated with epilepsy, especially regarding the temporal lobe origin, differs from schizophrenia by the preservation of affect and prominent paranoid ideation [13].

The last drawing of the presented patient expressed the advantage of her integrative treatment (Figure 4) by the normal paper size and content with humane figure in relaxation activity (playing the guitar). This varying of the size and motives of drawings could be speculated due to subclinical paroxysmal brain activities rather that they were only the products of the psychotic process, but repeated or continued electroencephalogram (EEG) were not obtained as evidences.

It has been reported that drawing may give insight into neurocognitive processes [14] and some specific artistic features by epilepsy syndrome were noted [8].

Epilepsy and psychosis comorbidity have great impact on a patient's lifestyle and therefore should be treated, but the side-effects of long-term medication have to be considered to.

Compared to other type of epilepsy free drawing is most sensitive to complex partial seizures with temporal focus [7]. It is a rare case, but drawing-induced epilepsy has been described with the focus in the right frontal lobe parietally spreaded, which is in accord with the current cognitive and neural network models of drawing [15].

The epilepsy of the presented patients was controlled with medication without registered seizures for a three years. She had not experienced, but was at risk of unacceptable side-effects from medication. An integrative neurological and psychiatry treatment approach was necessary due to psychotic comorbidity [1].

Epilepsy-associated stigma in adolescents and adults lead to social withdrawal and loneliness worldwide [16], but art work may arise understanding of these patients [17].

In this paper no generalisations was possible since it was a case study. One of the limitations of the case report is that the exact intellectual and cognitive functioning of the patient prior to the onset of epilepsy could not be elicited, so, the exact deterioration of the cognitive and intellectual impairment could not be ascertained. The reassessment by neuroradiological examination and EEG might highlight the underlying process.

Conclusion

Drawings may be useful self-expression of the unique self-concept of a patient with epilepsy and psychosis and may also give an insight into their perceptions, thoughts and feelings about themselves and outer world. A series of drawings for each patient is needed to record a possible variation and disturbance of immediate neurocognitive processes. Group sessions may additionally contribute to diminish stigma and enhance psychosocial reintegration of these patients.

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Clinical use of optical coherence tomography and fractional flow reserve

Klinička primena optičke koherentne tomografije i frakcione protočne rezerve

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Abstract

Introduction. The aim of each diagnostic method is to serve as a guide in deciding about the right patient treatment. During myocardial revascularization the decision to perform revascularization is usually not easy to make, especially in case of borderline stenosis. It has been proven that it is not enough to base morphological evaluation of coronary artery vessel stenosis solely on angiography. It is necessary to include additional modern diagnostic methods for functional analysis and detailed morphological analysis using fractional flow reserve (FFR) and optical coherence tomography (OCT), respectively. **Case reports.** In the first case report we showed the significance of morphological analysis using OCT and proved that it was not lumen stenosis. The second and the third case reports showed the complementarity between functional analysis (FFR) and morphological analysis (OCT) of stenosis in solving a complex coronary disease. The fourth case report showed the significance of OCT in dealing with the recurrent stent restenosis. **Conclusion.** By these short case reports we confirmed that percutaneous coronary intervention (PCI) guided by angiography is definitely not enough in deciding about myocardial revascularization especially in patients with a complex coronary disease. In certain cases FFR and OCT procedures can be complementary methods and improve quality of revascularization, particularly in case of recurrent in-stent restenosis.

Key words:
myocardial revascularization; diagnostic techniques and procedures; tomography, optical coherence; diagnosis, differential; drug-eluting stents; stents.

Apstrakt

Uvod. Cilj svake dijagnostičke metode jeste da bude vodič u donošenju prave odluke za lečenje bolesnika. Prilikom revaskularizacije miokarda donošenje odluke o revaskularizaciji nije uvek lako, posebno u slučaju postojanja graničnih sužnja. Dokazano je da morfološka procena stenoze koronarnog krvnog suda nije dovoljna samo na osnovu angiografije, te je neophodna upotreba savremenih dijagnostičkih sredstava za funkcionalnu analizu (npr. procena frakcione protočne rezerve − FPR) i detaljniju morfološku analizu primenom optičke koherentne tomografije (OKT). **Prikazi bolesnika.** U prvom prikazu bolesnika pokazali smo značaj morfološke analize upotrebom OKT na osnovu koga se zaključuje da se ne radi o suženju lumena krvnog suda, u odnosu na samu angiografiju. U drugom i trećem prikazu bolesnika ukazali smo na komplementarnost funkcionalne analize (FFR) i morfološke analize stenoza u rešavanju kompleksne koronarne bolesti, dok smo u četvrtom prikazu ukazali na značaj OKT prilikom rešavanja recidivantnih restenoza stenta. **Zaključak.** Ovom kratkom serijom bolesnika potvrdili smo da angiografski vođena perkutana koronarna intervencija (PCI) svakako nije uvek dovoljna za donošenje odluke o revaskularizaciji krvnog suda, pogotovo kod bolesnika sa kompleksnom koronarnom bolesti. FFR i OKT u pojedinim slučajevima mogu biti komplementarne metode i poboljšati kvalitet revaskularizacije, pogotovo ako se radi o recidivantnim *in-stent* restenozama.

Ključne reči:
miokard, revaskularizacija; dijagnostičke tehnike i procedure; tomografija, optička, koherentna; dijagnoza, diferencijalna; stentovi, lekom obloženi; stentovi.

Introduction

Each diagnostic method serves as a guide in deciding about the right patient treatment. During myocardial revascularization the decision to perform revascularization is usually not easy to make especially in case of borderline stenosis. Accordingly, it has been proven that it is not enough to base morphological evaluation of coronary artery vessel stenosis

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solely on angiography. The main disadvantage of coronary angiography in estimation of lesion severity is that it is based on subjective decision grounded on the experience of the interventional cardiologist, and sometimes it is also difficult to present and analyze lesion from several different angles. So, in some cases it is necessary to include additional modern diagnostic methods, thus enabling better morphological evaluation of stenosis as well as the assessment of its hemodynamic significance [1–3].

Fractional flow reserve (FFR) is an index of physiological significance for coronary blood vessel stenosis. It is defined as the ratio of a maximal blood flow achievable in the stenotic coronary artery relative to the maximal flow in the same artery if it is normal [4]. According to current guidelines for myocardial revascularization, FFR evaluation is indicated in patients undergoing diagnostic coronary angiography without prior non-invasive functional testing in the presence of borderline lesions and also in patients with multivessel disease (Table 1) [5].

Case reports

Case one

A 64-year-old male patient was admitted to the Institute of Cardiovascular Diseases of Vojvodina due to anginal discomforts, positive stress test and suspected stent restenosis.

Namely, the patient underwent PCI for two DES in the *ramus interventricularis anterior* (RIA) segment in 2010. He was rehospitalized in 2011 when recoronarography was performed and, due to stent restenosis, percutaneous transluminal coronary angioplasty (PTCA) was performed. Coronarography, performed again in 2014, registered previous stents and persistent stenosis immediately behind the second stent.

OCT was performed to assess the stenosis which angiographically appeared as borderline (Figure 1) and to analyze previously deployed stents.

Table 1

Recommendations for the clinical value of intracoronary diagnostic techniques according to the current guidelines for myocardial revascularization

Recommendations	Class	Level
FFR to identify hemodynamically relevant coronary lesion(s) in stable patients when evidence of ischaemia is not available	I	A
FFR-guided PCI in patients with multi-vessel disease	IIa	B
OCT in selected patients to optimize stent implantation	IIb	C

FFR – fractional flow reserve; OCT – optical coherance tomography; PCI – percutaneous coronary intervention.

