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World Immunization Week is celebrated in the last week of April each year with aim to raising awareness of the importance of immunization and collective actions needed to ensure that every person is protected from vaccine-preventable diseases. This year's theme: “Protected Together #VaccinesWork” should encourage the whole society to go further in efforts to increase immunization coverage for greater common good.

The Editorial Board of the Vojnosanitetski pregled invites all its readers and associates to join in this action, bearing in mind the recent epidemic of measles in Serbia, which included fatalities, as a result of non-vaccination or incomplete vaccination in the previous period.

Svetska nedelja imunizacije se obeležava svake godine u poslednjoj nedelji aprila sa ciljem podizanja svesti o značaju imunizacije i kolektivnih akcija potrebnih da se svakoj osobi obezbedi zaštita od bolesti koje se mogu sprečiti vakcinom. Ovogodišnja tema: "Zaštićeni zajedno - Vakcine deluju", treba da podstakne celo društvo da nastavi sa naporima za povećanje pokrivenosti imunizacijom, za veće opšte dobro.

Uredništvo Vojnosanitetskog pregleda poziva sve svoje čitaoce i saradnike da se uključe u ovu akciju imajući u vidu nedavnu epidemiju malih boginja u Srbiji, u kojoj je bilo i smrtnih slučajeva, kao posledice nevakcinisanja ili nepotpunog vakcinisanja u ranijem periodu.

ORIGINAL ARTICLES

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Neuropsychological parameters as possible indicators of speech fluency disorder in children

Neuropsihološki pokazatelji kao mogući indikatori poremećaja fluentnosti govora kod dece

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Abstract

Background/Aim. Speech disfluency (stuttering) is a multifactor disorder and its aetiology is a big unknown for the experts from various fields. Hemisphere dominance as the highest level in the process of integration of cortical functions is of special significance for the entire development. Praxis and gnosis related cortical organizers are the first to form; they become differentiated and functionally full in early childhood while the process of condensing is completed somewhere around the age of 7. Cortical activity organizers are definitely set at that age and from then on act from one hemisphere which becomes dominant for that function. Laterality is determined by hemisphere dominance, but it occurs as a special phenomenon and it is of great significance for personality. The aim of this research was to examine the influence and the relationship between hemisphere asymmetry on the occurrence of speech disfluency in children. **Methods**. Sixty children aged 5 to 7 years participated in this research. Thirty children suffer from speech fluency disorder (person who stutters – PWS) and they belong to the experimental group while thirty children are fluent speakers (person who does not stutter – PWNS) and they were the control group. Individual testing was used as a test method. Laterality assessment test was used as an instrument which consists of 5 sub-tests as follows: the assessment of hand-use laterality, the assessment of gestural hand-use laterality, the assessment of foot laterality, the assessment of auditory laterality and the assessment of visual laterality. **Results**. Gestural hand-use laterality and auditory laterality in the PWS examinees were considerably worse in comparison to the PWNS examinees ($\chi^2 = 11.80$, $p = 0.002$, and $\chi^2 = 10.90$, $p = 0.003$, respectively). Male examinees had worse scores in comparison with female examinees. **Conclusion.** There are certain changes in establishing a dominant hemisphere and differentiation of laterality in children who stutter in comparison with the children who are fluent speakers, which has been shown by statistically significant difference in accomplishments at the test of gestural hand-use laterality and the test of auditory laterality.

Key words:
stuttering; functional laterality; child.

Apstrakt

Uvod/Cilj. Disfluentan govor (mucanje) je multifaktorijalni poremećaj, a njegova etiologija velika nepoznanica za stručnjake različitih oblasti. Dominacija hemisfera kao najviši domet u procesu integracije kortikalnih funkcija, od posebnog je značaja za celokupni razvoj. Prvo se formiraju praksički i gnostički kortikalni organizatori koji se diferenciraju i dostižu svoju funkcionalnu punoću u ranijem detinjstvu, da bi se proces sažimanja dovršio negde oko sedme godine života. Tada se definitivno postave kortikalni organizatori aktivnosti koji od tada deluju iz jedne hemisfere i ona postaje dominantna za tu funkciju. Lateralizovanost je određena dominacijom hemisfera, ali se javlja kao posebna pojava i od velikog je značaja za ličnost. Cilj ovog istraživanja bio je da se ispita uticaj i odnos hemisferne asimetrije na pojavu disfluentnog govora kod dece. **Metode.** U istraživanje je bilo uključeno 60-toro dece, uzrasta od 5 do 7 godina. Tridesetoro dece je imalo poremećaj fluentnosti govora, (*person who stutters* – PWS), i oni su činili eksperimentalnu grupu, a tridesetoro dece je bilo fluentno u govoru, (*person who does not stutter*– PWNS), i ona su činili kontrolnu grupu. Primenjen je metod testiranja tehnikom individualnog testiranja. Kao instrument korišćen je Test za procenu lateralizovanosti koji objedinjuje pet testova i to: procenu upotrebne lateralizovanosti-ruke, procenu gestualne lateralizovanosti-ruke, procenu lateralizovanosti noge, procenu auditivne lateralizovanosti i

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procenu vizuelne lateralizovanosti. **Rezultati.** Gestualna lateralizovanost ruke i auditivna lateralizativnost kod ispitanika PWS bile su značajno lošije u odnosu na PWNS, ispitanike (χ^2 = 11,80, p = 0,002, odnosno χ^2 = 10,90, p = 0,003). Ispitanici muškog pola su postigli lošije rezultate na testovima u odnosu na ispitanike ženskog pola. **Zaključak.** Postoje izvesne promene u uspostavljanju dominantne hemisfere i diferenciranju lateralizovanosti kod dece koja mucaju u odnosu na decu koja su fluentni govornici, što je pokazala statistički značajna razlika u postignićima na testu gestualne lateralizovanosti ruke i testu auditivne lateralizovanosti.

Ključne reči:
mucanje; lateralnost; deca.

Introduction

The development of speech is usually monitored through the development of expressive and impressive speech. Impressive speech is decoding of perceptive speech which implies clear understanding of what has been said and expressive speech is language production. Speech and language functions are predominantly localized in the left hemisphere, while the right hemisphere is dominant for understanding of colour and tonality of verbal expressions, rough discrimination of the meaning of frequent words, and it also has a considerable role in learning of a new language, in other words, an irreplaceable role in the development of speech in childhood. The results of empirical and experimental studies on both healthy and sick people suggest that the left hemisphere in people is dominant for speech in 90% of cases and that the entire cortex participates in realization of language activities [1, 2]. The term fluency means the ability to generate new forms of speech in a given unit of time. Fluent speech implies easy, fluent and natural speech flow which unwinds without strain, interruption, hesitation, stopping and prolongation [3]. Speech disfluency (stuttering) means irregular pronunciation where speech tempo is noticeably disrupted as one of the basic suprasegmental speech structures. About 55 million people around the world stutter nowadays. The disorder occurs at all ages, but most frequently in children [4]. This communication disorder changes the speech accuracy, its rhythm, intensity, frequency, emotional colour and therefore the speech as whole [5–7]. Stuttering has negative influence on the general adaptation in society and nature, and this is why it should be considered and treated as a multidimensional problem [8]. There is evident difference in functional organization of the brain in persons who are disfluent speakers and those who are not. This organization includes a series of both cognitive and emotional processes [9, 10]. Some scientists claim that there are certain deviations in functioning of both cortical and subcortical parts of the brain in disfluent speakers in comparison with fluent speakers as well as considerable influence of hemisphere dominance on the appearance of stuttering [11].

Laterality is realized gradually in the course of central nervous system (CNS) maturation and gathering experiences acquired by perception, kinaesthesia, manipulative activities and finally cognition that this laterality occurred. In the following step of maturation, there is differentiation of laterality when laterality becomes dominant for one side and subdominant for the other side of the body (cognition that one extremity or sight organ are leading and thus is dominant over the other one). The assessment of laterality and dominant laterality points at the organization of ability of senses and movements in the function of voluntary motor activities and to the level of practognostic cortical organization in comparison to the development of hemisphere dominance [12]. Laterality is determined simultaneously with dominance determination. It is first estimated if the dominant side is always the same and stabilized in comparison with the subordinate one. After that conclusion, it is determined which side is stabilized as dominant one, and which always appears as subordinate.

Some researches have shown that there is a link between the occurrence of stuttering, hemisphere dominance and differentiation of laterality. Undifferentiated laterality was noticed in persons who stutter, although this claim was not supported nor proved by application of contemporary neuroimaging techniques by measuring metabolic activities at cell level [13]. Undifferentiated laterality was noticed at the level of upper limb, which was brought into connection with stuttering [14, 15]. Verbal organization and laterality of expressive speech centre in disfluent speakers also show certain differences in comparison with fluent speakers [16, 17]. The studies of auditory laterality in the examinees with disfluent speech showed results which confirmed aberrations from the results of fluent speakers and support undifferentiated auditory laterality of this population [18, 19].

Findings from modern neurodiagnostic techniques have implicated cortical and subcortical structures with PWS. Electroencephalographic measurements demonstrated greater activity in the nondominant right hemisphere in subjects with PWS during the speaking condition [20]. No such brain activity was reported in individuals without stuttering. Upon fluency improvement with treatment, this focused physiologic activity shifted to the left hemisphere [21–23].

The aim of this research was to examine the influence and the relationship of hemisphere asymmetry on the appearance of disfluent speech in children.

Methods

For the purpose of this research the sample of 60 children, aged 5 to 7 years, was formed. The research was carried out at the Institute for Psychophysiological Disorders and Speech Pathology "Dr Cvetko Brajović" and the Clinic of Neurology and Psychiatry for Children and Youth in Belgrade. The research was carried out in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. The Ethical Committee

approved the research, and taking into account that the research subjects were children, the informed consent was obtained from the parents/guardians.

Thirty children were selected according to the criteria of the presence of stuttering and they were monitored as an experimental group (E); the other thirty children were fluent speakers and this group was monitored as a control group (C). For both the experimental and control groups the additional criterion was defined – the absence of other impediments and disorders in intelligence, motor ability or sensory perception. The research was carried out from November 2014 until May 2015. In line with the set goal, we used the research method which included the method of documentation analysis and testing. Testing included the techniques of individual testing for the groups E and C. The instruments used in the research included specialized test for lateralization assessment (Bojanin, 1975). The lateralization test consisted of questions and tasks classified according to the assessment levels of and gesture laterality of extremities, sight and hearing. In order to assess the dominant hand-use laterality, a test consisting of 12 tasks was used and to assess the gesture laterality, a test consisting of 6 tasks was set by the trained measurer. The tested child was supposed to answer the questions by showing certain action or complete the specific task using the appropriate equipment offered. In the course of testing the dominant hand-use laterality of upper extremities, the measurers asked the following questions: "Show me how you comb your hair?" or "Show me how you hold the spoon?", and so on. It was recorded which hand the child used to show the action and based on the collected answers, it was assessed if the left or right hand was dominant, or if the child is ambidextrous. It was similar with the assessment of dominant gesture laterality of upper extremities. The tested child was asked "How would you put your fists together, one above the other?" and the child showed that spontaneously. Depending on which hand was above (in this case which fist was put as the upper fist), we recorded that this hand was gesture-dominant. By calculating the collected data we made the assessment regarding the dominant gesture lateraly.

The dominant laterality of lower extremities was assessed by movements made on an everyday basis in the social environment. There were 4 tasks. The tested child was supposed to perform the action according to the request "Show me how you kick the ball?" or "Show me how you stand on one foot". It was recorded which leg the child used to complete the task. By calculating the collected data, the dominant laterality of lower extremities was assessed.

The evaluation of dominant auditory laterality was made by searching for or by approaching the sound source. There were 4 tasks. The tested child was supposed to complete the task when asked to "Listen to this clock to hear if it is ticking" (we give a wrist watch to a child), and so on. It was recorded which ear the tested child used to complete the set task. By calculating all the data, the dominant ear was determined. The evaluation of dominant sight laterality was assessed by means of a cardboard with one hole, two distant holes and the telescope. There were 4 tasks. The tested child was supposed to complete the task upon the request "Look at me through this hole" (when the tester was holding the cardboard), or, "Take a cardboard and look at me through the hole". It was recorded which eye the tested child used to complete the task and by calculating the results, it was determined which eye was dominant during the task completion. The tests were performed so that the set tasks could be verbally repeated but not shown.

The collected data were processed using statistical analysis by means of parameter algorithms in Statistical Package for Social Sciences for Windows (SPSS) version 17. Out of the descriptive statistics measures, frequency and percentage were used as well as arithmetic mean with standard deviation. The differences between groups were determined by χ^2 test. Statistical significance was defined at the level of probability of null hypothesis $p \leq 0.05$ to $p < 0.01$.

Results

Sixty examinees, all aged 5 - 7 years, participated in the research. Within the experimental group there were 60% of male examinees, 40% of female examinees, while within the control group there were 56.7% of male examinees and 43.3% of female examinees. The groups were adjusted per sex ($p = 0.793$) (Table 1). The groups were also adjusted according to the average age ($p = 0.276$); the average age of the experimental group was 6.1 years, while the average age of the control group was 6.25 (Table 2).

Table 1

Structure of examinees with respect to the sex and age in children with speech fluency disorder (the Experimantal group – E) and those with fluent speech (the Control group – C)

Groups of exameenes	Gender, n (%)		Age (year)	Total
	males	females	r ± SD (min-max)	n (%)
Group E	18 (60)	12 (40)	6.10 ± 0.51 (5–7)	30 (100)
Group C	17 (56.7)	13 (43.3)	6.25 ± 0.46 (5–7)	30 (100)
Total	35 (58.3)	25 (41.7)	6.20 ± 0.50 (5–7)	60 (100)

n – number of respondents; min – minimum value of the variable in the sample; max – the maximum value of the variable in the sample; r – arithmetic mean (average value of the variable in the sample); SD – standard deviation (average deviation of the individual values of the variables in the sample).

Table 2

Differentiation of laterality in children with speech fluency disorder (the Experimantal group – E) and those with fluent speech (the Control group – C)

Parameters	Groups of exameenes, n (%)						*p*
	group E			group C			
	males	females	total	males	females	total	
	18 (60)	12 (40)	30 (100)	17 (56.7)	13 (43.3)	30 (100)	
Hand-use laterality							
left	5 (16.66)	7 (23.33)	12 (40)	4 (13.33)	8 (26.67)	12 (40)	
right	7 (23.33)	3 (10)	10 (33.33)	7 (23.33)	4 (13.33)	11 (36.67)	0.133
undiff.	6 (20)	2 (6.67)	8 (26.67)	6 (20)	1 (3.33)	7 (23.33)	
Hand- gestural laterality							
left	8 (26.67)	6 (20)	14 (46.67)	5 (16.67)	4 (13.33)	9 (30)	
right	7 (23.33)	4 (13.33)	11 (36.67)	12 (40)	9 (30)	21 (70)	0.002*
undiff	3 (10)	2 (6.67)	5 (16.67)	0 (0)	0 (0)	0 (0)	
Lower limb laterality							
left	7 (23.33)	5 (16.67)	12 (40)	9 (30)	7 (23.33)	16 (53.33)	
right	8 (26.67)	4 (13.33)	12 (40)	5 (16.67)	3 (10)	8 (26.67)	0.066
undiff	3 (10)	3 (10)	6 (20)	3 (10)	3 (10)	6 (20)	
Auditory laterality							
left	2 (6.66)	2 (6.66)	4 (13.33)	5 (16.67)	3 (10)	8 (26.67)	
right	8 (26.67)	6 (20)	14 (46.67)	12 (40)	10 (33.33)	22 (73.33)	0.003*
undiff	8 (26.67)	4 (13.33)	12 (40)	0 (0)	0 (0)	0 (0)	
Visual laterality							
left	4 (13.33)	5 (16.67)	9 (30)	8 (26.67)	6 (20)	14 (46.67)	
right	8 (26.67)	4 (13.33)	12 (40)	4 (13.33)	4 (13.33)	8 (26.67)	0.166
undiff	6 (20)	3 (10)	9 (30)	5 (16.67)	3 (10)	8 (26.67)	

undiff. – undifferetiated laterality; *values that show significant difference.

As for the test score of estimated upper limb laterality, in the test of hand-use laterality out of 12 trials only 2 resulted in statistically significant difference in the groups E and C, which in final consideration of the results did not produced statistically significant difference between them (χ^2 = 4.37, p = 0.133). In the examinees from the group E, gestural hand-use laterality ranges from undifferentiated laterality (ambidexterity), which was present in 5 examinees, left-hand gestural laterality was observed in 14 examinees and 11 examinees had right-hand gestural laterality. In the group C, none of the examinees had undifferentiated laterality, 9 examinees had left-hand gestural laterality while 21 examinees had right-hand gestural laterality. The data processing resulted in a statistically significant difference (χ^2 = 11.80, p = 0.002) between the groups E and C. As for the distribution according to sex, there were 8 male examinees in the group E with the left gestural hand-use laterality, 7 with the right, while 3 examinees did not have laterality differentiation. In the female examines 6 of them had left gestural hand-use laterality, 4 of them had the right, and 2 did not have laterality differentiation. According to the sex of examinees the distribution in the group C was as follows: 5 male examinees had left-hand gestural laterality, 12 of them the right, while undifferentiated laterality was not present in any of the examinees in this group. In the female examinees, 4 of them had left gestural hand-use laterality, ?9 of them had right while undifferentiated laterality was not present in any of the examinees in this group.

As for the laterality of the lower limb – foot, a statistically significant difference between the groups E and C (χ^2 = 0.80, p = 0.666) was not determined.

The distribution of the results for auditory laterality showed that 4 examinees in the group E had left auditory laterality, 14 had right and 12 had undifferentiated (ambidextrous) auditory laterality. In the group C, 22 examinees had right auditory laterality and 8 of them had left. Statistical processing showed a statistically significant difference between the groups E and C (χ^2 = 10.90, p = 0.003). With respect to the sex, there were 2 male examinees in the group E with left auditory laterality, 8 with the right, while 8 examinees did not have auditory laterality differentiated. As to the female examinees, 4 of them had left auditory laterality, 14 had the right, and 12 examinees did not have laterality differentiated. In the group C, the distribution according to the sex was as follows: 5 male examinees had left auditory laterality, 12 had the right while undifferentiated auditory laterality was not present in any of the examinees in this group. As to the female examinees, 3 of them had left auditory laterality, 10 of them had the right, while undifferentiated laterality was not present in any of the examinees in this group. The data processing regarding the estimation of visual laterality did not provide statistically significant difference between the groups E and C (χ^2 = 1.70, p = 0.166).