The FAME trial pointed out the advantage of FFR when deciding on the percutaneous coronary intervention (PCI) in reference to angiography (FAME I trial) used for drug-eluting stent (DES) implantation in patients with multivessel disease [6].

Optical coherence tomography (OCT) is a catheter-based invasive imaging system. Using light rather than ultrasound, OCT produces high-resolution *in vivo* images of coronary arteries and deployed stents. OCT is ideally suited to accurate detection of intraluminal structures. Plaque composition, including the presence of lipid pools and intraluminal thrombi, can also be determined. Also, this is the only technique capable of providing accurate measurements of the thickness of the fibrous cap and to detect even minor cap disruptions [5, 7–9].

Clinical indications and recommendations for OCT usage, except in selected patients to optimize stent implantation, are still not completely defined (Table 1). It still remains unclear when it should be used in clinical practice and whether its use would significantly improve the treatment. However, recent retrospective and observational studies show that clinical outcome can be improved when PCI is performed after OCT [10, 11].

The use of invasive hemodynamic (FFR) and morphological (OCT) diagnostic procedures in Cath Labs facilitates making the right decision on myocardial revascularization. It also enables assessment whether the optimal result is achieved after the stent implantation.

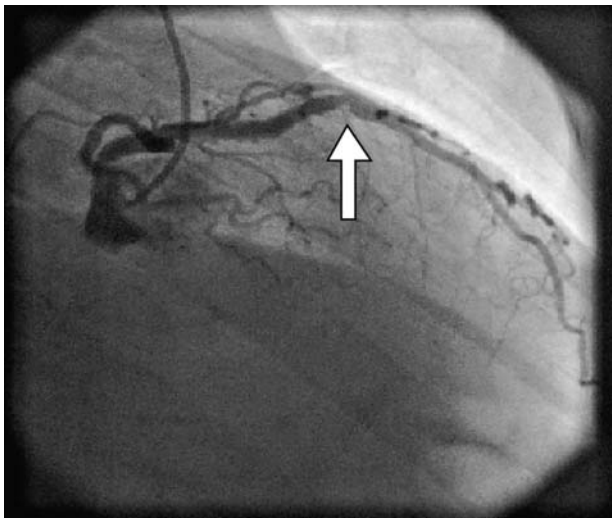

Fig. 1 − Angiography of acute coronary syndrome and suspected stenosis in the area behind the stent.

OCT showed that the deployed stents were well-expanded with a mild restenosis in some segments and with a small aneurysmal dilatation as a part of the artery remodeling after stent implantation (Figure 2). It also showed that it was not lumen stenosis behind the deployed stents, but a curve which probably

made it angiographically appeared as stenosis (Figure 2a). According to OCT finding there were no clear indications for PCI.

Case two

A 53-year-old male patient was admitted to the Institute of Cardiovascular Diseases of Vojvodina in 2014 as an elective for the invasive hemodynamic procedure based on positive stress test. In 2001 this patient underwent PCI with the placement of one bare-metal stent (BMS) at the proximal-medial segment of the RIA. Seven months afterwards the patient underwent recoronarography and PTCA of in-stent restenosis. In 2004 recoronarography was done again registering stent restenosis. It was followed by PTCA which gave optimal results. Due to the front wall non ST-segment elevation myocardial infarction (NSTEMI) the patient was rehospitalized again in 2011. Since it came to coronary disease progression, the patient underwent PCI of one DES distally from the previously deployed BMS. Because of the front wall STEMI induced by the restenosis in the area of BMS, the primary PCI was done in 2012 and DES was placed in RIA.

The final coronarography done in 2014 was presented in this paper. It registered several significant borderline stenoses along the RIA (Figure 3).

FFR analysis was done behind the distal stent giving the result of 0.65, and the repeated FFR analysis above the distal stent gave the result of 0.69. PTCA of proximal stents followed with the drug-eluting balloon (DEB). Control FFR analysis above the distal stent gave the result of 0.84, while FFR analysis below the distal stent resulted in 0.79. Therefore, a DES was implanted in that segment using the overlap technique. The final FFR of the distal area in reference to the deployed stents gave the result of 0.84 (Figure 4).

OCT analysis of the RIA was performed afterwards showing good stent expansion (Figure 5a) and the presence of a stable fibrous plaque with 50% stenosis, proximally from the deployed stents (Figure 5b). Since functional and morphological tests showed insignificant stenosis, it was decided not to perform PCI.

Case three

A 54-year-old male patient was transferred to our institution from the regional hospital for the invasive hemodynamic procedure. The patient was admitted to the regional hospital due to NSTEMI of the inferolateral region which was complicated by ventricular fibrilation. At one point ventricular fibrilation (VF) was stopped with a defibrillator.

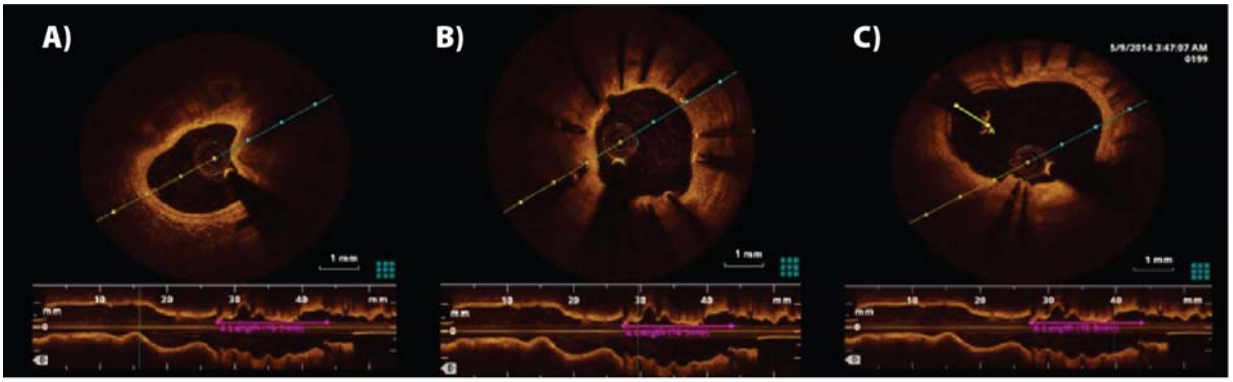

Fig. 2 − A) Optical coherence tomography (OCT) image of stenosis behind the stent; B) OCT image of a well-expanded stent with mild restenosis; C) Aneurysmal dilation of 0.98 mm as part of coronary artery remodeling after stent placement.

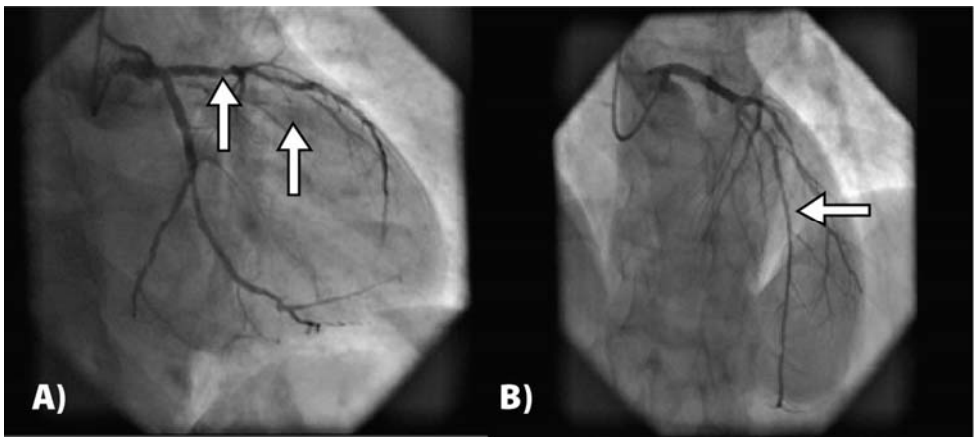

Fig. 3 − A) The first arrow showing stenosis above the previously deployed stents, while the second arrow shows the area of in-stent restenosis; B) The arrow showing peristent stenosis (immediately below the previously deployed stents) in the distal segment of the *ramus interventricularis anterior*.