Discussion

The scores obtained at the test of laterality were analysed collectively for both groups (30 PWS children and 30 PWNS children). Observation of dominant laterality of motor ability and senses suggests the dominance of the CNS functions. The problem appears when the result is ambivalent (ambidexterity), and this is not a physiological ambiva-

lence characteristic for children of 3 to 4 years of age. Harmonious laterality means identical dominant laterality at the level of a hand, eye, ear and foot. The category of inharmonious laterality consists of the examinees with complete disharmony in dominance of a hand, eye, ear and foot. In addition to this, the presence of undifferentiated laterality, i.e. the presence of ambidextrous children within the group is also disputable. It can be seen from the above-mentioned that at the test of gestural laterality of the upper limb – hand, the PWS examinees were statistically considerably worse. Unsuccessfulness of the PWS examinees reflected in overall larger number of examinees present in ambidexterity and the left-handedness. The results suggest that at the laterality test, the test of gestural hand-use laterality, in the PWS examinees, there were more examinees with undifferentiated laterality and the examinees with dominant left hand-use than in the PWNS examinees. According to our results, the children who stutter had mostly undifferentiated gestural hand-use laterality or left-hand gestural laterality. The distribution of the results in relation to the sex in the PWS in comparison with the PWNS examinees showed that undifferentiated gestural hand-use laterality appeared mostly in the male examinees. Unsuccessfulness of the male examinees in this task, reflected in totally larger number of the examinees in the ambidexterity group. Some findings reflected the imprecise functional connectivity within the right frontal cortex and incomplete segregation between the adjacent hand and mouth motor representations in stutterers during speech production. During speech production, the right motor-premotor cortex generated consistent evoked activation in fluent speakers but it was silent in stutterers [24]. There is increasing body of evidence supporting the various manners of linguistic information processing, both for perception and production, in those who stutter and in their peers who do not stutter. At cortical level there is increased activation of the right hemisphere present in the language centres and sound-processing centres. Therefore, it is necessary to stimulate the development of sensory and work capacities in the organization of therapy. In this way, there is influence on the development of perceptual attention and perception in general, the function of the hand being dominant in its significance, since it is by the hand exactly that concentration of all personal capacities is achieved, from perceptual attention, which cannot be separated from pure motor ability, to the higher cognitive functions, which speech actually is. Considering in detail the obtained research results, we can conclude that in children with fluent speech disorder hand-use and gestural hand-use laterality are not in agreement. This may mean that the environment enforced the hand-use laterality by daily manipulative activities and led to forced change of the dominant hand which might have created a “confusion” in the brain.

The results of the test of auditory laterality in the PWS examinees in comparison with the PWNS ones mean that the PWS examinees obtained worse scores. On the test of auditory laterality in comparison with the PWNS examinees. This can be seen in the overall number of examinees with undifferentiated auditory laterality. The distribution of the scores on this test related to the sex, showed that undifferentiated auditory laterality is more frequent in the PWS male examinees than in the PWNS examinees. In fluent speakers, the left auditory cortex is more sensitive to the side of stimulation (right versus left ear), whereas the right auditory cortex is more sensitive in the stutterers. The stutterers were also reported to have difficulties in sound localization [25].

In literature, the research which deals with the cause of stuttering shows that there is hypoactivity of the left hemisphere which is caused by reactive amygdala response. Considering that speech centres are localized in the left hemisphere, we can assume that its hypoactivity causes dysfunction of neuromotor processes and discoordination of speech motor ability based on motor programming which can represent a causal factor for occurrence of stuttering [26]. Some research where bioelectric activities of both hemispheres electroencephalography (EEG) were monitored find the cause of stuttering in suppressing the activity of alpha waves over the right temporal part which causes increased activity of the right hemisphere in this part [27, 28]. Accordingly, stuttering can also occur when both the input data from both hemispheres are processed and motor programming of the separate linguistic units are in the right hemisphere. These differences in processing can refer to the to process separate language aspects under certain circumstances. This shows the significance of linguistic division since it refers to motor programming in some PWS people [21, 29].

Conclusion

Our results suggest that 5 (16.67%) children who stuttered had mainly undifferentiated gestural hand-use laterality, while all children with fluent speech had gestural laterality differentiated. According to the results, auditory laterality was not differentiated in children with speech fluency disorder in 12 (40%) examinees, while in all children with fluent speech the auditory laterality was differentiated, which also showed that differentiation of auditory laterality was considerably better in children who were fluent speakers. The results suggest that it was the accomplishment of these two sub-tests (gestural hand-use laterality and auditory laterality) that showed significant difference between the children with fluent speech and the children with speech fluency disorder, which might perhaps be used to predict possible speech fluency dysfunction.

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Breath holding index in episodic primary headaches

Indeks zadržavanja daha u epizodičnim glavoboljama

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Abstract

Background/Aim. Examination of cerebrovascular reactivity in patients with primary headaches is focused mainly on migraine, while the smaller number of studies deals with tension-type and cluster headache, or comparison of cerebral haemodynamic in migraine and tension-type headache (TTH). In this study, we hypothesized that cerebrovascular reactivity differs among different types of episodic primary headaches. In order to prove that we aimed to compare the interictal cerebrovascular reactivity in patients with the episodic form of the three most common types of primary headaches using the breath holding test. **Methods.** Examination was performed in 243 patients, 100 migraineurs with aura (group I), 70 migraineurs without aura (group II), 38 patients with episodic tension-type headache (group III), 35 patients with episodic form of cluster headache (group IV) and 35 healthy controls (group V). The Doppler instrument was used for transcranial doppler (TCD) sonography and breath-holding test performance. Blood flow mean velocities (MV), pulsatility indices (PI) and breath-holding index (BHI) for middle cerebral artery among these groups were analyzed. **Results.** The mean velocities and pulsatility indices were not different in 4 groups of headache patients and controls. The BHI was found to be significantly greater in the migraineurs with aura (1.668 ± 0.269) compared with the patients with migraineurs without aura (1.411 ± 0.358, $p = 0.005$), tension type headache (1.401 ± 0.428, $p = 0.035$), cluster headache (1.203 ± 0.311, $p < 0.01$) and controls (1.195 ± 0.269, $p < 0.01$) showing an exaggerated reactivity to hypercapnia in patients with migraine with aura. **Conclusion.** In conclusion, our finding support the literature data that increased cerebrovascular reactivity is a feature of migraine with aura. Result of unchanged cerebrovascular reactivity in migraine without aura, cluster headache and tension-type headache is expected, still, it is possible that in future, using different technique, we will be able to put more light on vascular changes that are following different headache disorders.

Key words:
migraine with aura; migraine without aura; cluster headache; tension-type headache; breath holding; ultrasonography doppler transcranial.

Apstrakt

Uvod/Cilj. Istraživanja cerebrovaskularne reaktivnosti kod osoba sa primarnim glavoboljama fokusirana su uglavnom na migrenu, dok je manji broj studija bio usmeren na glavobolju tenzionog tipa ili poređenje cerebralne hemodinamike između migrene i glavobolje tenzionog tipa. Cerebrovaskularna reaktivnost kod osoba sa migrenom je dosta ispitivana, primenom različite metodologije i sa različitim zaključcima. Istovremeno, cerebrovaskularna reaktivnost kod osoba sa glavoboljom tenzionog tipa i klaster glavoboljom bila je predmet istraživanja značajno manjeg broja studija. Ovim istraživanjem, cilj nam je bio da uporedimo intraiktalnu cerebrovaskularnu reaktivnost merenu metodom zadržavanja daha kod bolesnika sa epizodičnim formama tri najčešća tipa primarnih glavobolja. **Metode.** Ispitivanje je sprovedeno kod 243 ispitanika, 100 osoba sa migrenom sa aurom (grupa I), 70 osoba sa migrenom bez aure (grupa II), 38 osoba sa epizodičnom glavoboljom tenzionog tipa (grupa III), 35 osoba sa epizodičnom formom klaster glavobolje (grupa IV) i 30 zdravih ispitanika u kontrolnoj grupi (grupa V). Ispitianicima je urađena transkranijalna Doppler sonografija i test zadržavanja daha, pri čemu su analizirane dobijene vrednosti srednjih brzina protoka, indeksa pulsatilnosti i indeksa zadržavanja daha (*Breath-holding index* – BHI), merenih i izračunavanih za srednju cerebralnu arteriju. **Rezultati.** Srednje brzine protoka i indeksi pulsatilnosti nisu se razlikovali među ispitivanim grupama. Vrednosti BHI bile su značajno veće u grupi ispitanika sa migrenom sa aurom (1,668 ± 0,269) u poređenju sa ispitanicima sa migrenom bez aure (1,411 ± 0,358, $p = 0,005$), glavoboljom tenzionog tipa (1,401 ± 0,428, $p = 0,035$), klaster glavoboljom (1,203 ± 0,311, $p < 0,01$) i zdravim kontrolama (1,195 ± 0,269, $p < 0,01$) ukazujući na povećanu reak-

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tivnost na hiperkapniju kod osoba sa migrenom sa aurom. **Zaključak.** Naši rezultati podržavaju hipotezu o postojanju interiktalne hipersenzitivnosti i slabosti habituacije kod osoba sa migrenom.

Ključne reči:
migrena sa aurom; migrena bez aure; klaster glavobolja; glavobolja, tenziona; zadržavanje daha; ultrasonografija, dopler, transkranijumska.

Introduction

Migraine and cluster headache, have been considered for the long time to be „vascular headaches“ [1] Today they are known to be „brain diseases“, shifting the primary patological process from vessels to brain tissue [2–4]. Changes in cerebral blood flow, after the period of silence are again in focus, now from the point of epiphenomena, or surrogat markers of headache and its progression [5].

Examination of cerebrovascular reactivity in patients with primary headaches is focused mainly on migraine, while the smaler number of studies deals with tension-type headache, or comparison between cerebral haemodynamic in migraine and tension-type headache (TTH) [6–11].

Cerebrovascular reactivity in patients with migraine have been widely examined by different metodology and different conclusions were brought. [12–18]. The key point of those differences was whether the vasoconstrictor or vasodilator stimuli was used. The results of several studies performed with vasoconstrictor stimuli indicated an increased cerebrovascular reactivity in patients with migraine. These conclusions had been limited by results showing an increased cerebrovascular reactivity exclusively in patients with migraine with aura. The results of the studies using vasodilator stimuli are contradictory, probably due to differences in methodology and patients selection.

Cerebrovascular reactivity in patients with tension-type headache has been the subject of a significantly smaller number of researches [8, 11, 14]. The difference in interictal cerebrovascular reactivity in patients with migraine without aura and episodic TTH was not found [9]. That kind of difference did not show neither by comparison of patients with TTH and healty controls [13, 14].

In patients with cluster headache cerebrovascular reactivity had been examined during the cluster period, after the inhalation of 100% oxygen [19]. In comparison to migrainers, patients with cluster headache had increased response. Comparative data about cerebrovascular reactivity in more than 2 types of episodic primary headaches does not exist.

In this study, we hypothesized that cerebrovascular reactivity differs among different types of episodic primary headaches. In order to prove that, we aimed at comparing the interictal cerebrovascular reactivity in the patients with the episodic form of the 3 most common types of primary headaches using the breath holding test.

Methods

Examination was performed at the Headache Center and Ultrasound Laboratory in the Neurology Clinic, Clinical Center of Serbia, Belgrade, over 5 groups of subjects were treated for the period of two years: migraineurs with aura (group I), migraineurs without aura (group II), patients with episodic TTH (group III), patients with episodic form of cluster headache (group IV) and healthy controls who had no history of headache (group V). Exclusion criteria were cardiovascular, cerebrovascular, or pulmonary disease, arterial hypertension, therapy with beta-adrenoceptor blockers or calcium antagonists in the last three months, comorbidity of 2 types of primary headaches.

All patients and healthy control subjects gave their informed consent to participate in the study and the study was approved by the Ethics Committee of Neurology Clinic, Clinical Center of Serbia, Belgrade.

The diagnosis of episodic primary headache was based on the International Classification of Headache Disorders criteria [20]. The Doppler instrument, RIMED Digi-Lite, a dual-channel transcranial Doppler (TCD) system, was used for TCD sonography and breath-holding test performance. Insonation was performed interictaly, throughout the temporal acoustic bone windows according to a standard approach using 2 MHz transducers to display flow through the middle cerebral artery (MCA). Bilateral monitoring of the MCA, from a depth of 45 mm to 65 mm, was performed with each probe held in place over the temporal bone by the head frame.

Cerebrovascular reactivity has been examined by breath-holding test, based on vasodilatator effect of hypercapnia resulted after 30 seconds of breath holding [21, 22].

Blood flow mean velocities and pulsatility indices were recorded before (MV1, PI1) and after (MV2, PI2) 30 seconds of breath holding.

Breath-holding index (BHI) was calculated for each MCA, using the formula [15]:

$$\text{BHI} = \frac{MV1 - MV2}{MV1} \; x \; \frac{100}{30}.$$

Mean value of BHI was calculated using the formula

$$\text{BHI} = \frac{BH1\,right + BH1\,left}{2}.$$

Blood flow mean velocities (MV), pulsatility indices (PI) and breath-holding index (BHI) for the middle cerebral artery among these groups were analyzed.

Statistical analysis was performed using the SPSS software version 17.0. Distrubution of parameters was assessed by Kolmogorov–Smirnov test. For multiple comparisons among the groups, ANOVA and Kruskal-Wallis test were used, with Tukey honest significance difference (HSD) test and Mann-Whitney test applied in *post hoc* analyses. The significance level was set at 5% ($p < 0.05$).

Results

A total of 243 patients were studied, including 100 migraineurs with aura (group I), 70 migraineurs without aura (group II), 38 patients with episodic tension-type headache (group III), 35 patients with episodic form of cluster headache (group IV) and 35 healthy controls (group V). Demographic features of examined groups are presented in Table 1.

The mean velocities and pulsatility indices were not different in 4 groups of headache patients and controls (Tables 2 and 3).

Mean velocities for MCA in examined groups are presented in Table 2.

Pulsatility indices for MCA in examined groups are presented in Table 3.

BHI was higher in patients with migraine with aura than in migraine without aura, episodic TTH, cluster headache and healthy controls (Table 4). No difference was found among the other groups regarding BHI.

Discussion

Diferent results of cerebrovascular reactivity in migraine might be caused by the diferences in patient selection and methodology. Beside heterogenous data, the result of higher cerebrovascular reactivity in migraine with aura has remained stable over decades of research [23]. Results of our study show that BHI is higher in patients who have migraine with aura than in patients with other types of primary episodic headaches, migraine without aura, episodic

Table 1

Demographic features of the examined groups

Features	MA n = 100	MO n = 70	TTH n = 38	CH n = 35	Controls n = 30
Male, n (%)	29 (29)	9 (12.9)	17 (44.7)	25 (71.4)	17 (56.7)
Age at the time of examination (years), r ± SD	33.75 ± 10.980	38.07 ± 10.136	41.08 ± 12.782	43.74 ± 12.195	35.23 ± 8.386
Age at the time of headache onset, (years) r ± SD	20.04 ± xx.953	20.33 ± 1.134	32.24 ± 1.806	34.23 ± 1.999	–

***MA – migraine with aura; MO – migraine without aura; TTH – tension-type headache; CH – cluster headache.**

Table 2

Mean velocities (MV) in arteria cerebrimedia in the examined groups

Groups	MV (cm/s), r ± SD	*p*-value
MA (n = 100)	56.395 ± 10.817	*vs* MO: 0.500; *vs* TTH: 0.649; *vs* CH: 0.320; *vs* C: 0.552
MO (n = 70)	57.100 ± 10.98	*vs* TTH: 0.789; *vs* CH: 0.157; *vs* C: 0.281
TTH (n = 38)	53.723 ± 11.937	*vs* CH: 0.957; *vs* C: 0.799; *vs*
CH (n = 35)	53.571 ± 11.146	*vs* C: 0.960
C (n = 30)	55.851 ± 11.927	

***MA – migraine with aura; MO – migraine without aura; TTH – tension-type headache; CH – cluster headache; C – controls; r – mean value; SD – standard deviation; MV – mean velocity.**

Table 3

Pulsatility indices (IP) in the examined groups

Groups	IP, r ± SD	*p*-value
MA (n = 100)	0.728 ± 0.175	*vs* MO: 0.061; *vs* TTH: 0.734: *vs* CH: 0.970; *vs* C: 0.552
MO (n = 70)	0.677 ± 0.120	*vs* TTH: 0.209; *vs* CH: 0.168; *vs* C: 0.168
TTH (n = 35)	0.648 ± 0.200	*vs* CH: 0.264; *vs* C: 0.911
CH (n = 35)	0.722 ± 0.117	*vs* C: 0.810
C (n = 30)	0.720 ± 0.155	

***MA – migraine with aura; MO – migraine without aura; TTH – tension-type headache; CH – cluster headache; C – controls; r – mean value; SD – standard deviation; MV – mean velocity.**

Table 4

Breath-holding index (BHI) in the examined groups

Breat-holding index	MA n = 100	MO n = 70	TTH n = 38	CH n = 35	Controls n = 30	*p* value
BHI (ACM), r ± SD	1.668 ± 0.269	1.411 ± 0.358	1.401 ± 0.428	1.203 ± 0.311	1.195 ± 0.269	< 0.01*

***MA – migraine with aura; MO – migraine without aura; TTH – tension-type headache; CH – cluster headache; BHI – breath-holding index; ACM – *a. cerebri media*; **Mann-Whitney Test, Asymp. Sig. (2-tailed): MA and MO, 0.005; MA and TTH, 0,35, MA and CH, < 0.01, MA and control, < 0.01; MO and TTH, 0.971, MO and CH 0.059, MO and control 0.080, TTH and CH 0.075, TTH and control 0.088, CH and control 0.912.**

TTH and cluster headache. These data confirm results of previous studies reporting an increased cerebrovascular reactivity exclusively in patients with migraine with aura in comparison with migraineurs without aura [12, 14]. Also, our data do not confirm literature reports of increased vasodilatatory response in migraine without aura [12]. This difference is just one among other, epidemiological and clinical differences between these two entities imposing the question older more than thirty years, whether the migraine with and without aura are two kinds of headache disorder [24]. In our group of patients with migraine with aura, one third of them had migraine without aura as well. Beside that, the cerebrovascular reactivity was significantly higher in this group of patients.