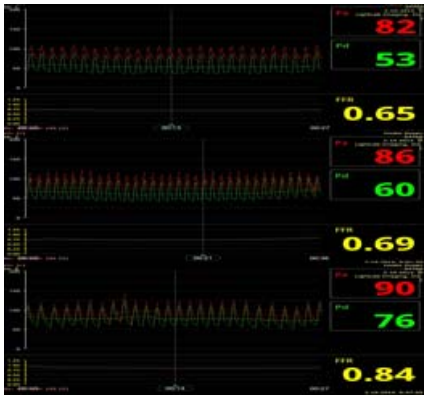

Fig. 4 − The first two and the final fractional flow reserve.

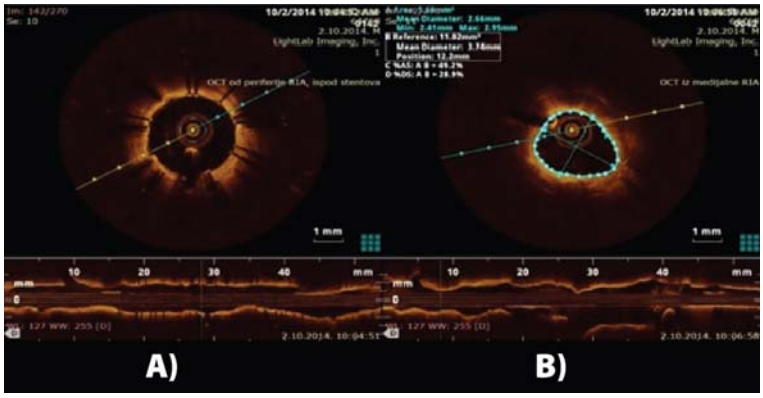

Fig. 5 − A) Optical coherence tomography (OCT) image showing good stent expansion; B) OCT image showing 50% stenosis in the area above the deployed stents.

The patient was transferred to our hopital after his condition was stabilized with medications. Coronarography, performed 18 days after the incident, registered occlusion in the proximal segment of *ramus circumflexus* (RCX) (Figure 6a) and, angiographically significant stenosis in the proximal segment of the RIA (Figure 6b). Figure 6c shows RCX after BMS implantation.

FFR on RIA was performed giving the result of 0.90 (Figure 7a).

To thoroughly assess stenosis and the plaque quality thought to be hemodynamically insignificant (angiographically estimated around 85%), OCT was performed. It showed that it was a stable calcified plaque with 72% stenosis, and lumen cross sectional area (CSA) stenosis of 3.54 mm^2 (Figure 7b).

Seven days after the intervention the control stress myocardial perfusion scintigraphy (SPECT) was done showing good perfusion in the vascular area of the RIA.

Case four

A 48-year-old male patient was admitted to the hospital as an elective based on positive stress test and suspected stent restenosis. This patient underwent primary PCI in October 2012 due to STEMI of the inferolateral region and two BMS

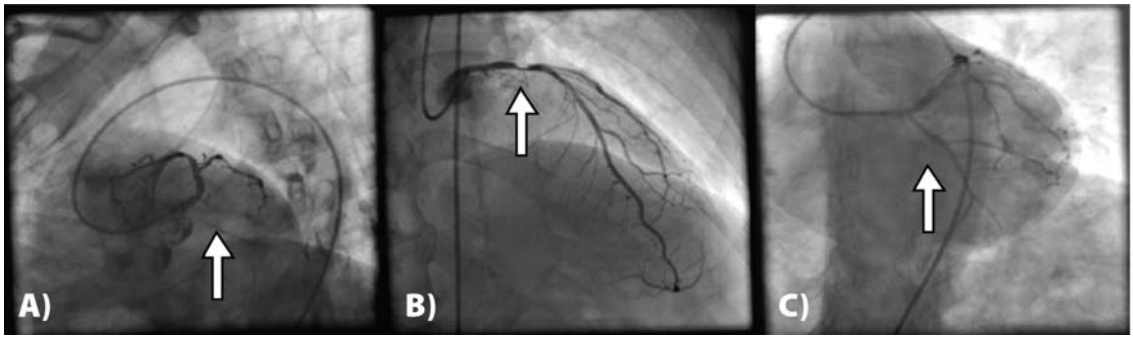

Fig. 6 − A) *Ramus circumflexus* (RCX) occlusion in proximal segment; B) Stenosis of proximal *Ramus interventricularis anterior* (RIA) segment; C) RCX after stent implantation.

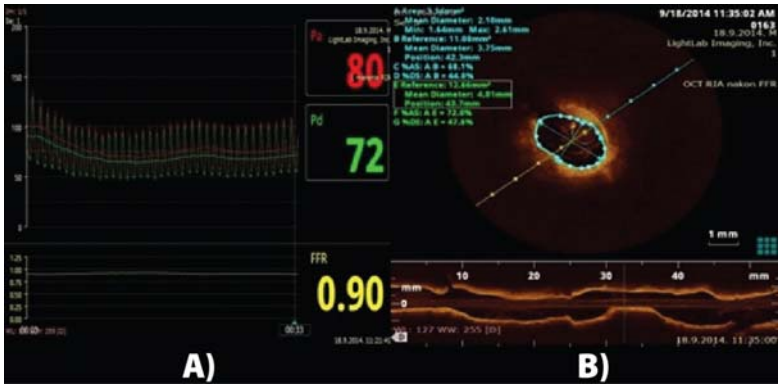

Fig. 7 − A) Fractional flow reserve (FFR) values of the *Ramus interventricularis anterior* (RIA) referential blood vessel diameter; B) Optical coherence tomography (OCT) analysis of the RIA shows 72% stenosis with cross sectional area (CSA) 3.54 mm^2.

were implanted in the right coronary artery (*arteria coronaria dextra* – ACD). In the second act of the same hospitalization PCI was performed and one BMS was placed in the RIA and one DES in the RCX. The patient was rehospitalized again in 2013 due to anginal discomforts. Recoronarography registered in-stent occlusion in ACD, while the RIA and RCX stents were perviously without any visible significant stenoses. During PCI two DES were implanted in ACD.

The suspicion of stent restenosis led to new recoronarography (6 months after the last PCI procedure) showing no signs of disease progression in the ACS system, while 90% restenosis was registered in the ACD in the proximal and medial segment, i.e. in the area of the previously deployed stents (Figure 8).

Balloon dilatation was performed due to restenosis in the medial segment of ACD while one DES was implanted in the proximal segment of ACD. OCT followed to check the PCI result. It showed that balloon dilatation did not give satisfactory results in certain segments (Figure 9a). Dilatation with a larger-diameter balloon was repeated until the optimal result was achieved (Figure 9b).

Control stress myocardial perfusion scintigraphy was performed 6 months after the last procedure because of the multiple restenoses in ACD giving a good result. It is our belief that OCT procedure helped us significantly showing that effects of balloon dilatation were not satisfactory. It resulted in repeated dilatation with a larger-diameter balloon until the optimal result was achieved.

Discussion

After coronarography it is not always easy to make decision on blood vessel revascularization. By these short case reports we pointed to the clinical use of FFR and OCT procedures as methods that enable more qualitative treatment of coronary disease in everyday practice.

Invasive functional assessment of stenosis has clearly defined its place in routine PCI procedures as was confirmed by the FAME trial. However, clear indications for the use of OCT in the everyday practice have not yet been established [6, 10].