Potential explanation for increased cerebrovascular reactivity in migraine, particulary with aura, lies in hypersensitivity and impaired habituation to stimuli [23, 25]. Literature data, in accordance with our results, suggest that the autoregulation disorder leading to inadequate, increased response of intracranial arteries to metabolic stimuli could be the key feature for increased cerebrovascular reactivity [25]. According to neurovascular coupling theory [26, 27] cerebral blood flow varies due to local cortical activity. Intraictally impaired cerebrovascular reserve could point to disfunction of vascular elements of neurovascular unit, meaning pericits, muscle and endothelial cells contained in the wall of small vessels [28]. Endothelial dycfunction in migraine is the new question arrised just few years ago, with increasing number of ongoing researches dealing with it [29].

To our best knowledge, this is the first study on cerebrovascular reactivity covering and comparing, at the same time, the episodic forms of the 3 most common types of primary headaches.

There are studies showing no difference in cerebrovascular reactivity in patients with migraine without aura in comparison to patients with tension-type headache and healthy controls[14]. Our study showed the same result, overcoming the limitation of broad overlap between migraine and TTH data that were presented in a report of Arjona et al. [14], with strict patient selection.

The results considering interictal cerebrovascular reactivity in patients with cluster headache, being significantly lower in comparison to migraine with aura, and showing no difference to migraine without aura, tension-type headache and healty subjects, are in accordance with conclusions of other authors [19] who found significant difference in vasomotor reactivity between CH patients and controls in response to hypocapnia only during the headache phase, with difference disappearing 30 min after the attack. Recent study showed that the BHI mesured after the oxygen inhalation is significantly higher in the cluster patients compared to the migraine patients which is the conclusion that our study could not support. Opposite to CO_2, a powerful vasodilatatory stimulus, oxygen is a powerful vasocontrictor and its inhalation just before the testing of cerebrovascular reactivity by breath-holding test, without doubt affects the test results. Beside vasoconstriction, with direct impact on vessels, 100% oxygen influence the cerebral blood flow indirectly, by inhibition of neurons in the trigeminocervical complex. This „oxygen inhibition“ of neuronal activation in the trigeminocervical complex is shown on an animal model for cluster headache, developed in order to reveal therapeutic effect of oxygen in cluster headache [30].

Conclusion

In conclusion, our finding support the literature data that increased cerebrovascular reactivity is a feature of migraine with aura. Result of unchanged cerebrovascular reactivity in migraine without aura, cluster headache and tension-type headache is expected, still, it is possible that in future, with different technique, we will be able to put more light on vascular changes that follow different headache disorders.

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Rhinoplasty without nasal packing and splinting

Rinoplastika bez tamponade nosa i bez udlage

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Abstract

Background/Aim. Most surgeons, at the end of corrective rhinoplasty, use endonasal tamponade and external splinting, primarily because of hemostasis and immobilization. Possible complications of this surgery are various. Pain, nose edema, palpebral swelling and ecchymosis, are the most common and usual. The aim of our study was to evaluate the incidence of nonaesthetic complications and the efficiency of corrective rhinoplasty without the use of tamponade and external splint. **Methods.** One hundred and fifty-one patients, who underwent primary corrective rhinoplasty without endonasal tamponade and without an external splint, were analyzed at the Clinical Centre "Kragujevac" in Kragujevac, Serbia, in the period 1999–May 2016. The surgeries were done by the same surgeon. Instead of a splint, sterile skin adhesive tapes were used. Study was prospective, consecutive case-series type. We analyzed the possible complications and subjective estimates of the patients who underwent primary corrective rhinoplasty in described fashion. In assessing postoparative pain, the visual analogue scale (VAS) in a range of 1 to 5 was used. Palpebral swelling and ecchymosis, were estimated 24h after surgery, by the Surgeon Periorbital Rating of Edema and Ecchymosis (SPREE) scale ranging from 0 to 5. The degree of restriction of nasal respiration was evaluated by the scale 1–4. The overall comfort of patients in the postoperative period was evaluated according to a scale: good, no opinion, bad. The patient satisfaction with the aesthetic result was analyzed on 7th and 30th day after surgery, by a scale from 1 (very satisfied) to 5 (very dissatisfied). **Results.** There were 151 patients aged between 18–47 years. Females were more frequent (72.18%). Most of the patients (40.39%) had moderate pain. None of the patients had neither severe nor the worst pain and 59 patients had no pain at all. Eyelid edema and periorbital ecchymosis were moderate in all patients (100%). The other complications did not occur, apart from one (0.66%) unilateral epistaxis, on postoperative day 10. Most of the patients (52.97%), immediately after surgery, could freely breathe through the nose. The general impression of the patient comfort after surgery was mainly good (74.17%). The majority of patients (52.28%), were satisfied with aesthetic result after 7 days, and 52.32% after 1 month. There were very satisfied patients: on day 7 - 27.15% and on day 30 - 39.73%. **Conclusion.** We concluded that the rhinoplasty without using tamponade and immobilization was safe, comfortable and economical. The degree of pain, edema and ecchymosis were low, as well as the incidence of other complications.

Key words:
rhinoplasty; surgical procedures, operative; postoperative period; postoperative complications; patient satisfaction.

Apstrakt

Uvod/Cilj. Većina hirurga, na kraju korektivne rinoplastike, koristi endonazalnu tamponadu i spoljašnju udlagu prvenstveno radi hemostaze i imobilizacije. Moguće komplikacije ove operacije su različite i najčešće su bol, otok nosa, i otok sa krvnim podlivima očnih kapaka. Cilj naše studije bio je da se proceni učestalost neestetskih komplikacija i efikasnost korektivne rinoplastike bez tamponade i spoljašnje udlage. **Metode.** Sprovedena je analiza sto pedeset i jednog bolesnika u Kliničkom centru "Kragujevac" u Kragujevcu, Srbija, u periodu od 1999. godine do maja 2016. godine, koje je operisao isti hirurg i uradio primarnu korektivnu rinoplastiku bez endonazalne tamponade i bez spoljašnje udlage. Umesto splinta, korišćene su adhezivne sterilne kožne trake. Prospektivnom studijom, tipa uzastopne serije slučajeva, analizirali smo moguće komplikacije i subjektivne procene bolesnika kod kojih je urađena primarna korektivna rinoplastika na opisani način. Za procenu postoperativnog bola korišćena je vizuelna analogna skala (VAS). Dobijeni rezultati rangirani su od 1 do 5. Za procenu otoka i krvnih podliva očnih kapaka 24 sata posle operacije, primenjena je skala hirurškog periorbitalnog rejtinga otoka i ekhimoza (SPREE). Rezultati su rangirani od 0 do 5. Stepen restrikcije disanja kroz nos evaluiran je u skali od 1 do 4. Opšti komfor u postoperativnom periodu operisani su oce-

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nili kao: dobar, neodređen, loš. Zadovoljstvo bolesnika estetskim rezultatom je analizirano sedmog i tridesetog dana od operacije, na skali od 1 (veoma zadovoljan) do 5 (veoma nezadovoljan). **Rezultati.** Analiziran je sto pedeset i jedan bolesnik, starosti od 18 do 47 godina, a najviše je bilo mlađih bolesnika (prosečno 23,19 godina). Zastupljenost ženskog pola (72,18%) je bila veća, Umeren bol imalo je 47,02% bolesnika. Nijedan od njih nije imao jake ili najteže moguće bolove, a 32,45% bolesnika nije uopšte imalo bolove. Otok očnih kapaka i periorbitalne zone bili su umereni kod svih bolesnika (100%). Ostale komplikacije nisu postojale, osim unilateralne epistakse kod jednog (0,66%) bolesnika, desetog postoperativnog dana. Najveći broj operisanih (52,97%) je odmah posle operacije mogao normalno da diše kroz nos. Bolesnici su opšti komfor nakon operacije uglavnom opisivali najvećom ocenom (74,17%). Većina bolesnika bila je zadovoljna estetskim rezultatom posle sedam dana (58,28%) kao i posle mesec dana (52,32%). Bilo je veoma zadovoljnih bolesnika, sedmog dana - 27,15% i tridesetog dana – 39,73%. **Zaključak.** Smatramo da je rinoplastika bez tamponade i imobilizacije bezbedna, ugodna i ekonomična. Intenzitet bola, otoka i ekhimoza je nizak, kao i učestalost drugih komplikacija.

Ključne reči:
rinoplastika; hirurgija, operativne procedure; postoperativni period; postoperativne komplikacije; bolesnik, zadovoljstvo.

Introduction

Rhinoplasty is one of the most common aesthetic surgeries. The most surgeons use nasal packing and external immobilization with splint because of hemostasis and fixation of the operated cartilages and bones [1–5]. In addition, nasal packing is used to prevent mucosal adhesions, and for that purpose, different materials are used, usually paraffin gauze [3–12]. For external fixation, plaster of Paris is most commonly used [3–23]. There are many complications in corrective rhinoplasty that may occur [1, 2, 4]. The postoperative period is accompanied by edema of the nose and glabella, with hematoma in the periorbital region, which are considered as less important, but represent common complications. After nasal tamponde, a patient breathes through the mouth, which is followed by drying of the upper respiratory tract and sometimes by a stimulatory cough. The patients describe the presence of nasal packing, especially its removal, as unpleasant and sometimes painful. There are cases of tamponade migration toward the pharynx causing palate irritation and vomiting as well as allergic or toxic complications. In addition, in a case of a postoperative infection, diagnosis is difficult. During external immobilization, skin lesion of the nose may occur due to compression which is noticed in practice only after the removal of the splint.

Assuming that tamponade of the nose and external immobilization in corrective rhinoplasty are not necessary and that some of the complications of this surgery can be brought in connection with these proceedings, we set a goal to perform corrective rhinoplasty without nasal packings and external immobilization with rigid materials and to analyze the results of these types of operations.

Methods

Prospective clinical study of the consecutive cases series was conducted at the Center for Plastic Surgery, Clinical Center “Kragujevac” in Kragujevac, in the period from 1997 to May 2016, after rhinoplasty without nasal packings and immobilization of the nose with rigid materials, but only with skin adhesive tapes.

All the surgeries were done by the same surgeon and under general endotracheal anesthesia. The closed method of rhynoplasty was used with intercartilagineous, transcollumelar and piriform incisions. Reduction of the lateral cartilage, caudal and dorsal edge of the septum, bone and cartilage resection of the “hump” were done as well as lateral osteotomy and resection of the lateral crus of alar cartilages. At the end of the surgery, all endonasal incisions were sutured with fast absorbable sutures 4-0 with a cutting needle 3/8, and sterile adhesive skin tapes were placed on the nose (Figure 1). None of the patients had a nasal tamponade or an external immobilization. Antibiotic therapy was not applied neither preoperatively, nor postoperatively. In the postoperative period, head and chest were elevated to 30°, hygiene and disinfection of the anterior part of the nasal tunnels were carried out and oral analgesics were prescribed

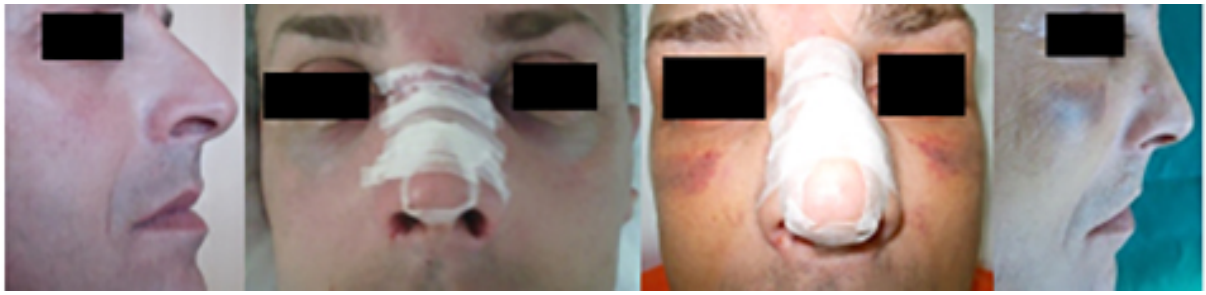

Fig. 1 – a) before surgery; b) 24h after surgery; c) day 3 after surgery; d) day 7 after surgery.

only in the case of the pain. The patients were dismissed from the hospital after 24 h. Check-ups were performed on postoperative days 3 and 7 when the adhesive tapes were removed. After that, check-ups were performed after 1 month, 3 months and 1 year. All patients were photographed before and after surgery (after 24 h and on the day 7).

The exclusion criteria in the study were: minors, patients with diseases or scarred skin of the nose, persons who previously had this surgery, people with severe forms of systemic diseases and psychiatric patients. Also, the exclusion criteria were cosmetic surgery patients who had, unrealistic requests, for example, thinking that the surgery would make him/her become another person or would solve all their problems, patients who wanted their nose look like some other person's nose, patients who wanted to undergo surgery because their family members pushed them to do so or patients who did not know what they exactly wanted.

In assessing the outcome, 6 following methods were used: analysis of the occurrence and type of postoperative complications; analysis of the pain intensity; a degree of eyelid edema and ecchymosis 24 h after the surgery; a degree of restriction of nasal respiration; the overall comfort of the patient after the surgery and patient satisfaction with the aesthetic result, on the day 7 after the surgery and after removing the skin strips and after 1 month.

In assessing posteparative pain, after 24 h, the visual analogue scale (VAS) was used, in a range from 1 to 10: no pain, mild pain, moderate pain, severe pain and the worst pain possible. Patients were asked "Do you have any pain?". If the answer was "Yes", patients were asked to record the pain level in the scale chart (Figure 2).

Palpebral swelling and ecchymosis, 24 h after the surgery, was recorded by the surgeon using a Surgeon Periorbital Rating of Edema and Ecchymosis (SPREE) scale, from 0 to 5 (Table 1, Figure 3).

The degree of restriction of nasal respiration was investigated by using the questionnaire with 4 possible answers: easy breathing through both nostrils, breathing

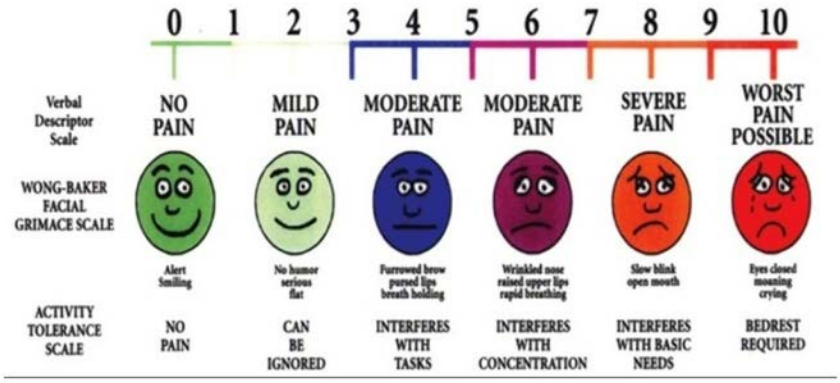

Fig. 2 – Pain assement by visual analog scales.

Table 1

Surgeon Periorbital Rating of Edema and Ecchymosis (SPREE) questionnaire within 24 h

Score	Description
0	No ecchymosis. No edema.
1	Up to medial one third of the lower and/or upper eyelid. No coverage of iris with eyelids.
2	Medial half of the upper and/or lower eyelid. Slight coverage of iris with swollen eyelids.
3	Up to the full length of the lower and/or upper eyelid. Full coverage of iris with swollen eyelids.
4	Entire part of the lower and upper eyelid and/or conjunctiva. Full coverage of eyes.
5	Extension of ecchymosis below the malar bone.

Fig. 3 – Degrees 1–4 in Surgeon Periorbital Rating of Edema and Ecehymosis (SPREE).

through one nostril, difficult breathing through both nostrils, inability to breathe through the nose.

The overall comfort of the patient after surgery was examinated so that the patients answered using these three options: good, no opinion, bad.

The satisfaction with the aesthetic result was assessed by the surgeon, after verbal communication with patients, on the day 7 after surgery, when the adhesive sterile skin tapes were removed, and after 1 month. The patient satisfaction was analyzed using 1 to 5 scale: very satisfied, satisfied, without opinion, dissatisfied and very dissatisfied (Table 2).

The results were analyzed by descriptive statistics, showing the percentage of certain categorical variables.

Results

The total of 151 rhynoplasties without nasal packing and without splinting were done. All the patients were Caucasians. The age ranged from 18 to 47 years and majority of patients were young (on average 23.18%). Females were significantly more prevalent (72.18%).

There were no complications in the immediate postoperative period of 24 h after surgery. In one (0.66%) female patient, unilateral epistaxis occurred at the 10th postoperative day and was resolved by a one-day tamponade (Figure 4).

The majority of patients (54.97%) had easy breathing through both nostrils, immediately after surgery. Thirty-three (23.84%) patients could easily breathe through one nostril and in 20 (13.25%) patients difficult breathing through the both nostrils was present. The smallest group consisted of 12(7.95%) patients, with inability of breathing through nose.

The general impression about the overall comfort after the surgery was mainly good in the majority (74.17%) of patients. Thirty-four (22.52%) patients had no opinion about postoperative comfort and 5 (3.31%) patient found it bad.

On the seventh day after rhinoplasty, when sterile skin adhesive tapes were removed, the majority (58.28%) of patients were pleased with the aesthetic result. Among them, 41 (27.15%) very were satisfied. The rest (14.57%) of the patients had no clearly defined opinions. There were no dissatisfied and very dissatisfied patients. After 1 month, the results were different (Table 2). There were 19 (12.6%) more very satisfied patients, 9 (5.96%) less satisfied ones and much less (12.58%) patients without opinion. Unlike the 7th postoperative day, 8 (5.29%) patiens were dissatisfied and 1 patient was very dissatisfied.

Discussion

The goal of any surgery, especially aesthetic surgery, is to achieve maximum result, without complications and with high comfort of the operated patient. Many patients, when they come for the first time to the surgeon with a desire to do rhinoplasty, are already informed about the unpleasant postoperative period, because of the tamponade, external immobilization, swelling, hematoma and pain. Some of these patients frequently asked questions related to the time of the tamponade removal. According to literature data, there are different opinions about it, and this period rates from 1 to 10 days [24].