OCT is a useful "imaging" method which enables better understanding of vascular biology of atherothrombosis and significantly assists in performing the PCI procedure [7]. Viceconte et al. [12] have shown that OCT can certainly be used when choosing a stent, as well as during stent positioning and expansion.

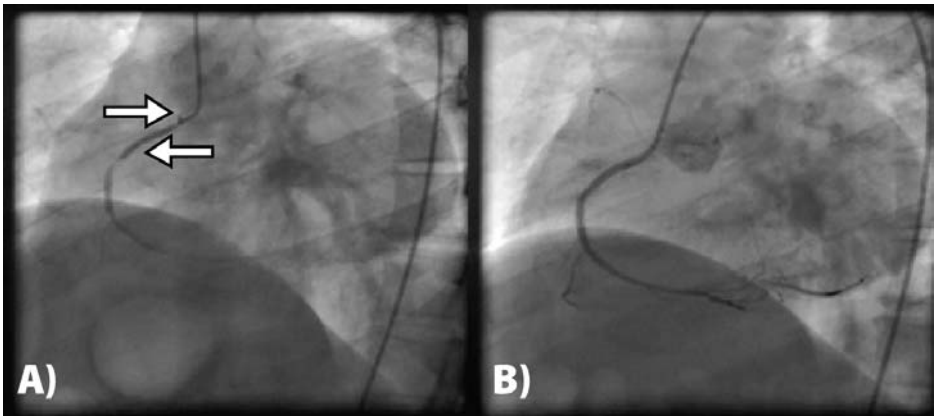

**Fig. 8 − A) Arrows showing restenosis in the previously deployed stents;
B) Final optimal result after the percutaneous coronary intervention (PCI) procedure deployed stents.**

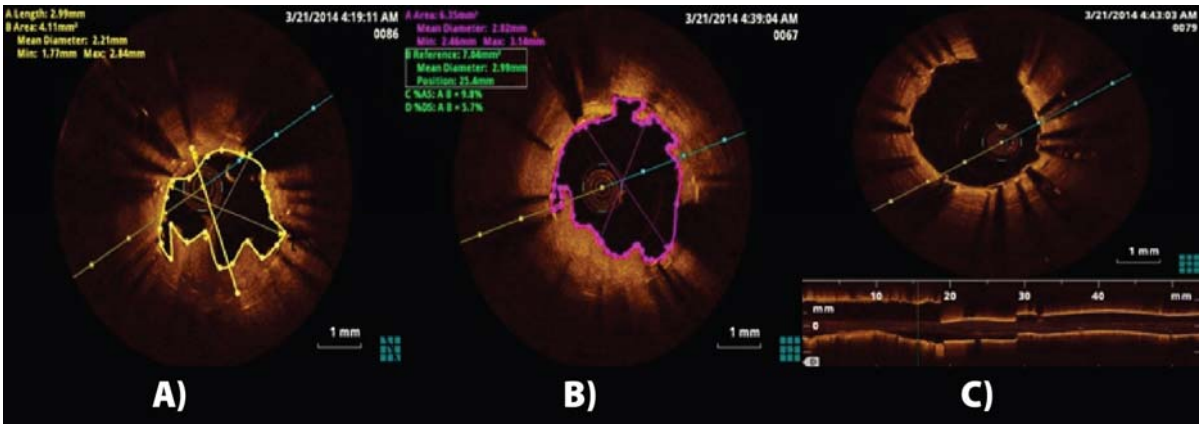

Fig. 9 − A) Image of insufficient postdilatation effect deployed stents, stent cross sectional area (CSA) stenosis 4.11 mm^2; B) A satisfactory result after postdilatation using a larger-diameter balloon, stents CSA stenosis 6.35 mm^2; C) A well-expanded stent.

Incomplete neointimal coverage after stenting is related to the increased risk of stent thrombosis. In their study Guagliumi et al. [13] and Finn et al. [14] and have shown that late thrombosis in a DES is related to uncovered stent struts registered by OCT.

A smaller study has shown that availability of morphological and functional data on stenosis received by FFR and OCT is of great significance especially in patients with multiple stenoses because it is impossible to decide what lesion to treat solely on FFR finding. Morphological OCT plaque analysis is significant when there exists suspicion on plaque rupture in the acute phase of coronary syndrome and FFR is not recommended [10].

OCT use proved to be highly significant in detecting a mechanism and severity of in-stent restenosis [15]. Secco et al. [16] showed in a study on 14 patients that the use of OCT in in-stent restenosis is of great significance. It enabled the use of larger diameter cutting balloon when the initial smaller diameter cutting balloon failed to give satisfactory results. It further enabled better expansion of a later deployed DES.

Early experiences with OCT-guided implantation of biodegradable vascular scaffolds (BVS) in dealing with complex lesions were promising [17].

We expect that the guidelines for the use of OCT in clinical practice will be more accurately defined when the FORZA study data are obtained [18].

In our study we presented a small series of patients with complex coronary disease in order to show the significance of FFR and OCT use in the routine clinical practice.

In the first case report it we showed that the angiographic finding can largely depend on blood vessel anatomy. Only after the OCT procedure performance it was clear that there were no stenosis but a blood vessel curve. Therefore, there was no need to perform FFR and PCI as was first considered during coronarography.

The third case report showed that it was not enough to base the decision on revascularization solely on angiography, although there had been angiographically registered 85% stenosis estimated by the experience of several interventional cardiologists, cardiac surgeons and computer programs (Radiant Dicom viewer and Microdicom).

After performing FFR we determined that it was not hemodynamically significant stenosis on the RIA, but we were not able to claim with certainty that it was a stable plaque until OCT was performed. After RCX recanalization we decided not to repeat FFR in the RIA because the result of intial measurement was 0.90, if the FFR result was below 0.90 we would have repeated FFR procedure. Based on our more than 10-year experience we do not repeat FFR in such cases. However, to be sure that RIA stenosis is insignificant we used other method as a control, so a week after the PCI procedure the patient underwent control SPECT which showed good perfusion in the vascular area of the RIA.

In the two reported patients there was recurrent stent restenosis. Using OCT we managed to perform satisfying balloon dilatation and stent implantation as well as to check afterwards whether the deployed stents were well-expanded, i.e. to exclude stent malposition. We believe that this precisely plays the crucial role in the prevention of new restenosis after DES implantation.

Conclusion

By these short case reports we confirmed that PCI guided by angiography is definitely not enough to decide on blood vessel revascularization especially in patients with a complex coronary disease.

In certain cases FFR and OCT procedures can be complementary methods and can improve the quality of revascularization, particularly in case of recurrent in-stent restenosis.

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PET/CT fusion in radiotherapy planning for lung cancer – Case reports

Fuzija PET/CT u planiranju radioterapije za karcinom pluća

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Abstract

Introduction. Application of imaging methods, namely computed tomography (CT), magnetic resonance imaging (MRI) and in recent years positron emission tomography/computed tomography (PET/CT), and the progress of computer technology have allowed the construction of effective computed systems for treatment planning (TPS) and introducing the concept of virtual simulation in 3D conformal radiotherapy planning. **Case report.** We hereby presented two patients with the diagnosis of non-small cell lung cancer who did PET/CT examination. Both patients had surgery earlier and local recidives are diagnosed with PET/CT. PET/CT of the first patient described the focus of intense ^{18}F-fluorodeoxyglucose (^{18}FDG) accumulation 2.99 × 2.9 × 2.1cm in diameter in the projection of soft-tissue volume in the left corner, at operating clips height, corresponding to metabolically active recurrence of the tumor. Mediastinum and right lung parenchyma were without focal accumulation of ^{18}FDG. Control PET/CT after 3 months was without detectable focus of intense pathological ^{18}FDG accumulation – good therapeutic response, (metabolic disease remission). On the other hand, in the second case PET/CT showed a focus of intense ^{18}FDG accumulation screening in the scar tissue of the apical part of the right lung, 20 × 16 mm, corresponding to metabolically active tumor recurrence. In the lung parenchyma on the left and in the mediastinum no visible focus of intense ^{18}FDG accumulation was descrbed. Radiography included using 3D conformal radiotherapy with fusion PET/CT scan and CT simulations. **Conclusion.** PET/CT provides important information for planning conformal radiotherapy, especially in dose escalation, sparing of organ at risk and better locoregional control of the disease.

Key words:
carcinoma, non-small-cell lung; radiotherapy, conformal; positron-emission tomography.

Apstrakt

Uvod. Primena visokorezolutivnih metoda snimanja, npr. kompjuterizovane tomografije (CT), magnetno-rezonantnog snimanja (MRI) I, poslednjih godina, pozitron-emisiona tomografija-kompjuterizovana tomografija (PET-CT), kao i konstrukcija efikasnih kompjuterskih sistema za planiranje radioterapije (TPS) omogućili su uvođenje koncepta virtuelne simulacije u planiranju 3D konformalne radioterapije (3D CRT). **Prikaz bolesnika.** U radu su prikazana dva bolesnika sa nemikroćelijskim karcinomom bronha kod kojih je načinjen PET/CT pregled. Oba bolesnika ranije su operisana a lokalni recidiv je otkriven primenom PET/CT pregleda. Nalazom PET/CT kod prvog bolesnika ustanovljeno je nakupljanje ^{18}F-fluorodeoksiglakoze (^{18}FDG), prečnika 2,9 × 2,9 × 2,1 cm u projekciji mekih tkiva u levom uglu u predelu operativnih klipseva. Opisana promena odgovarala je metabolički aktivnom recidivu tumora. Medijastinum i plućni parenhim desno bili su bez fokalne akumulacije ^{18}FDG. Kod drugog bolesnika ustanovljen je fokus intenzivnog nakupljanja u regiji ožiljka apikalnog segmenta desnog plućnog krila promera 20 × 16 mm, koji je prevashodno odgovarao metabolički aktivnom recidivu tumora. U parenhimu pluća sa leve strane i na medijstinumu sa leve strane nije ustanovljeno prisustvo aktivnih fokusa. Radioterapijski tretman planiran je 3D konformalnom tehnikom pomoću fuzije PET/CT nalaza i CT za planiranje radioterapije. **Zaključak.** PET/CT obezbeđuje važne informacije za planiranje radioterapije, posebno za eskalaciju doze, poštede rizičnih organa i bolje lokoregionalne kontrole bolesti.

Ključne reči:
pluća, nesitnoćelijski karcinom; radioterapija, konformalna; tomografija, positron-emisiona.

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Introduction

Application of imaging methods, namely computed tomography (CT), magnetic resonance imaging (MRI) and, in recent years, positron emission tomography-computed tomography (PET-CT), and the progress of computer technology have allowed the construction of effective computerized systems for treatment planning (TPS) and introducing the concept of virtual simulation in 3D conformal radiotherapy planning [1, 2].

The concept of 2D radiotherapy (defining the limits of the radiological fields according to bone structures) is now reserved for palliative radiotherapy. The golden standard for modern radiotherapy is 3D conformal radiotherapy. Planning 3D conformal radiotherapy based on CT simulation, which computer system makes into a 3D anatomical model reconstruction resulting in a virtual patient. The advantages of this method are complex analysis of the spatial relationship between the tumor and organs at risk, more precise definition of the tumor in all three dimensions, and air dose distribution whose shape corresponds to the form of tumor volume [3].

Furthermore, 3D conformal planning allows individualization in modeling the shape of the radiation field. This can be done because of using cone beams with different spatial orientation angles so that their central axes are not in the same plane [3, 4].

Isodose curves with high dose radiation are adjusted to the shape of the target volume which makes the best option of the dose distribution.

Application of conformal radiotherapy allows: dose escalation, better sparing of surrounding tissue, better locoregional control of the disease, lower rate of morbidity, i.e. complications in general. Imaging methods as CT and MRI have become the standard in the diagnosis, evaluation of the treatment answer, radiotherapy planning − target volume delineation. CT provides information about electronic density of the tissue, and they are used as the basis for calculating the tumor dose radiation in 3D RT [4, 5].

Nonetheless, in 3D conformal radiotherapy planning problems are often present. For example, sometimes it is impossible to distinguish benign lymphadenomegaly, or a seemingly normal lymph node. The explanation is in the poor contrast between the tumor and the surrounding area. Furthermore, criteria for involvement of the lymph node − size, structure − have not yet been defined. Subclinical, microscopic tumor spread around the gross tumor volume, is still based on empirical experience. It should be noted that inadequate margins around the tumor cannot be compensated with escalation in exposure [6].

The fusion of images in the system for planning with PET/CT is a new dimension in 3D radiotherapy planning. Image fusion of PET and CT results in the symbiosis between metabolically detected tumors (PET) and its anatomical and morphological boundaries (CT), and allows functional and biological evaluation of the tumor. The essential information for the definition of the target volume and organs at risk can be found on PET/CT images, but for delineation of the tumor this also must be projected to CT images. Biological processes that could be used are glucose metabolism, cell proliferation and hypoxia [7, 8].

There are various methods of PET and CT fusion. The most widely accepted is "visual fusion", where two scans are placed side by side, to compare and then integrate overlap [6]. This is performed by using hardware fusion and transport images in Focal Xi Over.4.6.

Case report

Case 1

A 74-year-old male patient had left pneumonectomy 15 months ago. Histopathological analysis confirmed that it was squamous cell carcinoma of the bronchus.

Control CT of the chest showed a tumor mass in the left tracheobronchial corner, described as a lymph node with perinodal growth (Figure 1).

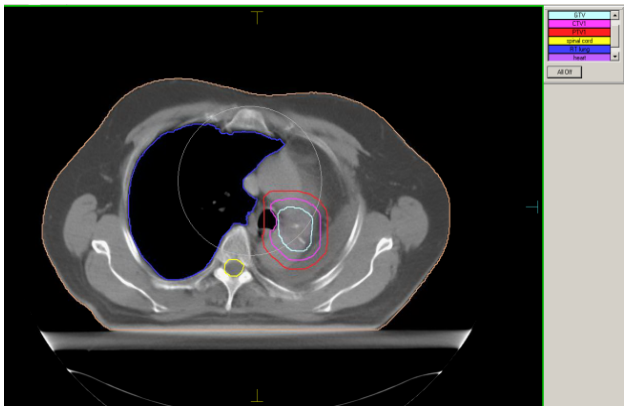

Fig. 1 – In the left corner the tracheobronchial tumor mass corresponding lymph node growth was seen.

PET/CT described the focus of intense ^{18}F-fluorodeoxyglucose (^{18}FDG) accumulation, 2.9 × 2.9 × 2.1 cm in diameter, in the projection of soft tissue volume in the left corner, at the operating clips height, corresponding to metabolically active recurrence of the tumor. The mediastinum and the right lung parenchyma were without focal accumulation of ^{18}FDG (Dg: *Recidivum anguli reg. tracheobronchialis*) (Figure 2).