Table 2

The patients satisfaction after 7th day and one month after surgery

Degree of satisfaction	Number of patients	
	7th day	One month
Very satisfied	41	60
Satisfied	88	79
Without opinion	22	3
Dissatisfied	0	8
Very dissatisfied	0	1

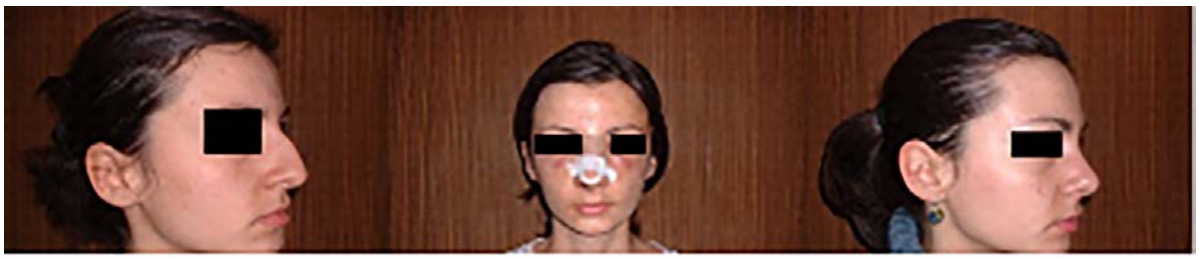

Fig. 4 – Left: patient before surgery; In the middle: 3rd postoperative day; Right: 7th postoperative day after tapes removal.

Analysis of the pain intensity, showed that most (40.39%) patients had mild pain and 39.07% of the patients had no pain at all. The smallest group (20.54%) had moderate pain. No patient had severe, nor the worst pain possible.

The extent of eyelid edema and periorbital ecchymosis, measured at the scale 0–5, was in all patients (100%) in the category 1: up to medial one-third of the lower and/or upper eyelid; no coverage of iris with eyelids.

The fact that the tamponade of the nose and external fixation in rhinoplasty are performed by using different materials, it can be concluded that there is no ideal method. For tamponade, the paraffine gauze is used, the gauze with antibiotic ointment or tampons made from other materials such as polypropylene [8], polyethylene oxide gel [9, 10], respiratory tubes, vaginal tampons [11], X-ray film [13] and others. For external fixation, nasal splints that are most commonly used are plaster of Paris, metal and various plastics [16–23].

There are many possible complications of rhinoplasty [1, 2, 4, 25–38]. Some of them are in fact inevitable, such as swelling of the nose and bruises in the periorbital region, and sometimes subconjunctival ecchymosis. Other complications include bleeding, dislocation of the bone and cartilage structures, mucosal synechiae and perforation of the nasal septum. As a result of the nasal tamponade, postoperative nasal obstruction may occur due to nasal valve collapse with inspirium or allergic rhinitis with chronic nasal mucosal edema. There are other rare complications, such as necrosis of the skin of the nose and eyelids, subcutaneous emphysema with possible propagation to the mediastinum, blindness due to central retinal artery occlusion, lesions of the lacrimal system, neuromas, numbness on the nose, hiposmia, rhinoliquorrhea, endocranial and some other complications like cardiac arrest due naso-cardiac reflex or discoloration of incisors. The second group of complications include those of aesthetic character and they are often a result of the disproportion of the cartilage-bone structure.

An extreme early complication of rhinoplasty is usually bleeding within the first 24 h of surgery. This is one of the reasons why most surgeons use tamponade and firm external fixation of the nose. In order to prevent dislocation of the operated structures, besides tamponade, most surgeons use splinting at the end of the surgery. In practice, we have noticed that this immobilization after a few days becomes inadequate, because of the resolution of edema, and that immobilization loses its meaning. There are described cases of skin roughness or erosion after the splint removal due to compression. Moreover, there are cases of conjunctival irritation with gypsum particles. According to relevant literature, there are numerous complications of endonasal tamponade [22–30]. Besides being uncomfortable for a patient, nose tamponade may cause difficulty in breathing, odor and pain. There may also occur hypoxia, obstructive apnea, prolonged rhinorrhea, pressure changes in the middle w reduced drainage in the case of the wounds infection. There are findings that suggest foreign body reaction, formation of mucous cyst, allergic complications and paraffin cyst when paraffin tamponade is used and endonasal incisions are not completely sutured. An alergic complications are possible as well as toxic shock syndrome (TSS). Toxic shock syndrome is an acute, drastic, multisystem disease that can occur in various pathological conditions. It has been described after corrective rhinoplasty too, mainly when the nose was tamponaded [33–36]. TSS is usually manifested by hypotension, nausea, vomiting, diarrhea and erithrodermia.

After tamponade of the nose, migration is possible towards the pharynx because of the inspirium reflex, vomiting may also occur as well as aspiration or ingestion of tampons and even bowel perforation [29–38]. Sometimes, we can see in practice, that some patients try to take out the tamponade in the postoperative period. According to some studies [39–44], there were significantly more postoperative pain, headache, epiphora, dysphagia, and disturbed sleep at night after surgery in patients whose nose was tamponaded. After a few days, due to potentiated rhinorrhea, nasal tamponades and poor hygiene, an unpleasant odor started to appear. In addition, the length of the tamponade itself is inconsistent, and is rather based on an individual assesment of the surgeon [24].

The removal of a nasal packing is uncomfortable and mostly painful. There are recommendations to use some kind of anesthesia during the removal of the tamponade [25–27]. Besides, the nose tamponade is often the reason for preventive use of antibiotics, which sometimes results in longer hospitalization.

Stucker and Ansel [38] were the first to suggest that nasal packing should not routinely be used because of possible complications. Guyuron [39] has released the results of a study on patients who underwent septorhinoplasty and concluded that complication rate was lower in those with the nose tamponade. Several studies were done later with suggestion that the nose tamponade did not give an effect [40–44]. Camirand [41] and Camirand et al. [42], concluded that it was not necessary to do even external nasal immobilization and that the septoplasty could be done in the same way.

In our study, epistaxis did not occur immediately after the surgery in any of the patients.

Splint is used to immobilize the bone and septum and to decrease the pain and swelling. Our results are the same as the results of Camirand [41] and Camirand et al. [42], because there were no bone and septal displacements or excessive pain and swelling. The placement of adhesive sterile skin tapes was quite enough for adequate nose fixation, because the nose does not have joints. Good example in support of this opinion is a fact that even when a fracture without dislocation of the bone that has the joints, such as the phalanx of a hand, fixation with the tape to the adjacent finger is quite sufficient. Nasal packing is used to maintain immobilization and hemostasis, to prevent bleeding, septal hematoma and necrosis as well as to prevent synechiae between the septum and lateral nasal wall. Our results differ from those of Guyuron [39], but they are the same as results of many other researchers [41–51] because postoperative bleeding occurred in only 1 patient on the day 10 after surgery, when the most surgeons already removed internal packing. Also, there were no cases of the bone and septal displacements, septal hematoma, septal necrosis and synechiae. We found that this technique of rhinoplasty reduced pain, extent of eyelid edema and periorbital ecchymosis and discomfort of the patients. This is in correlation with the results of many other authors [38, 41–51]. We suggest that corticosteroid therapy and the use of lidocaine in prevention of the edema and ecchymosis in rhinoplasty [46, 48, 52] are not necessary, when using this technique of rhinoplasty.

We believe that the prevention of bleeding after rhinoplasty, apart from general surgical principles such as meticu-

lous surgical technique, osteotomy with as less trauma as possible, is very important, by using the small diameter osteotomes, but large enough for the purpose of surgery. In the same meaning, digital compression, for a few minutes immediately after the osteotomy, may be very useful. We believe that it is important that all of the endonasal incisions should be sutured at the end of surgery. We also believe that it is important that during the surgery and on waking up from anesthesia, the patient has controlled blood pressure and elevated head. Of course, preoperative selection of the patients is very important, as it is for any cosmetic surgery, especially in the context of understanding the possible complications and importance of cooperation, in order to prevent certain complications.

Conclusion

On the basis of our results, we can conclude that the nose tamponade and external immobilization are not necessary in corrective rhinoplasty. Comfort of surgery is higher, and because the patient can breathe through the nose, oxygenation is better. There is less pain, swelling and ecchimosis of the nose and the surrounding regions. Also, incidence of complications is lower. The method we have described is considerably more economical because the operative time is shorter, the usage of medical supplies is lesser, there is no need for antibiotics and other preventive therapy and period of hospitalization is shorter.

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The assessment of personality dimensions, tobacco smoking and depression among treatment-seeking male alcoholics

Procena dimenzija ličnosti, pušenja duvana i depresije kod lečenih muških akoholičara

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Abstract

Background/Aim. The co-occurrence of depression and tobacco smoking among treated alcoholics is frequent, but understudied. Some findings suggest that there are some shared etiological factors, but a few clinical researches of personality dimensions among patients with these comorbidities were done. The personality dimensions, the pattern of cigarette use and depression and correlation of personality and depression among inpatient alcoholics were explored. **Methods.** One hundred primary male inpatient alcoholics were consecutively recruited. The eighty-six of them completed study and were compared with 30 age-matched, healthy male subjects. A semi-structured clinical interview related to sociodemographics, the pattern of cigarette and alcohol use and family history data was applied. According to cut-off on the Hamilton Depression Rating Scale (HDRS), the alcoholics were divided into depressive and non-depressive subgroups resulting in half of alcoholics in each subgroup. The Eysenck personality questionnaire (EPQ) was completed. Student's *t*-test for differences and Pearson's test for correlation were used. **Results.** There were no significant sociodemographic differences between groups. Alcoholics were more frequent smokers (86% vs. 50%). They did not start drinking earlier, but they started smoking earlier, with higher daily cigarettes use than controls. On average, alcoholics had mild depression after detoxification. The personality dimensions did not show differences between groups, except neuroticism. The neuroticism showed significantly higher level among alcoholics vs. controls (12.72 ± 5.19 vs. 5.00 ± 3.36 respectively) and among depressive vs. non-depressive alcoholics (15.07 ± 4.89 vs. 10.37 ± 4.40 respectively). The depression correlated only with neuroticism (r = 0.487, p < 0.001). **Conclusions.** The majority of detoxified alcoholics were smokers who started smoking earlier, with mild depression and higher neuroticism compared to controls. Our results suggest that the alcoholics with high neuroticism may experience higher depression and may require more intensive integrative treatment.

Key words:
alcoholism; men; smoking; depression; neurotic disorders; comorbidity.

Apstrakt

Uvod/Cilj. Kod lečenih alkoholičara česta je, mada nedovoljno ispitana, udružena pojava depresije i pušenja cigareta. Neke studija pokazuju da postoje zajednički etiološki faktori kod bolesnika sa navedenim komorbiditetom, ali su dimezije ličnosti retko istražene. U radu su ispitane dimenzije ličnosti, depresija, obrasci upotrebe alkohola i cigareta, kao i povezanost dimenzija ličnosti i depresije kod hospitalizovanih alkoholičara. **Metode.** U studiju je bilo uključeno 100 konsekutivno hospitalizovanih alkoholičara. Ispitivanje je završilo 86 alkoholičara koji su bili poređeni sa trideset zdravih, kontrolnih ispitanika. Ispitanici su bili muškog pola, upareni po starosti. Sociodemografski podaci, obrasci upotrebe alkohola i cigareta i podaci iz porodične anamneze prikupljeni su pomoću semistrukturisanog upitnika. Posle četiri nedelje apstinencije, alkoholičari su na osnovu cut-off skora na Hamiltonovoj skali za depresiju (HAMD) razvrstani u dve grupe: depresivni i nedepresivni alkoholičari. U svakoj grupi se nalazila polovina ispitanika. Primenjen je Ajzenkov upitnik ličnosti (EPQ). Međugrupne razlike ispitane su pomoću Studentovog *t* -testa, a korelacije pomoću Pearson-ovog testa. **Rezultati**: Nije bilo značajnih

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sociodemografskih razlika između grupa. Alkoholičari su češće pušili više cigareta dnevno nego kontrolni ispitanici (86% vs. 50%). Alkoholičari nisu ranije počeli da piju, ali su ranije počeli da puše, sa većim brojem cigareta dnevno nego kontrolni ispitanici. Nakon detoksikacije alkoholičari su imali depresiju koja je bila prosečno blagog stepena. Osim neuroticizma, nije bilo razlike u dimenzijama ličnosti između grupa. Neuroticizam je bio značajno veći kod alkoholičara u odnosu na kontrolne ispitanike (12.72 ± 5.19 vs. 5,00 ± 3.36) i kod depresivnih u odnosu na nedepresivne alkoholičare (15.07 ± 4.89 *vs* 10.37 ± 4.40). Depresija je bila značajno povezana samo sa neuroticizmom ($r = 0.487$, $p < 0.001$). **Zaključak.** Većina detoksikovanih alkoholičara su pušači, ranije počinju da puše, depresivni su u blagom stepenu i imaju visok neuroticizam u poređenju sa kontrolnim ispitanicima. Naši rezultati ukazuju na to da alkoholičari sa visokim neuroticizmom mogu razviti težu depresiju i zahtevati intenzivnije integrativno lečenje.

Ključne reči:
alkoholizam; muškarci; pušenje; depresija; neurotski poremećaji; komorbiditet.

Introduction

There is increasing interest of researchers and clinicians in heterogenity of alcohol dependent patients, especially of those with comorbid conditions. The findings from epidemiological study on the prevalence of lifetime alcohol dependence was 12.5% and the prevalence of nicotine dependence among alcoholics (Alc) was 45%, which is over two times higher than in general population [1]. Smoking is a major health issue in persons with a lifetime history of depression who are twice as likely to smoke as those who do not suffer from depression [2]. Co-occurance of mental disorders, such as alcohol use disorders (AUD), major depression, and nicotine dependence are increasingly common among patients in the clinical settings but understudied and present challenges for treatment [3].

The complex, self-sustaining relationship between alcohol and tobacco dependence and depression is partly explaned by self-medication attempt by a person who may smoke or drink to alleviate depression [4]. On the contrary, the long-term use of alcohol and nicotine can decrease levels of brain serotonin production and thus might worsen depression [5]. The comorbidy of alcoholism and depression is high and may be related to effects of alcohol on neurotransmitters involved in mood regulation, but nicotine has been demonstrated antidepressant effects due to counteracts alcohol-induced depression in preclinical as well as clinical studies [6]. This interaction may also be a contributory factor to drinking-smoking comorbidity [7]. Post-treatment depressive symptoms are related to shorter periods of abstinence and more frequent drinking [8].

Comorbidity of alcohol dependence with other substance abuse appears as a part to unique etiology factors underlying each substance use disorder. However, comorbidity of alcoholism with anxiety, mood and personality disorders were explained by shared aethiological factors, but a few clinical studies of personality traits among patients with these comorbidities were done [9].

Some researches reported that smoking behavior in general population is linked to personality traits and negative emotionality, but it is unknown whether these traits are related to alcoholics who smoke [10]. Previous research has indicated that specific relations exist between individual personality traits and alcoholism [11]. Exploration of these complex associations among drinking, smoking and depression is important because depression can complicate comorbid alcohol and nicotine dependences by exacerbating the negative affect during early abstention from one or both drugs [12].

The aim of this study was to explore the personality traits, cigarette smoking and depression among treatment-seeking Alc and correlations between personality traits and depression.

Methods

Subjects and procedures

The cross-sectional study was performed at the Clinic for Psychiatry of the Military Medical Academy (MMA), Belgrade. The sample included consecutively recruited 100 treatment-seeking male inpatient Alc, aged between 25 and 60 years. The total final sample consited of 86 Alc who completed study. Inclusion criteria were alcohol dependence syndrome diagnosed according to the Diagnostic and Statistic Manual of Mental Disorders (DSM)-IV (American Psychiatric Association, 1994) [13]. They were assessed at a baseline and compared to 30 age-matching male controls (Cont) which were consecutively recruited among persons undergoing periodical routine examination in the MMA. All subjects received complete medical, neurological and psychiatric examinations to confirm good health condition. Exclusion criteria for Alc were any other current DSM-IV Axis I diagnoses assessed by the Structured Clinical Interview for DSM-IV, a history of significant medical illness, the use of other psychotropic drugs or substances except for tobacco smoking. Control subjects did not meet current or lifetime abuse or dependence criteria for alcohol or any other illicit drug and Axis I psychiatric diagnosis according to structured clinical interview (SCID).

All participants were explored for demographic characteristics, the patterns of alcohol and cigarette use, the family history, personality dimensions and depression. In additional analysis, the Alc were reassessed for depression after 4 weeks of in-patient treatment and were categorized into depressive (Ad) and non-depressive (And) subgroup. The 50% of total Alc were scored above cut-off score on Hamilton Depression Rating Scale [14] (HDRS) resulting in 43 subjects in each subgroup. The subgroups were compared for baseline characteristic.

All subjects signed written informed consent prior entering the study and all study procedures were approved by the Local Ethics Board.

Measures

On the baseline, a trained psychiatrist interviewed subjects by semi-structured clinical interview for collecting sociodemographic characteristics, the paterns of alcohol and cigarette use and family hystory data. The pattern of alcohol use included the alcohol use in years, the drink number per week for the past year and the number of treatments. The smoking status was evaluated by years of daily smoking and daily cigarettes number. The family history of alcoholism and depression (FH+) was explored.

Michigan alcoholism screening test (MAST) [15] is 25-items screening tool for alcohol use disorder. The cut-off score sum, MAST score < 3, is related to no alcohol use disorder.

Depression was assessed by indepedent trained rater who applied the 21-item HDRS [14]. The HDRS is semi-structured interview which score sum ranges from 0 to 63 and indicates condition with no depression (scored 0–7) and 3 degrees of depression severity: the mild depression (scored 8–16), moderate depression (scored 17–24), and severe depression (scored 25 to 63).

The personality dimensions were measured by self-administered Eysenck Personality Questionnnaire (EPQ) [16]. The EPQ consists of 90 true-false self-descriptive items and covers 4 dimensions: extraversion/introversion (E), neuroticism (N), psychoticism (P) and control (C) or lie scale. The neuroticism refers to the stability/instability dimension of personality and assesses the general emotional over-responsiviness, anxiety and worryng. Extraversion describes sociable, uninhibited personality. The psychoticism is related to more bizarre personality characteristics, such as being distant, cold, insensitive, absurd, and unable to empathize with others. The control, lie scale highlights the social desirability and it was introduced later in an attempt to measure to what extent subjects were deliberately attempting to control their scores [17].

Statistics

For all variables, descriptive statistics was applied and data expressed as mean ± standard deviation (SD). The difference between groups was estimated by the Student's *t*-test and χ^2 test. Corellation was tested by Pearson's correlation coefficient. SPSS for Windows was used and the *p* values of 0.05 or below were defined as statistically significant.

Results

The differences between alcoholics and controls

Demographic data did not show significant differences between groups.

The mean age (± SD) of the Alc and control subjects (Cont) was 43.29 (± 7.32) years and 43.33 (± 7.10) years, respectively (t = 0.028; n.s). The groups were similar in terms of years of education: Alc vs. Cont – 13.72 ± 1.95 vs. 13.47 ± 2.28, n.s.; 83.7% of Alc and 93.3% of Cont subjects were married.

The significant difference for MAST score between Alc (19.01 ± 10.64) and Cont (1.30 ± 1.12) was found (t = 15.192, $p < 0.01$). The majority of Cont were social healthy drinkers (90%) and only 10% were sober non drinkers. The Alc smoked cigarettes more frequently (86.01%) than the Cont (50%).

The data from the pattern of alcohol use and cigarettes use were presented in Table 1.

While Alc and healthy Cont did not differ in years of drinking, the group of Alc had more lifetime smoking in comparison with the healthy Cont. The Alc consumed more drinks and more cigarettes daily (Table 1). The Alc had significantly more frequent family history of alcoholism, but not of depression and suicide in comparison with the Cont (Table 1).

The personality traits assessed by the EPQ showed significant difference only for neuroticism which was more prominent among Alc vs. Cont 12.72 ± 5.19 vs. 5.00 ± 3.36 (t = 9.292; $p < 0.01$). There were not significant differences in other personality dimensions (Figure l).

Table 1

The patterns of alcohol drinking and cigarettes smoking and family history (FH+) between the alcoholics and controls

Parameters	Alcoholics	Controls	t (χ^2)	p
	x̄ ± SD	x̄ ± SD		
Years of alcohol use	25.47 ± 8.35	26.40 ± 8.64	-0,367	n.s.
Alcohol units *per* week	65.52 ± 27.49	4.20 ± 5.53	19.585	< 0.01
Max alcohol units *per* occasion	13.93 ± 5.04	2.57 ± 1.72	18.114	< 0.01
Daily cigarettes number	27.91 ± 17.29	14.17 ± 14.39	3.903	< 0.01
Years of smoking	20.20 ± 10.46	11.93 ± 12.26	3.297	< 0.01
FH+, % (n)				
FH+ for alcoholism	67 (77.9)	8 (26.7)	25.554	< 0.01
FH+ for depression	8 (9.3)	0 (0)	2.997	n.s.
FH+ for suicide	9 (10.5)	5 (16.7)	0.806	n.s.

n.s. – non-significant; r – mean; SD – standard deviation.

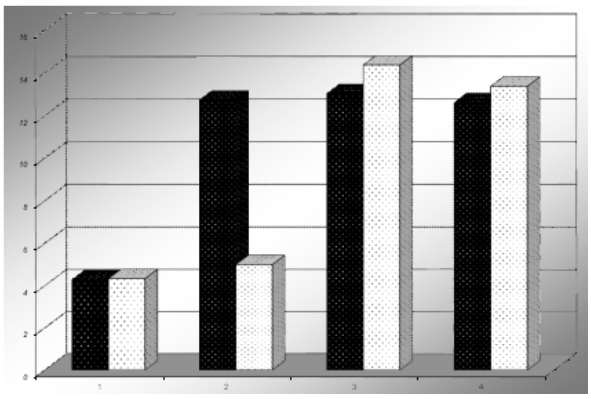

Fig. 1 – Personality dimensions assessed by Eysenck personality questionnaire (EPQ) among alcoholics and controls.

1 EPQ Psychoticism 3 EPQ Extraversion
2 EPQ Neuroticsm 4 EPQ Control scale (lie)
■alcoholics □controls

The Alc were depressed. The mean HDRS sum score was 15.37 ± 6.20 for the Alc and 1.43 ± 1.55 for the Cont with significant difference between groups (t = 19.219, p < 0.01). The mean HDRS sum scores for the Ad and And subgroups at a baseline was 18.67 ± 5.60 and 12.07 ± 4.89, respectively (t = -5.822, p < 0.01).

The differences between depressive and nondepressive alcoholics

The Ad had longer lifetime of smoking and more number of treatments comparing to And near statistical significance difference, but there was no difference in daily cigarettes smoking (Table 2).

The personality traits differences between Ad and And subgroups showed that only for the control (lie) scale there was no significant difference. The most prominent difference was higher neuroticism among Ad vs. And 15.07 ± 4.89 vs. 10.37 ± 4.40, respectively (t = -4.684, p < 0.01). Ad had lower extraversion and higher psychoticism than And (p < 0.05) (Figure 2).

Relationship between personality dimensions and depression measured in Ad recorded the positive and significant correlation between the mean HDRS sum score

Table 2

The pattern of cigarettes smoking and alcoholism, the number of treatment and MAST score differences between the non-depressive (And) and depressive (Ad) alcoholics

Parameters	And (n = 43)	Ad (n = 43)	*t*	*p*
	r ± SD	r ± SD		
Daily cigarettes number	27.21 ± 16.27	28.60 ±18.43	-0.372	n.s.
Years of smoking	18.1 ± 10.46	22.28 ± 10.16	1.872	0.065
Number of treatment	1.14 ± 0.41	1.37 ± 0.66	- 1.968	0.052

MAST – The Michigan Alcholism Screening Test; r – mean; SD – standard deviation.

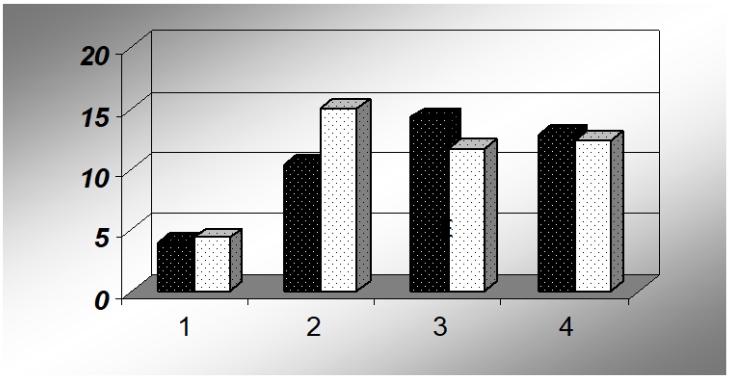

Fig. 2 – The personality dimensions assessed by Eysenck personality questionnaire (EPQ) among depressive and nondepressive alcoholics.

1 EPQ Psychoticism 3 EPQ Extraversion
2 EPQ Neuroticism 4 EPQ Control scale
□ non- depressive alcoholics
■depressive alcoholics

and the EPQ Neuroticism dimension (r = 0.487; p < 0.001), without significant correlation between other EPQ dimensions and depression. Analysis of relations between each EPQ dimensions showed only negative correlation between Neuroticism and Extraversion (r = -0.310; p < 0.05).

Discussion

This study explored the male alcoholics personality and sociodemografic heterogeneity in early alcohol recovery. The gender and individual differences may impact susceptibility to AUD and other addictions. However, the majority of studies included mainly male subjects and gender was not taken into consideration in the analysis [18]. In this paper all participants were male, and there were no significant demographic differences between the groups. The majority of Cont were socal drinkers (90%) and 50% of them were daily smokers. Among Alc 86% were smokers and they smoked more cigarettes daily than Cont. These findings were in concordance with previous research in which smoking prevalence was estimated in 80% of Alc in the clinical samples [19].

Early age at onset is an index of high liability to illness and may increases risk of illness in relatives in many biomedical disorders [20]. The age when alcohol was used for the first time was reported as a risk factor for the development of the AUD [21]. In this paper the Alc had significantly longer duration of lifetime smoking than the Cont, which means that they started smoking earlier in adolescence with almost two-fold higher number of daily cigarettes use than the Cont. Nicotine addiction problems developed rapidly in adolescents and it was most expressed in vulnerable persons who had other substance use disorders or psychiatric illness. These findings are of interest since psychiatric comorbidities are associated with less favorable prognosis [22].

There is growing interest in research and treatment of comorbid AUD and tobacco dependence. Some investigations showed that alcoholics-smokers have greater dependence severity than non-smokers suggesting careful assessment of both on admission [23]. Alcoholics- smokers evidenced shorter alcohol treatment duration and poorer outcome comparing to their non-smoking counterparts [24]. The debate about treating tobacco dependence during early alcohol abstinention have been going on. Some researches showed that concurrent treatment did not increse risk of alcohol relapse and suggested that integrating smoking cessation services in treatment program during early alcohol remission are needed to enhance smoking cessation outcomes in this population [25].

The family history of alcohol dependence among first-degree relatives was significantly more frequent among the Alc (77.9%) comparing to the Cont (26.6%). The male gender and FH+ in the AUD inherited high risk factors which contibute to heterogenity of Alc [26, 27]. Also, adult psychopathology was proposed as risk factors for the AUD, especially depression, anxiety and personality disorders [28]. Nicotine dependence is considered as a general marker of psychiatric comorbidity and the patients suffering from alcohol and nicotine dependence should be carefully assessed for other mental disorders [29]. There are findings that negative affect is a strong relapse predictor [30]. The association of smoking and depression, explored in researches, showed that former smoking and persistent smoking could predict all depression dimensions [31]. On the baseline, the total Alc sample revealed the average mild depression score compared to normal mood level among the Cont and after 4 weeks of abstention the half of Alc (Ad subgroup) showed persistent depression according to cut- off score on the HDRS. The findings from general population survey suggested that gender and measurements were the key issues in interpreting the relationship between depression and alcohol. This relationship was stronger in women than in men only when major depression diagnose was measured, but not when only recent depressed affect was measured [32].

When exploring drinking pattern in 3 urban Eastern European populations it was found that drinking problem was associated with approximately two-fold increase of risk for depressive symptoms in both sexes [33]. The central serotonergic [5-hydroxytryptomine (5-HT)] function was related to alcohol dependence between both genders with positive family history of alcoholism. Among them the gain-of-function of serotonin transporter polymorphisms (5-HTTLPR) genotype was related to higher score of depression and neuroticism, so that this may contribute to a compensatory drinking for these affective tendencies [34]. For comorbid alcoholism and smoking, various explanations were provided including genetics, pharmacodynamic and pharmacokinetic interactions such as rewarding and mood effects [35]. Chronic heavy alcohol use may precipitate depressive-like behavior, however nicotine may block the depressogenic effects of alcohol [6]. The early recovery Alc with comorbid depression were in higher risk for tobacco smoking for mood modulation than Alc without depression [36]. The depression was associated with relapse to drinking and there was a need for early recognition and concurrent treatment among Alc [25]. Individual differences may have an impact on susceptibility to addiction [37]. Neuroticism was useful marker of non-specified general risk for common mental disorders and it was a product of genetic and environmental factors with heritability estimated range from 40% to 60% [38, 39]. In this paper, the EPQ personality dimensions did not expressed significant differences between the Alc and Cont groups except for neuroticism which was more than two-fold scored higher among Alc. However, the depressive alcoholics subgroup was characterised by significantly higher neuroticism and psychoticism, but lower extraversion when compared to the non-depressive alcoholics. The most prominent differences were registered for neuroticism which was three-fold vs. two-fold higher among Ad vs. And compared to Cont (15.07 ± 4.89 vs. 10.37 ± 4.40 vs. 5.00 ± 3.36, respectively). These results were consistent with findings of other researches that the alcohol dependent patients showed high neuroticism, extroversion, anxiety, depression as com-

pared with the healthy control subjects [10, 38]. The alcohol-dependent patients also obtained significantly higher scores on neuroticism dimension [40]. This indicated that they were significantly more emotional, frequently anxious and/or depressed, moody and tense. Similar results were reported in earlier studies [18].

Exploring the relationship between personality dimensions and depression among Ad, it was showed that only neuroticism significantly correlated with depression (r = 0.487; $p < 0.001$). Other authors suggested that since depression in male Alc was more related to neuroticism, strategies for tailored stress or mood management would be useful [38]. The researches of association between alcoholism and personality reported that the persons with high neuroticism/negative emotionality may be the most vulnerable to alcoholism [41]. Furthermore, in this paper the results indicated negative correlation between neuroticism and extraversion, without significant differences in other personality dimensions. The prominent extraversion was characterized by sociability, activity, assertiveness and under-arousal, thus substance use disorders may be considered as a form of stimulation [42].

The expression of personality traits may be influenced by other factors, thus potentially biasing the results. The gender differences in personality traits across cultures showed significantly higher impulsivity and lower neuriticism among men than women [43]. Also, the age of participants should be taken into consideration because a personality may not be fully established before the age of 30 years [37]. In this paper all subjects were middle aged male, so the gender and age differences did not influence the personality traits results. When consider personality vulnerability and depression among Alc there was a need to take into account the previous research which suggested that treatment-seeking Alc often had greater alcohol-related problems and psychiatric distress than those who did not seek treatment [28].

There are several limitations of the findings in this study. The cross-sectional design used relatively small sample, thus the observed differences in personality traits do not provide explanation whether they are the causes or consequences of alcohol dependence development. Furthermore, the inpatients are likely to have more severe psychopatology and comorbities comparing to general population. Also, patients' personality traits scores were not pre-morbid, and chronic AUD may modify the assessment of personality traits. The larger prospective study with both gender subjects is needed for further research of complex interplay between alcoholism, tobacco smoking, depression and personality traits. Thus, these findings might inform early interventions and treatments that target Alc at risk for developing persistent depression in the early alcohol recovery.

Conclusion

These findings showed that male Alc significantly earlier started smoking more daily cigarettes and were significantly more depressive, with prominent neuroticism compared to healty subjects. The primary male treatment-seeking alcoholics characterised with higher neuroticism may experience persistent depression, thus requiring more intensive interventions and relapse prevention approaches.

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Expression of p63 as predictive and prognostic factor in advanced non-small-cell lung cancer

Ekspresija p63 kao prediktivnog i prognostičkog faktora kod uznapredovalog nesitnoćelijskog karcinoma pluća

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Abstract

Background/Aim. Serbia belongs to the group of countries with a high lung cancer incidence and mortality rate. p63 gene plays an important role in development of lung cancer and immunohistochemical expression of p63 is considered to be a reliable marker for squamous histology. The results of some *in vitro* studies show a significant association of p63 expression and cisplatin chemoresistance. The aim of this study was to estimate the significance of p63 expression as predictive and prognostic factor in advanced non-small-cell lung cancer (NSCLC). **Methods.** Expression of p63 in 85 NSCLC (stages III, and IV) was investigated by the use of immunohistochemistry. Four weeks after the completion of 2 cycles of platinum-based doublet chemotherapy all the patients were evaluated based on the treatment response. Kaplan-Meier analysis with log-rank tests were used for overall survival (OS) and progression free survival (PFS) calcultations. **Results.** The expression of p63 was present in 49.4% of the patients out of whom 38.8% were with positive expression (p63+) and 10.6% of the patients were with weak expression (p63+-). Positive expression of p63 was seen in 93.9% of squamous cell carcinomas (SQCC), 5% of adenocarcinomas (AC), and in no patient with not otherwise specified (NOS) NSCLC. Weak expression of p63 was found in 12.5% of AC, 25% of NOS and only in 3% of SQCC. Analysis of the impact of the presence of p63 expression on the initial response to chemotherapy showed no statistical significance. The patients with weak p63 expression had a significantly shorter OS than the patients with no p63 expression ($p = 0.049$), and the tendency of shorter OS than the patients with p63 expression ($p = 0.068$). **Conclusion.** This study shows that p63 expression has no predictive significance for tumor response to initial chemotherapy regimen gemcitabine/cisplatin or paclitaxel/cisplatin observed in advanced NSCLC. Weak expression of p63 have a negative prognostic effect in stage III and IV NSCLC.

Key words:
carcinoma, non-small-cell lung; neoplasm staging; immunohistochemistry; disease progression; predictive value of tests.

Apstrakt

Uvod/Cilj. Srbija se ubraja u grupu zemalja sa visokom incidencom i stopom mortaliteta od karcinoma pluća. Značajnu ulogu u nastanku karcinoma pluća ima gen p63. Imunohistohemijska ekspresija p63 je značajan marker za dijagnostiku skvamocelularnih carcinoma (SCK) pluća. Rezultati nekih *in vitro* istraživanja ukazuju na značajnu vezu ekspresije p63 i rezistencije na cisplatin. Cilj ovog istraživanja bio je da se proceni značaj ekspresije p63 kao prediktivnog i prognostičkog faktora kod uznapredovalog nesitnoćelijskog karcinoma pluća (NSĆKP). **Metode.** Imunohistohemijski je analizirana ekspresija p63 kod 85 NSĆKP pluća u III i IV stadijumu bolesti. Četiri nedelje nakon završetka 2 ciklusa hemioterapije na bazi platinskog dubleta vršena je procena odgovora na terapiju. Preživljavanje bez progresije bolesti i dužina preživljavanja izračunavani su primenom Kaplan-Meierove analize i log rang testa. **Rezultati.** Ekspresiju p63 imalo je 49,4% bolesnika. Pozitivnu ekspresiju (p63+) imalo je 38,8%, a slabu ekspresiju (p63+-) 10,6% bolesnika. Pozitivna ekspresija je ustanovljena kod 93,9% SCK, kod 5% adenokarcinoma (AC) i nijednog neklasifikovanog (NK) NSĆKP. Slaba ekspresija je nađena kod 12,5% AC, 25% NNS i kod 3% SCK. Analizom uticaja prisustva ekspresije p63 na inicijalni odgovor na hemioterapiju nije utvrdjena statistička značajnost. Bolesnici

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sa slabom ekspresijom p63 imali su značajno kraće vreme ukupnog preživljavanja u odnosu na bolesnike bez ekspresije p63 (p = 0.049) i tendenciju kraćeg vremena ukupnog preživljevanja u odnosu na bolesnike sa ekspresijom p63 (p = 0.068). **Zaključak.** Ovim istraživanjem ustanovljeno je da ekspresija p63 nema prediktivni značaj za odgovor na inicijalnu hemioterapiju po gemcitabin/cisplatin ili paklitaksel/cisplatin protokolu kod uznapredovalog NSĆKP. Slaba ekspresija p63 ima negativan prognostički značaj u III i IV stadijumu NSĆKP.

Ključne reči:
pluća, nesitnoćelijski karcinom; neoplazme, određivanje stadijuma; imunohistohemija; bolest, progresija; testovi, prognostička vrednost.

Introduction

Lung cancer is the most frequently diagnosed cancer and the leading cause of cancer death among males worldwide. Among females, lung cancer is one of the leading cause of cancer death in more developed countries, and the second leading cause of cancer death in less developed countries. In males, the highest lung cancer incidence rates are in Europe, Eastern Asia, and Northern America [1]. Serbia befalls in the group of Central and South Eastern European countries with the high lung cancer incidence and mortality rate [2, 3]. Non-small-cell lung cancer (NSCLC) accounts for 80–85% of lung cancers, while small-cell lung cancer has been decreasing in frequency over the last two decades [4]. NSCLC is usually in an advanced stage not amenable to surgical resection when first diagnosed. About 40% of patients with newly diagnosed NSCLC first present with locally advanced disease, and most are inoperable [5]. The median survival of patients with untreated metastatic NSCLC is only 4 to 5 months, while the 1-year survival rate is only 10% [6]. In spite of the progress of targeted therapy, platinum-based doublet chemotherapy still represents the standard of initial care for advanced NSCKC [7].