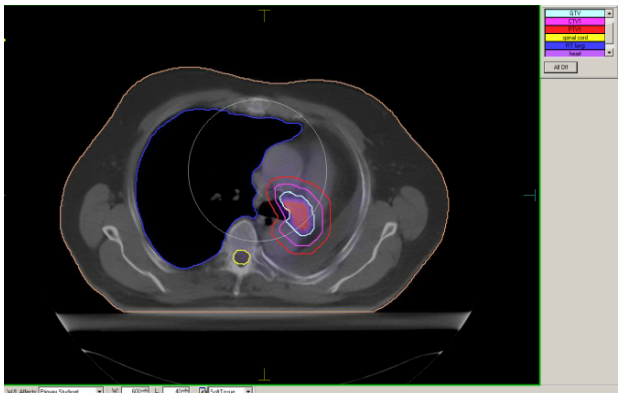

Fig. 2 – Positron emission tomography/computed tomography (PET/CT) − solitary focus of intense ^{18}F-fluorodeoxyglucose (^{18}FDG) accumulation.

We made a radiotherapy plan using 3D conformal radiotherapy with fusion PET/CT scan and CT simulations. The daily dose was 60 Gy/30 fractons with 2 Gy. Control PET/CT after 3 months was without detectable focus of intense pathological ^{18}FDG accumulation – good therapeutic response – metabolic disease remission (Figure 3).

Case 2

A 53-year-old female patient in 2002 had right upper lobectomy with resection of the parietal pleura. Histopathological analysis confirmed adenocarcinoma. After receiving postoperative chemotherapy and radiotherapy the patient was monitored through regular controls.

CT of the chest done on March 16, 2012 described an oval soft tissue mass in the scar tissue of the apical part of the right lung, 20 × 16 mm, no signs of mediastinal and hilar lymphadenomegalia or increase in lgl axillary and infraclaviculary gl. PET/CT done on March 28, 2012 showed a focus of intense ^{18}FDG accumulation screening in the scar tissue of apicall part of right lung, 20 × 16 mm, corresponding to metabolically active tumor recurrence. In the lung parenchyma on the left and in the mediastinum no visible focus of intense ^{18}FDG accumulation was described (Figure 4). Control PET/CT done 3 months after the radiotherapy was described as a metabolic disease remission.

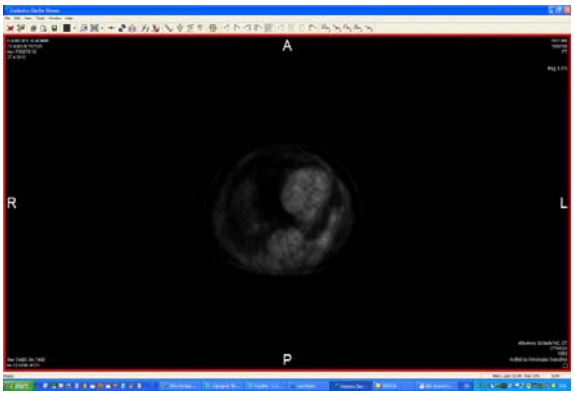

Fig. 3 − Control results of positron emission tomography/computed tomography (PET/CT) after 3 months without detectable focus of intensive pathological accumulation of ^{18}F-fluorodeoxyglucose (^{18}FDG).

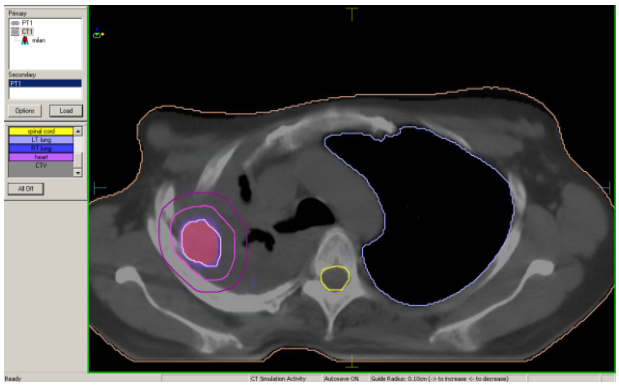

Fig. 4 − Positron emission tomography/computed tomography (PET/CT) findings (March 28, 2012) − the focus of intense ^{18}F-fluorodeoxyglucose (^{18}FDG) accumulation in the projection described soft-tissue changes in the scar tissue of the lung top right.

Discussion

Indications for using ^{18}FDG PET/CT in lung tumor are evaluation of solitary lung nodules (sensitivity and specificity of the method is over 90%), determination of staging, determination of the status of mediastinal lymph nodes (the reliability of PET/CT is 92%, while of CT it is merely 75%), planning radiotherapy and evaluation of the therapeutic response [6, 9]. The advantage of PET/CT is that this imaging can determine the primary tumor close to the atelectatic lung area. Data from the literature suggest that PET/CT provides high sensitivity and specificity and could be applied for early diagnosis of lung cancer and locale reccurence [10, 11].

This improvement in diagnostic accuracy will have direct impact on designing therapeutic strategies and on radiation treatment planning [12]. Some autors confirm in their retrospective studies that shortfall of CT alone in staging and guiding treatment decisions in patients with non-small cell lung carcinoma. It also strengthens the previously known better sensitivity and specificity of PET/CT as compared to conventional CT benefits [5, 13].

Few studies on radiotherapy planning show that the addition of PET/CT information is associated with smaller size of gross tumour volume (GTV), clinical target volume (CTV), planning target volume (PTV) when compared with standard conformal 3D radiotherapy. In radiotherapy planning this allows dose escalation with slightly lower doses on organs at risk with promising high curability rates [14, 15].

In both presented cases CT of the chest did not show reccurence, but PET/CT detected a metabolically active focus. This suggestes that implementation of PET/CT as a result have a possibility for the earler diagnosis. According to the data this is not only the adventige of PET/CT, the major one is maybe incorporaction of PET/CT in radiotherapy planning.

Many studies show that when PET is used, radiotherapy fields or target volumes or estimates of GTV are different from the treatment fields or volumes that would have been drawn if PET had been unavailable [11, 16, 17].

Conclusion

PET/CT provides important information on planning conformal radiotherapy, evaluation of the effects of chemotherapy, in making decisions of the amount of therapy dose: lower dose, in cases of microscopic residue of the disease, higher doses, in cases of large residual tumor.

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Conflicting interests in biomedical research and medical practice

Suprotstavljeni interesi u biomedicinskim istraživanjima i medicinskoj praksi

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A conflict of interest exists when decisions made by a person or institution are affected by direct financial interests or by non-financial issues, such as personal relationships, business associations, and membership in political, national or other groups. These circumstances favor secondary interests over primary interests in medicine and can influence contributions to science and health. Regardless of individual life experiences and personal background, physicians and other health professionals should strive to remain conflict-free.

This paper illustrates conflicts of interests in biomedical research and medical practice caused by financial and non-financial influences. It also assesses how medical journals, professional organizations, healthcare, and government examine these issues.

Presented examples of potential conflicting interests are related to healthcare industry, psychiatry and psychology (e.g., 'enhanced interrogation techniques'), sports medicine, and in the publication process of biomedical journals (authors, editors and peer reviewers). In order to avoid potential conflicting interests, today majority of medical journals request from the authors to disclose any such interests in a written statement on a form prepared by the ICMJE on the journal. This disclosure includes payments for consulting, speaking, honoraria, research support, personal relationships, and institutional conflicts of intersts that may influence the work presented in the submitted manuscript. The editor will decide on potentially relevant conflicting interest in the disclosure and publish it.

Conflicted interests and healthcare industries

Financial collaboration of individual researchers and/or medical institutions with the healthcare industry (pharmaceutical, medical device, and biotechnology companies) frequently benefits both medical practice and scientific research. For example, such collaborations have yielded new drugs (e.g., ACE inhibitors, angiotensin receptor blockers, aliskiren) and monoclonal antibodies (e.g., abciximab, rituximab used for the treatment of non-Hodgkin's B cell lymphoma and other malignancies), as well as medications for human immunodeficiency virus (HIV) infection and various medical devices, especially those used in surgery and cardiology (e.g., catheters, cardiac pacemakers, venous filters, heart valves, annuloplasty rings).

The pharmaceutical and health product industries are highly profitable and influential, and financial relationships in these collaborations, like those involving tobacco and food industries could influence professional judgment and threaten objectivity. Between 1998 and 2005 the pharmaceutical and healthcare industries spent $612 million on lobbying, and $19 billion on marketing in the USA. Thus far, medical and psychological associations have failed to prevent the influence of these entities on the health care system.

Clinical trials and similar studies dependent upon financial support by the industry are more likely to produce favorable results for their sponsors than studies without industrial ties. Ultimately, discrepancies due to financial influence or conflicting interests could expose a large number of patients to less effective or unsafe clinical care and contribute to the loss of public trust in medicine. Publication of biased clinical research fails to maintain high-quality evidence based medicine (EBM) and clinical recommendations, which are important educational tools for clinicians [1]. Regardless of the direct impact on clinical care, conflicts of interest in nonclinical research are also dangerous. They undermine scientific judgment and damage integrity of the research process, thus inhibiting improvement of clinical application.

Representatives of healthcare industry may attract practicing physicians with offers that promise to advance medical knowledge and patient care. However, the professionalism of participating physicians may be threatened when conflicts of interests arise. These conflicts could be as varied as accep-

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tance of company gifts, sponsored travel to meetings, drug samples, fees for promotional speaking, or publishing on behalf of a particular company. More importantly, commercial interests could influence which products a physician prescribes, uses, or recommends. Physician ownership of health care facilities or his own practice presents a conflict of interest that is not easy for government to limit. Participation of community-based and/or academic physicians, residents, and fellows in industry-sponsored trials has the potential benefit of education, yet conflicts of interest can arise when payments to participants exceeds actual expenses or when a clinical study is designed to alter prescribing habits rather than to collect information.

Local medical associations sometimes regulate the gifts that a doctor can accept from industry, but their guidelines are only recommendations. Medical institutions (university hospitals and community-based health institutions) would be wise to include codes for the acceptance of gifts in contracts made between physicians and the institution. Most American hospitals allow gifts ranging from $5 to $25. The US Federal law does not limit the financial support of industry to individual physician, but industry must report each gift or payment to a physician with a value greater than $10. This information is made public for every physician registered in the country. The Open Payments program, introduced in 2013, publishes financial relationships between the medical industry and physicians on free website and includes consulting, speaking, honorariums, gifts, travel, lodging and research support. Various specialties are included: medicine, dentistry, dental surgery, and osteopathy, optometry, chiropractic medicine, and podiatry. Cardiovascular specialists (78%) and neurosurgeons (77%) are most likely to receive general payments (per physician median $100, interquartile range $31–$273). The least likely are pathologists (9%) [2].

In sports medicine, especially at the highest competitive level, physicians frequently have dual responsibilities: one to the athlete (as a patient) and second to his team [3]. Clinical decisions should always protect the player for his long-term health interest. However, a player's short term interest to win, pose conflict of interests for practicing physician. Drug misuse (doping) in elite sport is much bigger problem that faces a physician who is responsible for health of the athlete. To eliminate such temptation of the athletes, physicians should follow the World Anti-Doping Code.

Conflict of interests and psychology

Conflicts of interests in psychiatry and psychology are not different from those in other areas of medicine. Problems arise when industries such as tobacco, food, and pharmaceuticals fund activities to promote marketing, influence regulations, or advance industry interests.

Both public and biomedical media have addressed potentially damaging financial relationships between scientists and industry. One story concerned a senior researcher at the National Institutes of Health (NIH) who accepted unauthorized fees from 25 pharmaceutical and biotechnology companies in addition to government sponsored research involving drugs. A second example was that of a senior scientist, a head of the geriatric psychiatry at the NIH, who admitted that he failed to disclose $285,000 in fees from a pharmaceutical company [4].

Conflicts of interests in practical psychology can raise serious ethical issues. For example, B.F. Skinner, a neobehaviorist, thought that famines, wars, and the threat of a nuclear war could be solved by human behavioral modification [5], and his plan for social engineering attracted funding from the American government *via* the Human Ecology Society. Neuroscientists, philosophers, and psychologists worked together on this controversial issue; one result was the theory of "learned helplessness" devised by Martin Seligman [6]. He discovered that giving dogs repeated shocks resulted in a state of passivity. This observation was translated to humans, who "in the face of events that seem uncontrollable, experience disruptions in motivation, emotion, and learning that amount to a sense of helplessness."

Seligman's theory became the basis for "enhanced interrogation techniques," developed by two psychologists (Bruce Jessen and James Mitchell) and sponsored by the Central Intelligence Agency (CIA) with $81 million [7]. These techniques (sleep deprivation, hypothermia and waterboarding) are considered to be torture. According to an FBI interrogator, [8] this form of torture yields no more information on crucial intelligence issues than can be obtained by more humane methods. Enhanced interrogation techniques were used on prisoners in Guantanamo Bay and Iraq, despite clear evidence that they were ineffective. The enormous financial gains enjoyed by participating psychologists in collaboration with government "to prevent terrorists from succeeding their goals" clearly constitute a conflict of interests [7].

Conflict of interests in publishing

A notorious example of how conflicts of interest can affect dissemination of medical knowledge began when it was revealed that an adviser to a review board for the safety of pharmaceutical products in Canada had financial ties with producers of calcium-channel antagonists. These financial relationships resulted in an article favorable to the manufacturer's positions on the safety of calcium-channel antagonists [9], although another, more careful study showed that calcium-channel antagonists considerably increased the risk of myocardial infarction in hypertensive patients treated with these medications. A television documentary, *The Fifth Estate*, suggested that the patients were not protected from dangerous adverse effects of calcium channel blocking drugs. This particular case raised a serious question of objectivity in assessing the safety of drugs, and the International Committee Medical Journal Editors (ICMJE) found an effective way to prevent a potential conflicting interests [10].

It is well known-that all participants in the publication process of biomedical journals (authors, editors and peer reviewers) may have potential financial or non-financial interests related to the manuscripts under consideration. The author who submits a manuscript is responsible for accuracy and reliability of the presentation. In order to avoid any un-

due influence related to this contribution, most medical journals ask the authors to sign a form prepared by the ICMJE or the journal. This disclosure includes all financial, personal relationships, and institutional conflicts of interests that could influence the work presented in the submitted manuscript. The editor will decide if there are relevant conflicts of interest in the disclosure and indicate this under the heading 'Competing interests' or 'Conflict of interests' or 'Declaration of interests statement'. This section is generally placed above the references, or rarely as a footnote on the first page of the paper. Some journals publish this information under the title 'Additional information and declaration' where in addition to the competing interests, one can find funding, author contributions, and supplemental information.

The examples below show how various journals report conflicts of interest. Instead of author names, the journal uses initials in order to save space, and the entire section is usually printed in a smaller font than used for the body of the paper. Some journals report the exact amount of money the author received during the last three years in relation to his manuscript.

Examples

Author disclosure: Nothing to disclose.

Declaration of Interests: None declared

Conflict of interests: The author reported no conflict of interests.

Disclosures: the authors declare no conflicts of interest, financial or otherwise.

Conflict of interest statement: None declared.

Conflict of interest statement: KFC is a co-Editor-in Chief of an online journal *Cough*. He was a co-organizer of the Fourth International Cough Symposium in 2006 that received educational grants from AstraZeneca, GlaxoSmithKline, and Novartis. He declares no other conflict of interest. IDP was one of the developers of the Leicester cough questionnaire. He receives occasional payments for the use of the questionnaire in commercially sponsored clinical trials. He declares that he has no other conflict of interest for this Series. SW declare no competing interests.

Conflict of interests: KHM has received unrestricted research support from Gilead Sciences. CB declares no competing interests. CB is a President of the International AIDS Society; KHM is a member of the International AIDS Society Governing Council.