Small biopsy specimens are the primary method for the diagnosis in the majority of lung cancer patients. In 2011 the new lung cancer classification was developed by the International Association for the Study of Lung Cancer, American Thoracic Society and European Respiratory Society, and it provides for the first time a proposed set of terms and criteria for all major histologic types of lung cancer in small byopsies and cytology [8]. One of the major changes in this approach to classification of lung cancer is greater use of special stains to classify difficult cases further into adenocarcinoma (AC) or squamous cell carcinoma (SQCC) and one of the important recommendations is to preserve as much tissue as possible for molecular testing in small biopsies. At present time, thyroid transcription factor (TTF-1) appears to be the single best marker for adenocarcinoma and p63 is a reliable marker for squamous histology [8]. When morphology and/or immunohistochemistry (IHC) are not clear to recognize AC or SQCC, carcinoma should be termed as NSCLC Not Otherwise Specified (NSCLC-NOS) [8].

p63 is a member of p53 genes family. Its basic role is to form squamons epithelial phenotype [9]. The p53/p63/p73 family binding sites modulate promoter activity of miRNAs of the miR-200 family which are known regulators of cancer stem cells and epithelial-mesenchymal transitions [10]. p63 is located in chromosome 3q27-29. p63 has 6 different isotypes. One of the isotypes – Tap63 activates p53 reporter genes and makes the cell turn to apoptosis. The isoform ΔNp63 suppresses transactivation of p53 and triggers cell proliferation [11]. Although ΔNp63 and TAp63 splice variants are expressed in NSCLCs, ΔNp63α is the predominant isoform, and in contrast to TAp63 is selectively expressed in SQCC [12]. Of clinical relevance is the fact that Tap63 is induced by many chemotherapeutic agents and that inhibiting Tap63 function leads to *in vitro* chemoresistance [13]. On the other hand, *in vitro* studies show ΔNp63 expression as a regulator of increased cell survival and cisplatin chemoresistance [14].

The aim of this study was to estimate the significance of p63 expression (determined by immunohistochemistry) as predictive and prognostic factor in advanced NSCLC.

Methods

Study design and patients selection

This prospective study included 85 patients. The study was approved by the Ethical Committee of the Military Medical Academy (MMA) in Belgrade. The patients were included in the study only if they met the following criteria: older than 18 years; the histological diagnosis of NSCLC, stage IIIa if inresectable or inoperable, IIIb or IV, according to World Health Organization (WHO) Tumor-Node-Metastasis (TNM) classification; adequate bone marrow reserve (white blood cell count ≥ 3.5×10^9/L ; platelets ≥ 100×10^9/L hemoglobin ≥ 100 g/L); adequate liver and renal function (bilirubin < 1.5 times than normal; aspartate aminotransferase (AST) and alamine aminotransferase (ALT) < 3 times than normal; normal serum urea and creatinine levels); no central nervous system metastasis; no prior chemotherapy or radiation therapy; Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 2 [15].

All the patients had available diagnostic tissue specimens obtained by bronchoscopy, and were diagnosed and treated at the Pulmonology Clinic of the MMA between 2011 and 2015. The diagnosis of lung cancer was made by endobronchial biopsy by fiberoptic bronchoscopy, or tru-cut biopsy.

Biopsy specimen was fixed with 5% buffered formalin solution, dehydrated, paraffin-embedded in Leica ASP 300. Paraffin-embedded tissue sections were cut to 4 micron thick tissue sections using a microtome and applyed to Superfrost+ glass slides. Immunohistochemical staining was preformed following the Dako immunohistochemistry protocol

(Glostrup, Denmark). To unmask epitop for p63 Target Retrieval Solution pH 9.0 (Dako catalog number S2367) was used, followed by heating in a microwave. Anti-human p63 protein (Dako catalogue, number M 7247, Clone 4A4, 1:300 dilution) was used as a primary antibody. For visualization we used EnVisionDetection Systems Peroxidase (Dako catalogue number K5007) and chromogen DAB Liquid (Dako catalog number K3466), and than observed by light microscopy. p63 expression was graded as negative (-) if there was no reactivity; weak (+-) if there was up to 10% positive staining tumor cells; and positive (+) if there was more than 10% positive tumor cells.

The patients were randomised to receive either gemcitabine/cisplatin (GC) or paclitaxel/cisplatin (PC). Gemcitabine 1000–1250 mg/m² was given on the days 1 and 8, and cisplatin 75 mg/m² was given on the day 1 of a 21-day cycle. Paclitaxel 135 mg/m² was given on the day 1, and cisplatin 75 mg/m² was given on the day 1 of a 21-day cycle.

Four weeks after the completion of 2 cycles of chemotherapy, all the patients were evaluated according to treatment response. The responses were categorized as: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) according to the Response Evaluation Criteria in Solid Tumors (RECIST) [16]. Further treatments of all the patients were in acordance with the National Comperhensive Cancer Network guidelines [7].

All data analyses were processed using the Statistical Package for Social Sciences, version 18 (SPSS, Chicago,IL). Data are presented as mean ± standard deviation (SD), and median with 95% confidence interval. The normality of the data was assessed using Kolmogorov-Smirnov test. The difference between the groups was tested by Student's *t*-test (alternatively Mann-Whitney test) or by one-way analysis of variance (ANOVA) or alternatively Kruskal-Wallis test. χ^2 test were used to detect singificant differences among the frequences of some categories. Kaplan-Meier analysis with Log-rank tests were used for Overall Survival (OS) and Progression Free Survival (PFS). A *p*-value of 0.05 or less was considered indicative of a statistically significant difference.

Results

A total of 85 patients were included in the study. Their basic demographic characteristics are shown in Table 1. The mean age of the patients of both sexes was 62.9 years (median 63 years), women 64.6 years (median 66 years), and for men 62.5 (median 63 years). Using *t*-test for independent characteristics it was found that there was no statistically significant difference in age between the sexes. Most patients (66 or 77.6%) at the time of the diagnosis had ECOG PS 1. PS 0 had 14 (16.5%) and PS 2 5 (5.9%) of the patients. AC was diagnosed in 40 (47.1%) of the patients, NOS NSCLC was diagnosed in 12 (14.4%), and SQCC in 33(38.8%) of the patients (Table 2). When it comes to age at the diagnosis there was no statistically significant difference among the three histological types of cancer (ANOVA).

At the time of the diagnosis most of the patients (45.9%) had T2 disease; 28.2% had T3, 16.5% T4 and 9.4% had T1 disease. N2 disease had 58.8% of the patients; the same percentage (16.5%) had N1 and N3 and 8.2% of the patients had N0 disease. No distant metastasis was found in 50.6% of the patients, M1a disease was found in 17.6% of the patients, whereas M1b disease was detected in 31.8% of the patients. Most of them, 42 (49.4%), were in clinical stage IV of the disease, followed by 31 (36.5%) in stage IIIa, while the smallest number of the patients, 12 (14.1%) were in clinical stage IIIb.

Table 1

Basic demographic data of patients

Gender	Number (%) of patients	Age, (years) r ± SD	Median	Minimum	Maximum
Male	69 (81.2)	62.5 ± 9.1	63.0	43	79
Female	16 (18.8)	64.6 ± 6.8	66.0	53	75
Total	85 (100)	62.9 ± 8.7	64.0	43	79
t-test		$p = 0.400$			

r ± SD – mean ± standard deviation;

Table 2

Histological type of carcinoma and patients ages

Histological type of carcinoma	Number (%) of patients	Age, (years) r ± SD	Median	Minimum	Maximum
AC	40 (47.1)	62.9 ± 9.0	65.5	43	77
NOS	12 (14.1)	61.2 ± 9.2	62.0	50	79
SQCC	33 (38.8)	63.4 ± 8.3	64.0	43	77
Total	85 (100)	62.9 ± 8.7	64.0	43	79
ANOVA		$p = 0.756$			

r ± SD – mean ± standard deviation; AC – adenocarcinoma; NOS – not otherwise specified; SQCC – squamous cell carcinoma; ANOVA – Analysis of variance.

The expression of p63 was present in 42 (49.4%) of the patients out of whom weak expression (p63+-) in 9 (10.6%) of the patients, positive expression (p63+) in 33 (38.8%) of the patients. Positive expression p63 was seen in 31 of the patients with SQCC (93.9% of p63 +; and 93.9% of SQCC), 2 patients with AC (6.1% of p63+; and 5% of AC), and in no patient with NOS NSCLC (Figures 1 and 2). So, there was a statistically significant difference between SQCC and AC, as well as between SQCC and NOS NSCLC ($p < 0.001$). Weak expression of p63 was found in 5 of the patients with AC (55.6% of p63+-; 12.5% of AC), 3 patients with NOS (33.3% of p63+-; 25% of NOS) and only 1 with SQCC (11.1% of p63+-; 3% of SQCC) (Table 3).

There was a favorable response to chemotherapy in 63 (74.1%) of the patients, of which a partial response in 39 patients (45.9% compared to the total number of patients), stable disease in 24 (28.25%) of the patients while 22 (25.9%) of the patients had disease progression. Gender had no significant effect on the response to chemotherapy. A total of 75% of women had a favorable response to chemotherapy, and 73.9% of the male population.

The studied patients had ECOG PS estimated in the range of 0 to 2. There was a significant influence of PS on the response to chemotherapy. A correlation of PS 2 score with adverse responses was statistically significant ($p = 0.015$). In the group of patients with an adverse response to chemotherapy, 80% showed PS score 2, accounting for 18.25% of the total number of patients.

There was no statistically significant effect of the histological type of the tumor, nor T disease on the response to chemotherapy. There was a statistically significant correlation of negative responses to chemotherapy with N2

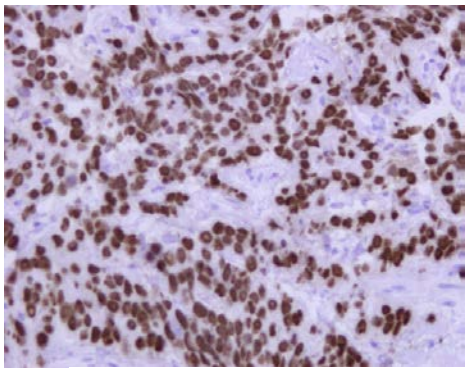

Fig. 1 – Squamous cell carcinoma/ p63 positive (p63 immunohistochemistry H&E, ×200).

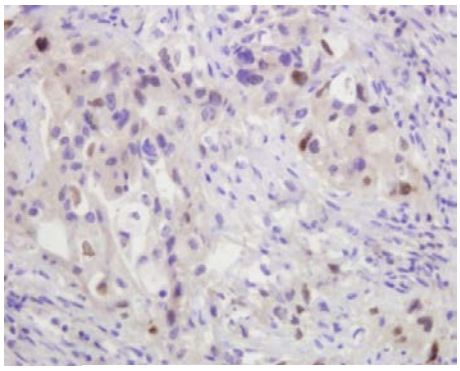

Fig. 2 – Adenocarcinoma/p63 weak positive (p63 immunohistochemistry H&E, ×200).

Table 3

P63 expression in relation to the histological type of carcinoma

p63 expression	Histological type of carcinoma			Total
	AC	NOS	SQCC	
p63-				
number of patients	33	9	1	43
(%) in p63-	76.7	20.9	2.3	100
(%) in histological type	82.5	75.0	3.0	50.6
p63+				
number of patients	2	0	31	33
(%) in p63+	6.1	0.0	93.3	100
(%) in histological type	5.0	0.0	93.3	38.8
p63+-				
number of patients	5	3	1	9
(%) in p63+-	55.6	33.3	11.1	100
(%) in histological type	12.5	25.0	3.0	10.6
Total				
number of patients	40	12	33	85
(%) in p63	47.1	14.1	38.8	100
(%) in histological type	100	100	100	100
χ^2 test			p <0.001*	

AC – adenocarcinoma; NOS – not otherwise specified; SQCC – squamous cell carcinoma.

There were no statistically significant differences in the distribution of chemotherapy protocols in relation to the histopathological type of cancer ($p = 0.116$), as well as in relation to the stage of the disease (p=0.203). The expression of p63 with respect to the stage of the disease showed no statistically significant differences ($p = 0.256$).

disease (p = 0.050). Compared to the group of patients with PD, 81.8% had N2 disease. There was no statistically significant difference among M0, M1a and M1b diseases as well as among IIIa, IIIb and IV stages of the disease, regarding the initial response to chemotherapy.

Analysis of the impact of the presence of p63 expression on the initial response to chemotherapy showed no statistical significance.

It turned out that there was no statistically significant difference in the studied patients regarding the initial response to chemotherapy when comparing the two applied chemotherapy regimens (gemcitabine / cisplatin and paclitaxel / cisplatin). Partial response to the regimen GC was found in 44.3% of the patients and to the regimen PC in 50% of the patients, SD in the group that received GC had 26.2% of the patients and in the group which received PC 33.3% of the patients. The progression of the disease in the group with the regimen GC was found in 29.5% and with the regimen PC in 25.9% of the patients.

Analysis of the progression-free survival and overall survival was related to 68 patients (56 male and 12 female). By the end of the monitoring period, 6 (10.7%) men and 4 (33.3%) women survived. A total of 17 of the patients continued treatment in other oncology centers, so that the analysis of PFS and OS did not apply to them. The median PFS time could not be determined because the number of end-point events did not reach half the total number.

The analysis of the impact of gender on PFS showed that there were no statistically significant differences. The estimated mean PFS in men was 14.25 months and 11.42 months in women. Log-rank (Mantel-Cox) test showed a statistically significant difference in favor of females regarding the overall survival time (p = 0.037) (Table 4).

Comparing the relationship of ECOG PS and PFS, there was a statistically significant difference between the PS 2 and PS 1 (mean 3.4 months *vs* 14.8 months).

The patients with PS 2 had a significantly shorter OS than the patients with PS 0 (mean 9.40 months/median 7.00 months *vs* mean 19.52 months) (p = 0.031) and the tendency of shorter OS than the patients with PS1 (mean 16.35 months/medain 13.00 months) (p = 0.059).

The results of PFS analysis compared to histological type of carcinoma showed no statistically significance difference. When it comes to OS, however, the patients with NOS lived significantly shorter as compared to the patients with SQCC (mean 10.33 months/median 9 months *vs* 17.14 months/median 14 months), (p = 0.027). There was also a tendency of shorter OS compared to the patients with AC, but with no statistical significance (p = 0.09) (Figure 3).

There were no statistically significant differences between the III and IV stages of the disease in terms of PFS. OS was statistically more significant in the patients with IIIb than in those with clinical stage IV of the disease (mean 24.66 months/median 21 months *vs* mean 13.81 months/median 12 months) (p = 0.008).

Table 4

Overall survival (OS) in relation to the patients gender

Gender	OS (months)	
	mean (95% confidence interval)	median (95% confidence interval)
Male (n = 56)	15.25 (12.82–17.69)	13.00 (10.93–15.06)
Female (n = 12)	22.00 (15.61–28.38)	19.00 (7.11–30.88)
Total (n = 68)	16.49 (14.09–18.88)	13.00 (11.03–14.97)
Log-Rang (Mantel-Cox)	p = 0.037*	

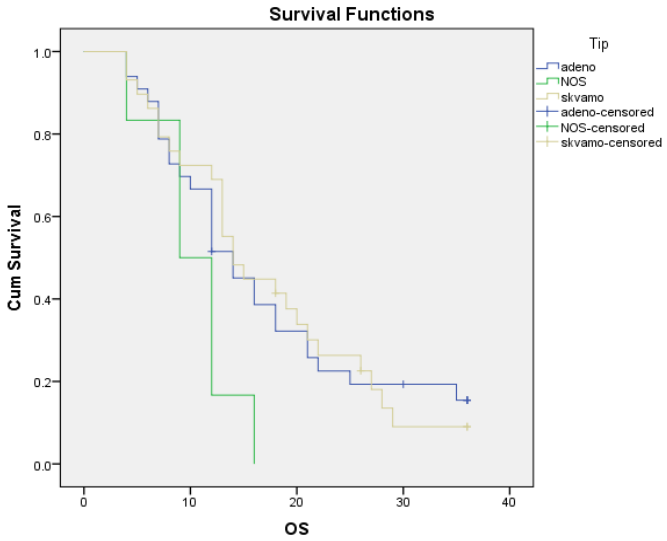

Fig. 3 – Kaplan-Meier estimates of overall survival (OS) according to the histological type of carcinoma.
NOS – not otherwise specified.
Estimation is limited to the longest survival time if it is censored.

The ratio of the expression of p63 had no statistically significant influence on PFS, but when it comes to OS there was a statistically significant difference. The patients with weak p63 expression had a significantly shorter OS than the patients with no p63 expression (p = 0.049), and the tendency of shorter OS than the patients with p63 expression (p = 0.068) (Table 5, Figure 4).

There were no statistically significant differences in PFS and OS regarding the two initially applied chemotherapy regimens.

Table 5

Overall survival (OS) in relation to the p63 expression

P63 expresion	OS (months)	
	mean (95% confidence interval)	median (95% confidence interval)
P63- (n = 33)	17.36 (13.78–20.95)	14.00 (10.99–17.01)
P63+ (n = 29)	16.75 (13.16–20.35)	14.00 (11.36–16.46)
P63+ (n = 6)	10.17 (6.76–13.58)	9.00 (5.23–12.77)
Total (n=68)	16.49 (14.11–18.88)	13.00 (11.03–14.97)
Log-Rang (Mantel-Cox)	P63	
	P63+ *vs* P63-	*P = 0.049**
	P63+ *vs* P63-	P = 0.806
	P63- *vs* P63+	P = 0.068

***statistically significant**

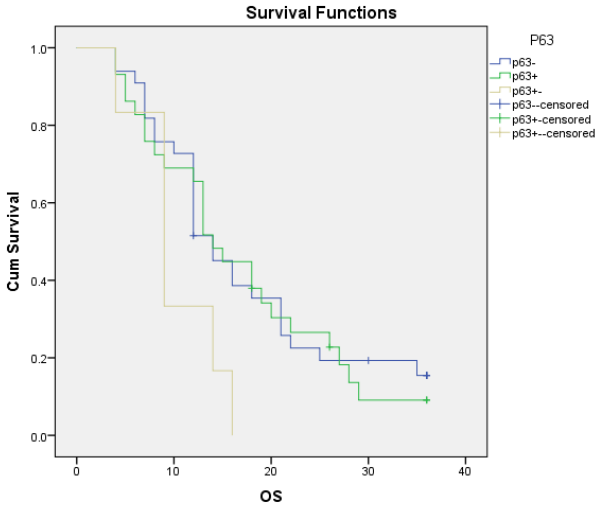

Fig. 4 – Kaplan-Meier estimates of overall survival (OS) according to the p63 expression. Estimation is limited to the longest survival time if it is censored.

Discussion

Despite the progress of the targeted molecular therapies, the most common treatment of advanced NSCLC in clinical practice is the use of chemotherapy in the first-line. Standard first-line chemotherapy regimens include combinations of third-generation agents with either cisplatin, or carboplatin. All the forms of anticancer therapy have side effects. On the other hand, the presence or development of resistance to chemotherapeutic agents is one of the major problems. That is why the search for predictors of response and for prognostic factors is still ongoing. The aim of our study was to determine whether p63, which is usually used for diagnostic purposes for NSCLC from small tissue samples, could be used as predictive or prognostic factor.

In this prospective study, analyzing a homogenous, well-defined patient population, we estimated the predicitive and prognostic significance of p63. Of the total number of 85 patients, there were 4.3 times more men than women. This ratio is in line with the epidemiological situation in the world and in our country [2, 3, 17]. Adenocarcinoma was diagnosed in 47.1% of the patients, squamous cell carcinoma in 38.8% of the patients, while unclassified non-small-cell lung cancer was diagnosed in 14.4%. The percentage of NOS in this study is similar to that published by Collins [18]. In his work, based on immunohistochemistry panel, 85% of the patients had AC or SQCC, whereas 15% of the patients were diagnosed with NOS NSCLC. On the other hand, Sigel et al. [19] are of the opinion that it is allowed to only 7% of all NSCLC from samples obtained by bronchial biopsy and cytological samples (after morphological examination, IHC staining and mucin) to remain unclassified. However, despite the use of the sophisticated methods of analysis small tissue samples NOS NSCLC diagnosis in practice occurs in about 10% to 30% [20], as in this study.

There was a highly significant expression of p63 in SQCC in comparison to AC and NOS NSCLC. Of the total number of the patients with the expression of p63, 93.6% had SQCC. On the other hand, of the total number of the patients with weak expression of p63, 55.6% had AC. Within the group of patients with SQCC, 97% had expression or weak expression of p63, and in the group with the AC expresion or weak expresion of p63 had 17.5% of the patients. p63 may show patchy and/or weak staining in 20%–30% of adenocarcinomas [8]. Bir et al. [21] in 2014 published similar results, according to which 24 out of 25 (96%) of the SQCC patients were p63 positive, and in 25%, 6 of 20 patients, AC showed weak p63 staining. According to the results of Yaman et al.[22] published in 2015, p63 staining was positive in 87.5% of SQCC and in 4.3% AC.

There was a statistically significant effect of PS as the response to chemotherapy. In the group of patients with adverse response to chemotherapy (regardless of which of the two regimes applied), 80% belonged to the group of patients with PS 2. Cuyún Carter et al. [23] published a comprehensive review of non-genetic prognostic and predictive factors that had an impact on the outcome of advanced NSCLC. The results of 54 studies, published from January 2000 to November 2010, were analyzed. Two out of ten studies examined and confirmed the importance of PS as predictive factor. The results of this study are also in favor of the importance of PS as a predictive factor.

It has been confirmed that there is a statistically significant correlation of negative responses to chemotherapy with N2 status. Of the patients with PD, 81.8% had N2 disease. According to the literature, the N status is significant to the prognosis [24]. When we analyze the effect of the presence and the level of p63 expression in the patients with advanced NSCLC on the response to chemotherapy, we found no statistically significant difference.

Gender had no significant effect on PFS, but there was a statistically significant difference in favor of females when looking at OS. Of the 45 studies, 17 (38%) confirmed a significant advantage of women in relation to better outcome [23].

Of the 49 studies on evaluating the PS as prognostic factor, the results of 36 (73%) confirmed a significant correlation of ECOG PS and clinical outcomes, and that a lower ECOG PS score is associated with better outcomes [23]. In this study, as expected, the patients with PS2 had the worst prognosis.

Of the 31 studies examining the importance of histology type as prognostic factor, 4 studies (12.9%) show an advantage of adenocarcinomas compared to other histological types of NSCLC [23]. Our group of patients with NOS histological type had the shortest time for OS, that is statistically significantly lower than that in SQCC, and the tendency of lower OS compared to AC.

Of the 38 studies that compared IIIB and stage IV disease, 21 (55%) show a significant association of a lower stage with a better outcome, as in our study [23].

According to the results of this study, expression of p63 did not influence PFS. However, in relation to the OS, there was a statistically significant difference between the patients with tumors with weak expression p63 (p63 +-) and the patients with no expression of p63 (10.17 months *vs* 17.36 months). There is also a tendency for better outcome in the patients with p63 expression as compared to the patients with weak expression. Ma et al. [25] explored the significance of p63 expression in SQCC of the lung in 76 patients in the early stage of disease (I, II, and IIIA-only T4N0). Based on the postoperative follow-up the obtained results show that there was a correlation of high expression of p63 with a better prognosis. Barlisi et al. [26] show in their study that in the squamous cell carcinoma p63 amplification and staining intensity are associated with better survival independently on the stage and the degree of differentiation of the tumor. On the contrary Yaman et al. [22] found no significant effect of p63 on survival.

The key limitation of this study is the relatively small number of patients. Another important limitation are small diagnostic samples of tumor tissue or metastatic lymph node because such samples may be histologically different from the rest of the whole tumor.

Conclusion

Expression of p63 is significantly more common in SQCC than in AC and NSCLC NOS. P63 has no predictive significance for tumor response to initial chemotherapy regimen GC or TC observed in the III and IV NSCLC clinical stage. Patients in stage III and IV NSCLC with low expression of p63 have worse prognosis than patients without p63 expression. They also have the tendency to worse prognosis compared to patients with p63 expression. This data give rise to additional investigation, which may provide the foundation for generating more effective therapeutic strategies in NSCLC.

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Transient elastography for noninvasive assessment of liver fibrosis in patients with primary biliary cirrhosis

Tranzijentna elastografija u neinvazivnoj proceni fibroze jetre kod bolesnika sa primarnom bilijarnom cirozom

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Abstract

Backgrund/Aim. In recent decades noninvasive methods for the assessment and monitoring of liver fibrosis have been developed and evaluated in numerous chronic liver diseases. The aim of this study was to evaluate the diagnostic accuracy of noninvasive markers for fibrosis assessment transient elastography (TE) and biochemical markers using liver biopsy as reference in patients with primary biliary cirrhosis (PBC). **Methods.** One hundred and twenty-two patients underwent both liver biopsy and blood tests on the same day and TE in a month following the biopsy and the tests. Liver biopsies were reviewed by a single pathologist using the METAVIR scoring system for assessment of liver fibrosis. Aspartate aminotransferase (AST), platelet ratio index (APRI), Forns scores, AST and alanine transaminase (ALT) ratio and TE were compared with liver fibrosis stage in order to determine the best noninvasive marker of liver fibrosis. **Results.** There was a statistically significant difference ($p < 0.05$) for the APRI score, Forns index and TE according to stages of liver fibrosis. TE showed superior diagnostic performance when compared to other surrogate markers of liver fibrosis that were investigated. Optimal cut-off for TE were 4.25 and 5.9 kPa for diagnosing the presence of fibrosis and distinguishing mild/moderate and advanced stages of fibrosis respectively. The areas under the receiver operating characteristic (AUROC) of TE were 0.963 and 0.865, respectively. **Conclusion.** Based on our investigation the APRI score, Forns index and TE adequately predict fibrosis stage in patients with primary biliary cirrhosis, but the most sensitive and specific parameter appears to be TE. Using noninvasive markers and methods in the evaluation of patients in daily clinical practice may reduce, but not eliminate, the need for invasive diagnostic procedures.

Key words:
liver cirrhosis; biopsy; blood chemical analysis; biological markers; elasticity imagine techniques; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Prethodnih decenija otkrivene su neinvanzivne metode za procenu i praćenje fibroze jetre kod hroničnih bolesti jetre. Cilj ove studije bila je procena dijagnostičke preciznosti neinvanzivnih metoda za određivanje fibroze jetre [tranzijentna elastografija (TE) i biohemijski markeri], pri čemu je kao zlatni standard korišćena biopsija jetre kod bolesnika sa primarnom bilijarnom cirozom. **Metode.** U studiju su bila uključena 122 bolesnika kod kojih su istog dana urađene biohemijske analize i biopsija jetre, a mesec dana kasnije urađena je TE. Za procenu fibroze jetre korišćen je METAVIR skor, a sve preparate biopsija proverio je jedan patolog. APRI skor – odnos aspartat aminotransferaze (AST) i trombocita, Forns indeks, odnos AST i alanin transaminaze (ALT) i TE poređene su sa stepenom fiboze jetre dobijene na osnovu biopsija jetre u cilju dobijanja najboljeg neinvanzivnog markera u proceni fibroze jetre. **Rezultati.** Dokazana je statistička značajnost ($p < 0.05$) za APRI skor, Forns indeks i TE za procenu stepena fibroze jetre. TE je imala najbolji dijagnostički učinak u poređenju sa ostalim markerima koje smo istraživali. Optimalne granične vrednosti za TE bile su 4.25 i 5.9 kPa za dijagnozu fibroze jetre i razlikovanje slabe/umerene i uznapredovale fibroze. Površina ispod krive operativnih

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karakteristka (AUROC) za TE bila je 0.963 i 0.865. **Zaključak.** Na osnovu rezultata ove studije proizilazili su APRI skor, Forns indeks i TE adekvatni dijagnostički markeri fibroze jetre kod bolesnika sa primarnom biijarnom cirozom, ali je TE najsenzitivniji i najspecifičniji parametar. Koristeći neinvanzivne parametre i metode u svakodnevnoj kliničkoj praksi može se smanjiti, ali ne i potpuno izbaciti, potreba za invanzivnim dijagnostičkim procedurama.

Ključne reči:
jetra, ciroza; biopsija; krv, hemijske analize; biološki pokazatelji; elasticitet, tehnike snimanja; osetljivost i specifičnost.

Introduction

Primary biliary cirrhosis (PBC) is a slowly progressing autoimmune disease of the liver that primarily affects middle aged women with an annual incidence ranging from 0.7 to 49 cases per million [1]. Histologically, PBC is characterized by portal inflammation and immune-mediated destruction of intrahepatic bile ducts resulting in further hepatic damage, fibrosis and liver cirrhosis [2].

Liver biopsy remains the gold standard for the liver fibrosis assessment, but it is an invasive and expensive procedure, associated with a low, but negligible risk of complications and mortality [3, 4]. Moreover, the accuracy of liver biopsy in assessing fibrosis has been questioned because of sampling errors as well as intraobserver and interobserver variability [5].

In the last decade, numerous noninvasive methods for the assessment of liver fibrosis were developed and evaluated. Ideally, the test should be reliable, fast, reproducible, easily applicable in every day clinical practice as well as acceptable for patients and reliable for both prognosis and staging of liver disease.

However, most of these methods have been extensively studied in viral hepatitis, but not much has been done regarding patients with PBC [6–9]. The aim of this work was to compare the diagnosis accuracy of liver stiffens – transient elastography (TE) with simple and routinely available blood markers: aspartate aminotransferase (AST) to platelet ratio index (APRI), the Forns index, AST to alanine transaminase (ALT) ratio using liver biopsy as the reference in patients with PBC.

Methods

Patients

This study included 122 prospectively selected patients who were diagnosed with PBC at the Clinic for Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade from June 2009 to January 2011. The diagnosis of PBC was based on at least 2 out of 3 criteria including elevated serum alkaline phosphatase (ALP), presence of serum antimitochondrial antibodies (AMA) and liver histology consistent with PBC. The diagnosis was confirmed on the basis of the presence of a typical clinical picture, biochemical (elevated ALP ≥ 1.5 times the upper normal value for over 24 weeks) and serological markers (AMA in serum ≥ 1:40) as well as characteristic histological findings on liver biopsy in absence of extrahepatic biliary obstruction. Histological staging was classified ranging from portal tract inflammation with predominantly lymphoplasmacytic infiltrates and septal and interlobar bile duct loss (stage I) to cirrhosis (stage IV). On the same day, each patient underwent blood testing and liver biopsy, while liver stiffness measurements (LSM) using the TE technique were carried out during the following month. Exclusion criteria were presence of ascites, obesity (body mass index > 30 kg/m^2), hepatocellular carcinoma, hepatothropic virus infection, history of alcohol abuse, and all other causes of chronic liver injuries.

Surrogate markers of liver fibrosis

The following serum parameters were examined by venous blood sampling and were processed in our hospital labaratory: AST measured in (IU/L), ALT mesured in IU/L, gamma-glutamyl transpeptidase (GGT) in IU/L, ALP in IU/L, platelets (Pt ×10^9/L), total bilirubin measured in μmol/L, albumins in g/L, cholesterol measured in mmol/L, and prothrombin time (PT – normal range 9.5–13.5 s). On the basis of these biological tests, we calculated the following scores for predicting liver fibrosis: AST/ALT ratio, APRI score = [(AST/upper limit of normal AST) ×100] / number of platelets (10^9/L) [9, 17] and Forns score = 7.811 - 3.131 × ln [number of platelets (10^9/L)] × 0.781 ln [GGT (U/L)] + 3.467 × ln [age (years)] – 0.014 [cholesterol (mg/dL)] [10].

Liver biopsy

Percutaneous liver biopsy was performed on each patient and specimens were routinely processed. Only specimens at least 2 cm long were selected and used for this investigation. Adequate biopsy specimens were obtained from 122 patients. Sections were analyzed independently by a single experienced pathologist unfamiliar with the patients clinical details and results of the noninvasive methods. Liver fibrosis was evaluated semiquantitatively according to the METAVIR scoring system. Fibrosis was scored on a scale of 0–4 as follows: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis; F4 = cirrhosis. Histological evaluation included grade of inflammation, ductopenia, bile duct inflammation and destruction, cholestasis and ductal proliferation.

Liver stiffness measurement

Liver stiffness was measured by transient elastography using a FibroScan® (EchoSens, Paris, France) equipped with

an M probe. The measurements were obtained from the right lobe of the liver. The patients lay in the dorsal decubitus position with the right arm maximally abducted, through the intercostal spaces between 25 mm and 65 mm from the skin surface. Only examination with 10 valid measurements at a success rate of at least 60% (ratio of the number of successful attempts over the total number of attempts) and an interquartile range less than 30% were considered reliable and kept for statistical analyses. The final result was the median of 10 valid measurements and was expressed in kPa.

Statistical analysis

We used methods of descriptive and analytical statistics. Basic descriptive statistics included means, standard deviations, ranges and percentages. Normal distribution of continuous data was tested using the Kolgomorov-Smirnov test. Analysis of variance (ANOVA) or Kruskal- Wallis test were used for assessment of differences among groups. The diagnostic performance of noninvasive liver assessment methods were performed by receiver operating characteristic (ROC) analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS®, version 14.0).

Ethics

This study was approved by the Ethical Committee of our hospital and all patients signed informed consent before inclusion into the study.

Results

Patients

From the total of 122 patients, 106 (86.5%) of the patients were women with a mean age of 57.40 ± 8.92 years. Their clinical details are summarized in Table 1. All the patients were treated with UDCA (ursodeoxycholic acid) after determining the diagnosis of PBC, 23 (19.2%) patients were without fibrosis (F0), 38 (30.8%) patients had mild fibrosis (F1), 12 (9.6%) patients moderate (F2), 16 (13.5%) patients advanced fibrosis (F3), and 33 (26.9%) patients had liver cirrhosis (F4).

Noninvasive serum parameters

The values of noninvasive serum markers were compared for each histological fibrosis stage (Table 2). We found a difference among APRI, the Forns index and TE

Table 1

Baseline clinical, biochemical and histological characteristics of patients with liver fibrosis (n = 122)

Variables	r ± SD (mediana, min-max)
Age, (years)	57.4 ± 8.9 (58; 42–75)
Alkaline phosphatase (IU/L)	137.9 ± 87.6 (98; 28–351)
Gamma-glutamyl transpeptidase (IU/L)	123.1 ± 142.0 (68; 13–603)
Aspartate aminotransferase (AST), (IU/L)	48.0 ± 30.3 (46; 14–176)
Alanine aminotransferase (ALT), (IU/L)	50.8 ± 27.7 (46; 18–158)
Bilirubin (μmol/L)	13.8 ± 8.3 (11.2; 3.5–36.5)
Platelets (10^9/L)	209.1 ± 87.2 (212; 52–422)
Albumine (g/L)	39.5 ± 4.6 (40; 28–51)
Protrombin time (PT), s	90.2 ± 18.2 (93; 43–125)
Cholesterol (mmol/L)	5.1 ± 1.4 (5.43; 2.33–8.73)
AST/ALT	0.9 ± 0.3 (0.97; 0.41–1.70)
AST platelet ratio index	0.6 ± 0.7 (0.40; 0.10–2.60)
Forns index	6.0 ± 2.1 (5.47; 2.75–10.85)
Transient elastography (kPa)	9.6 ± 6.9 (6.8; 3.2–30.7)
METAVIR scoring system, n (%)	
F0	23 (19.2%)
F1	38 (30.8%)
F2	12 (9.6%)
F3	16 (13.5%)
F4	33 (26.9%)

r ± SE – mean ± standard deviation; n (%) – number (percentage) of patients.

Table 2

Surrogate markers of liver fibrosis

Variables	METAVIR Score (r ± SD)				
	F0 n = 23	F1 n = 38	F2 n = 12	F3 n = 16	F4 n = 33
AST/ALT[a] (IU/L)	0.9 ± 0.2	1.0 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	1.0 ± 0.3
APRI*[b] (IU/L/10^9)	0.2 ± 0.1	0.5 ± 0.6	0.4 ± 0.2	0.9 ± 0.9	1.0 ± 0.7
Forns index*[a]	4.3 ± 0.9	5.8 ± 2.0	5.4 ± 0.5	7.0 ± 3.0	7.4 ± 2.4
TE (kPa) *[b]	5.5 ± 0.2	6.4 ± 2.1	9.3 ± 4.3	10.8 ± 3.9	17.7 ± 7.8

***Statistically significant differences; [a]One way ANOVA; [b]Kruskal -Wallis test; AST – aspartate aminotransferase; ALT – alanine aminotransferase; APRI – AST platelets index; TE – transient elastography.**

according to stages of liver fibrosis. AST/ALT ratio did not show any significant difference. For these parameters, we calculated sensitivity, specificity and Area under the receiver operating characteristic (AUROC), as presented in Table 3 and Figure 1, as well as best cut-off values for determining the presence of liver fibrosis (Table 4 and Figure 2). A cut-off value of 0.255 for the APRI score as well as the Forns index cut-off value of 4.168 were statistically significant for predicting existence of liver fibrosis. Also, we recognized a statistically significant possibility for distinguishing patients having mild-to-moderate fibrosis (F1 or F2) and advanced fibrosis (F3 or F4) as presented in Tables 5 and 6, and Figures 3 and 4, respectively.