Publishing a statement on conflicting interests alerts readers to recognize influence of financial or non-financial factors on the validity of research. However, it may affect readers in the opposite direction, causing them to find the article less interesting, relevant, or important than if published without declarations. In general, disclosure of potential conflicts of interest reduces bias and increases the transparency of scientific research.

When the editor of a medical journal concludes that the findings and conclusions of the authors are accurate and truthful, and that the paper may be interesting to readers, the process of manuscript evaluation moves ahead to select papers that contribute to science or improve medical practice. The peer-review process that includes the assessment and critique of the work is a crucial step in the evaluation [11, 12]. The reviewer (referee, manuscript assessor) advises the editor on the quality, originality, and suitability of the paper for publication. When a reviewer requests revision, a valid critique may improve the manuscript before publication, but a reviewer may also recommend rejection or acceptance of the paper without revision. It remains important for reviewers to know potential conflicting interests that could impact the conclusions of a paper under review. However, in a journal that is published in a local language for a "small scientific community" [13] the reviewer's report might be uncritically positive or negative. Therefore, it falls to the editor to assess the objectivity of the selected reviewers.

Some journal editors ask the authors to recommend potential reviewers, and also to indicate those who should be excluded from the assessment of their manuscript. A reviewer should also disclose potential conflict of interests that could influence his review. Many journals use a blind review system, where author(s) and reviewer(s) remain unknown to each other. Nonetheless, editors should be alert to potential conflicts of interest and provide this information as needed to achieve the best assessment of the manuscript.

Consluson

Conflict of interests (both financial and non-financial) may influence medical practice, and reporting and evaluating of medical reasearch. Transparency is important for fidelity of scientific journals, researchers, and physicians. Professional and scientific cooperation between practicing and academic physicians with industry should be open and disclosed. It is the responsibility of medical journals, professional organizations, health care providers and government to create adequate safe-guards in the form of specific codes or rules that apply to collaborations with business interests. Simply becoming aware of the problem may help to prevent or reduce conflicts of interests.

Conflict of interests

The author of this paper accepted travel and local living expenses as Editor-in-Chief of the *Scripta Medica* (Banja Luka) from 2010 to 2013.

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BOOK REVIEW/PRIKAZ KNJIGE

ANTIBIOTICI
Priručnik za kliničku primenu

Autor: Veljko Mirović
Izdavač: Data Status
Mesto i godina izdanja: Beograd, 2015.
Štampa: SP PRINT, Novi Sad
Tiraž: 300
ISBN: 978-86-7478-413-6

Antibiotici su jedna od najvažnijih grupa lekova koji se, nažalost, neretko nepotrebno propisuju i koriste. Zbog toga, svaka publikacija koja donosi preporuke o njihovoj racionalnoj upotrebi, bazirane na savremenim podacima iz relevantne stručne literature, predstavlja značajan doprinos promovisanju medicine zasnovane na dokazima koja danas predstavlja *conditio sine qua non* medicinske struke i nauke.

Prošle godine, u izdanju „Data Status"-a izišla je knjiga našeg poznatog mikrobiologa, prof. dr Veljka Mirovića, pod naslovom „Antibiotioci/ Priručnik za kliničku medicinu". Kako se iz samog naslova vidi pisan je, prvenstveno, za lekare-praktičare, dajući jasne smernice za primenu pojedinih grupa antibiotika i njihovih glavnih predstavnika u pojedinim indikacijama.

U knjizi, koja sadrži 383 strane, obrađene su sledeće grupe antibiotika: penicilini (prirodni penicilini, antistafilokokni penicilini, aminopenicilini, karboksipenicilini, ureidopenicilini, amidinopenicilini), kombinacije penicilina sa inhibitorima beta-laktamaza (amoksicilin/klavulanska kiselina, ampicilin/sulbaktam, piperacilin/tazobaktam, tikarcilin/klavulanska kiselina), cefalosporini (antistafilokokni cefalosporini, cefalosporini koji deluju na enterobakterije, antipseudomonasni cefalosporini, cefalosporini koji deluju na MRSA, kombinacije cefalosporina sa inhibitorima beta-laktamaza), monobaktami (aztreonam), karbapenemi (imipenem, meropenem, doripenem, ertapenem), aminoglikozidi (gentamicin, amikacin, tobramicin, netilmicin, streptomicin, kanamicin, spektinomicin), tetraciklini (tetraciklin, oksitetrciklin, doksiciklin, minociklin, tigeciklin), hloramfenikol i tiamfenikol, rifamicini (rifampicin, rifabutin, rifapentin, rifaksimin), makrolidi (eritromicin, azitromicin, klaritromicin, roksitromicin, fidaksomicin), linkozamidi (klindamicin), ketolidi (telitromicin), streptogramini (kvinapristin/dalfopristin, pristinamicin), nitroimidazoli (metronidazol, tinidazol), glikopeptidi i lipoglikopeptidi (vankomicin, teikoplanin, daptomicin, dalbavancin, oritavancin), oksazolidinoni (linezolid), fluorohinoloni (ofloksacin, norfloksacin, levofloksacin, moksifloksacin, gemifloksacin), sulfonamidi (trimetoprim/sulfometoksazol), nitrofurantoin, metenamin-maleat i metenamin-hipurat, fosfomicin, fusidinska kiselina, polimiksini (kolistin i polimiksin B), antibiotici za lokalnu primenu (bacitracin i gramicidin, mupirocin, novobiocin) i antimikobakterijski antibiotici I (izonijazid, rifampicin, etambutol, streptomicin, pirazinamid) i II linije (rufabutin i rifapentin, novi hinoloni, kapreomicin, viomicin, amikacin i kanamicin, p-aminosalicilna kiselina, cikloserin, etionamid, tiacetazoin, beta-laktami). U prikazu svakog pojedinog leka dat je kratak opis njegovog antimikrobnog spektra, farmakokinetičkih osobina, neželjenih dejstava, načina primene i doziranja, te indikacionog područja, potkrepljeno odgovarajućim podacima iz literature, uglavnom iz poslednjih 5–10 godina. Tekst je pisan lepim, ujednačenim i jasnim stilom, tako da se „lako" čita.

Na samom početku knjige, dat je spisak korišćenih skraćenica i akronima, spisak skraćenica za neke od časopisa koji su citirani u popisu literature, kao i Indeks kliničkih indikacija za primenu antibiotika, navedenih abecednim redom, sa oznakom stranice u knjizi gde se pojedina indikacija,

odnosno primena određenog antibiotika u toj indikaciji, pominju. Ovaj Indeks uveliko olakšava snalaženje u knjizi, pogotovo u situaciji kada se želi brzo doći do potrebnog podatka o prevenciji ili lečenju određene infekcije.

Na kraju knjige dat je spisak najznačajnih publikacija samog autora, prof. dr Veljka Mirovića, iz kojih se može sagledati uže područje njegovog stručnog i naučnog rada u oblasti kliničke mikrobiologije.

S obzirom na to da se antibiotici koriste gotovo u svim granama medicine, ova knjiga može da se preporuči svim zdravstvenim radnicima, bez obzira na specijalnost i mesto rada u sistemu zdravstvene zaštite, ali i studentima fakulteta iz medicinskog naučnog polja, kako na dodiplomskim, tako i na poslediplomskim oblicima školovanja.

Knjiga je štampana u tiražu od 300 primeraka, ali izdavač najavljuje i njeno elektronsko izdanje, što će, svakako, omogućiti njenu širu dostupnost svim zainteresovanim čitaocima.

prof. dr Silva Dobrić
Medicinski fakultet VMA
Univerzitet odbrane u Beogradu

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References

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Examples of references:

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

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

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Primeri referenci:

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

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

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

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

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

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