Table 3

Area under the reciever operating characteristic (AUROC) curve for surrogate markers of liver fibrosis (fibrosis: no vs. yes)

Surrogate markers of liver fibrosis	AUROC	Asymp significance	95% Confidence interval (bound: lower-upper)
AST/ALT (IU/L)	0.588	0.390	0.419–0.758
APRI (IU/L/10^9)	0.782	0.006*	0.649–0.915
Forns index	0.806	0.003*	0.685–0.927
TE (kPa)	0.963	0.000*	0.000–1.000

***Statistically significant; AST – aspartate aminotransferase; ALT – alanine aminotransferase; APRI – AST-platelet index; TE – transient elastography.**

Table 4

Sensitivity and specifity for surrogate markers of liver fibrosis (fibrosis: no vs. yes)

Surrogate markers of liver fibrosis	Cut-off	Sensitivity	Specifity	Asymp significance	AUC (95% CI)
AST/ALT	0.917	0.571	0.600	0.403	0.586 (0.388–0.783)
APRI	0.255	0.810	0.600	0.046*	0.705 (0.510–0.899)
Forns index	4.168	0.881	0.500	0.063	0.690 (0.486–0.894)
TE (kPa)	7.250	0.929	1.000	0.000*	0.964 (0.001–0.999)

AUC – area under the curve; for other abbreviations see Table 3;*statistically significant.

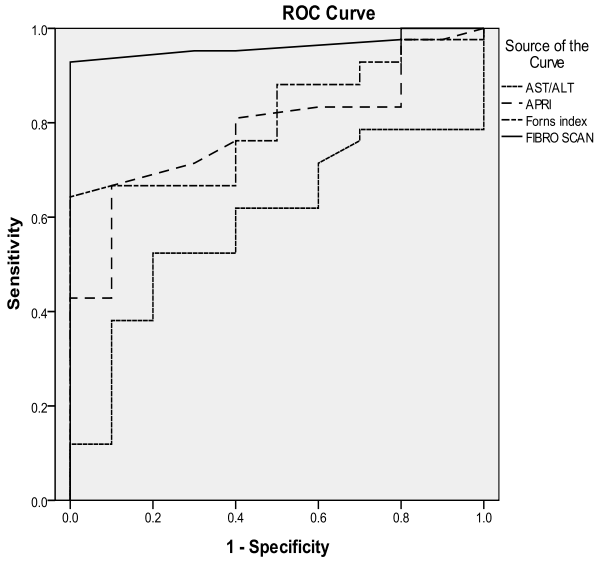

Fig. 1 – ROC curve for surrogate markers of liver fibrosis. ROC – receiver operating characteristic. For abbreviations see Table 3.

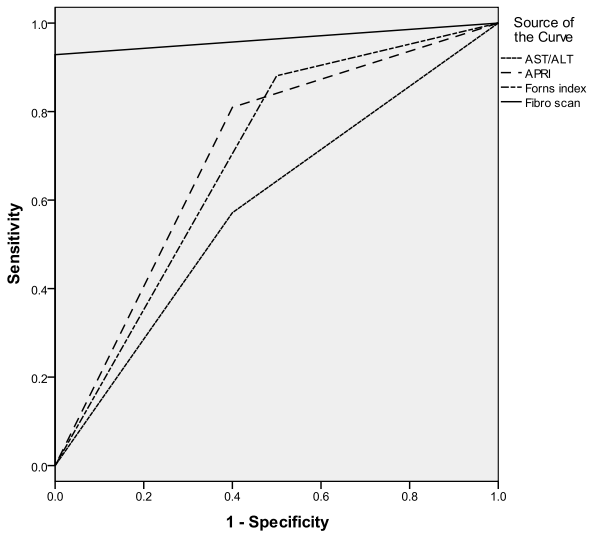

Fig. 2 – ROC curve for surrogate markers of liver fibrosis with cut-off. For abbreviations see Table 3.

Table 5

Area under the curve (AUC) for surrogate markers of liver fibrosis (fibrosis: stage I–II vs. III–IV)

Surrogate markers of liver fibrosis	AUC	Asymp significance	95% Confidence interval (bound: lower-upper)
AST/ALT	0.428	0.480	0.235–0.621
APRI	0.601	0.323	0.396–0.806
Forns index	0.676	0.084	0.495–0.858
TE (kPa)	0.865	0.000*	0.747–0.984

For other abbreviations see Table 3;*statistically significant.

Table 6

Sensitivity and specifity for surrogate markers of liver fibrosis (fibrosis: stage F – I-II vs. F – III-IV)

Surrogate markers of liver fibrosis	Cut-off	Sensitivity	Specifity	Asymp significance	AUC (95% CI)
AST/ALT	1.010	0.500	0.615	0.572	0.558 (0.358–0.757)
APRI	0.297	0.667	0.577	0.233	0.622 (0.429–0.814)
Forns index	5.460	0.750	0.654	0.048*	0.702 (0.522–0.882)
TE (kPa)	9.900	0.917	0.692	0.003*	0.804 (0.659–0.950)

AUC – area under the curve; for other abbreviations see Table 3; *statistically significant.

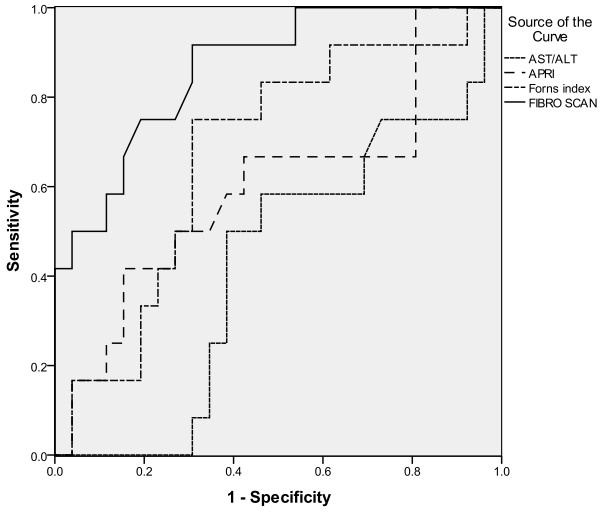

Fig. 3 – ROC for surrogate markers of liver fibrosis, fibrosis: stage F – I-II vs. F – III-IV. For abbreviations see Table 3.

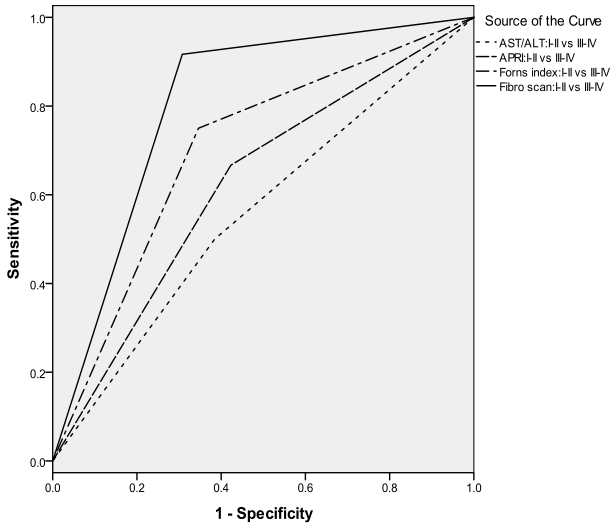

Fig. 4 – ROC curve for surrogate markers of liver fibrosis: stage F – I-II vs. F – III-IV with cut-off. For abbreviations see Table 3.

Transient elastography findings

The values of TE ranged from 3.2 to 30.7 kPa, median 6.8 kPa, mean 9.6 ± 7.0 kPa. A statistically significant difference was found between TE and fibrosis stage of liver disease (Tables 2 and 3). TE was found to be accurate in diagnosing the presence of liver fibrosis. For cut-off value of 7.25 kPa, it showed 92.9% of sensitivity and 100% of specificity (AUROC 0.964). These results were the best when compared to other surrogate markers of fibrosis. The optimal cut-off values for distinguishing patients having mild-to-moderate fibrosis (F1 or F2) and advanced fibrosis (F3 or F4) were 9.9, with 91.7% of sensitivity and 69.2% of specificity and AUROC 0.865 (Tables 5 and 6, and Figures 3 and 4, respectively). These results were superior in comparison to other surrogate markers for liver fibrosis.

Discussion

Primary biliary cirrhosis occurs worldwide with a female to male ratio of 9:1. The diagnosis of PBC is based on criteria which include elevation of liver enzymes, positive AMA test and positive liver biopsy. Widespread use of screening laboratory tests has led to an increase of PBC diagnosis frequency while the disease is in asymptomatic stage. Liver biopsy has been considered to be a gold standard for the diagnosis of PBC, even though fibrosis in PBC is patchy in distribution within the parenchyma and many patients are reluctant to experience repeated biopsies which limits our ability to monitor disease progression and effects of treatment.

In recent decades, a lot has been done in order to find adequate noninvasive markers for the assessment of liver fibrosis. The ideal characteristics of such markers would be: specificity for liver fibrosis; providing measurement of: stage of fibrosis, fibrogenesis activity; not influenced by comorbidities (e.g. renal, reticulo-endothelial); known half-life; known excretion route; sensitivity and reproduciblity [11]. Direct markers are markers of fibrogenesis, measurable in peripheral blood as a direct expression of either the deposition or removal of ECM in liver (several glycoproteins, the collagen family, the collagenases and their inhibitors and a number of cytokines connected with the fibrogenetic process). Indirect markers of liver fibrosis are routinely performed blood tests. The diagnostic performance of most direct and indirect markers of liver fibrosis has been widely investigated in all common etiological forms of chronic liver diseases, but not as much in patients with PBC. Unfortunately, there are currently no serum surrogate markers of liver fibrosis routinely recommended in PBC.

Our study is the PBC specific and it was conducted on particulary homogenous study population recruited from a single center. On the same day, each patient involved in this

study, underwent blood tests and liver biopsy, while TE was performed in the following month.

In this study we investigeted 3 noninvasive markers, available and routinely used in every day clinical practice. The AST/ALT ratio, although widely used, did not show any statistical significance.

As far as we know, there are few published studies assessing the APRI score in the PBC patients [7, 9, 11, 12]. Obara et al. [13] showed that the APRI score can predict fibrosis F ≥ 2 (AUROC O.77) in patients with nonviral liver diseases. In our study that was not the case. The APRI score did not show statistically significant difference in distinguishing patients with mild-to-moderate fibrosis and advanced fibrosis, perhaps because the study population was not the same. In our study, the APRI score showed statistically significant difference in presence and stages of liver fibrosis (AUROC 0.782). A cut-off value of 0.255 (sensitivity 81%, specificity 60%, AUROC 0.705) could distinguish patients who did not have (F0) and those who had liver fibrosis (F1).

In our study, the Forns index, although not so widely used and investigated in cholestatic liver disease, did correlate with the presence and stages of liver fibrosis (AUROC 0.806). The optimal cut-off for F ≥ 3 was 5.46 (sensitivity 75%, specificity 65.4%, AUROC 0.702).

In 2012, a retrospective study was conducted in China [14]. It included 73 patients with PBC and assessed the diagnostic value of noninvasive markers of liver fibrosis in PBC based on coventional laboratory results (platelet count, serum cholinesterase, albumin, HDL-C and prothrombin time activity). According to this study, the established noninvasive model could accurately distinguish pathological changes of early stage of PBC (stage I–II) from advanced stage (III–IV).

TE is a novel, noninvasive method used for evaluation of fibrosis in chronic liver disease. Published meta analyses have shown that TE is a reliable method for diagnosing liver cirrhosis [15–17]. The effectiveness of TE is well established in patients with chronic hepatitis C [18], but it has not been used widely in assessing fibrosis in nonviral liver disease. In the past few years, a very small number of studies investigating the effectiveness of TE in evaluation of patients with PBC was published.

A study conducted in Spain including 80 patients with PBC, showed statistically significant correlation between TE and liver biopsies [6], while another study from Italy conducted on 120 patients with PBC proved that TE is a simple, reliable and useful method for assessing liver fibrosis [7].

Coprechot et al. [8] assessed 140 patients with PBC in order to define the diagnostic performance of TE and the time course of changes of fibrosis progression as well as prognosis in a monitored cohort of ursodeoxycholic acid (UDCA)–treated patients followed up for five years. Their results showed that TE is one of the best current surrogate markers of liver fibrosis in PBC. In a five year period while on the treatment, liver stiffness appeared to remain stable in most noncirrhotic patients, whereas it significantly increased in patients with cirrhosis. The study did not find evidence that combination of TE and noninvasive markers significantly improved diagnostic accuracy.

Present findings strongly suggest that monitoring of TE in patients with PBC provides significant prognostic information in comparison with classic serum prognostic markers and that it may be used to predict outcome and select high-risk patients for further clinical trials [8].

In addition, TE efficiency was validated by comparing it to other imaging techniques. Friedrich-Rust et al. [9] compared TE, magnetic resonance imaging (MRI) and spectroscopy MRI, and serum markers in 45 PBC patients. They showed that MRI and TE can be used with comparable results for the assessment of liver fibrosis in patients with PBC and that the two techniques seem to supplement each other.

In our study, TE appears to be the best surrogate marker for assessment of liver fibrosis in patients with PBC. When compared to other noninvasive liver markers, TE was shown to have the highest sensitivity and specificity.

Conclusion

Assessment of liver fibrosis by TE is an easy, rapid, effective, and safe noninvasive method with high sensitivity and specificity. Using noninvasive markers and methods in evaluating patients in daily clinical practice may reduce, but still not eliminate, the need for invasive diagnostic procedures.

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Development of a scale for evaluating the severity of disfigurements caused by injuries disease or surgery

Razvoj skale za procenu stepena naruženja koja su nastala zbog povreda, oboljenja i operacija

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Abstract

Background/Aim. Medico-legal aspect of physical disfigurements has been subject of few scientific researches. The aim of this study was to establish a standardized instrument for forensic evaluation of all kinds of physical disfigurements, which has not existed until now. **Methods.** The research was conducted in 3 phases: the first one – drafting a list of disfigurements; the second phase – evaluation of the draft of the disfigurement list provided by 11 experts (plastic surgery lecturers) by the use of the Delphy method; the third one during which 51 medical doctors, members of Serbian Association of Plastic, Reconstructive and Aesthetic Surgery were evaluating the list of disfigurements. **Results.** Totally 176 individual physical disfigurements were described in the first phase of research. In the second phase, 217 disfigurements were established which were classified into 5 degrees of severity 0 – none/very small, 1 – small, 2 – medium, 3 – large, 4 – very large disfigurements. In the third phase, 217 disfigurements were evaluated. **Conclusion.** The first 4-point ordinal scale was established, rating 217 disfigurements, which showed high level of compatibility in practice and which can be used as an instrument for standardization of disfigurements in giving of forensic expertise.

Key words:
forensic medicine; surgery, plastic; cicatrix; compensation and redress; weights and measures; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Sudsko-medicinski aspekt telesnih naruženja do sada je bio predmet malog broja naučnih istraživanja. Cilj ovog rada bio je da se ustanovi standardizovan instrument za sudsko-medicinsku procenu svih vrsta telesnih naruženja koji do sada nije postojao. **Metode.** Istraživanje je realizovano kroz tri sukcesivne faze: prvu – formiranje nacrta liste naruženja nastalih kao posledica povreda, operacija, oboljenja i lečenja; drugu – procena nacrta liste naruženja od strane 11 eksperata (nastavnika plastične hirurgije) primenom Delfi metoda; treću - u kojoj je prethodno formirana lista naruženja procenjivana od strane 51 lekara, članova strukovnog udruženja hirurga specijalista plastične i rekonstruktivne hirurgije. **Rezultat.** U prvoj fazi istraživanja opisano je 176 pojedinačnih telesnih naruženja hipotetički razvrstanih u četiri stepena gradacije. U drugoj fazi formirano je 217 naruženja koja su razvrstana u pet stepeni 0 – bez ili veoma malo, 1 – malo, 2 – srednje, 3 – veliko i 4 – veoma veliko naruženje. U trećoj fazi, 217 naruženja je ponovo ocenjeno, pri čemu je stepen usaglašenosti sa drugom fazom bio visok a to je pokazao Mann-Whitney test. **Zaključak.** Istraživanjem u tri faze konstruisana je petostepena ordinalna skala sa 217 naruženja koja je pokazala visok stepen usaglašenosti među kolegama i koja se može koristiti kao instrument za standardizovano, unificirano merenje naruženja u sudskim veštačenjima.

Ključne reči:
medicina, sudska; hirurgija, plastična; ožiljak; naknada i obeštećenje; mere i merenja; ankete i upitnici.

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Introduction

The term disfigurement is defined as an impairment of previous external appearance or body harmony, i.e. the harmony of a body part of an individual or as an impairment of any body function which is reflected in physical appearance [1]. In legal terminology, disfigurement refers to any damage suffered by a patient. It represents the link between medical and legal science, both in practical and theoretical terms [1]. Living with a disfigurement represents an emotional, social and cultural challenge, since physical appearance is, after all, important in relations with other people [2]. The damaged ones often claim financial compensation for mental anguish suffered because of disfigurement [1]. It is believed that mental anguish here arises from the uncomfortable feeling of inferiority and suffering of disfigured individuals, which are particularly caused by reactions and behavior of their social environment (pitiful, repulsive, contemptuous attitude) [3]. The mission of experts is to evaluate disfigurements, in the most objective manner possible. The expert report submitted to the Court of Law is compiled based on a detailed clinical examination and it includes expert opinion – expertise [1]. Although disfigurements are often clear and obvious, it is difficult to establish objective criteria for disfigurement description and evaluation, due to a wide range of existing situations referring to such individuals [1]. Therefore, disfigurement and disfigurement expertise remain an inadequately resolved problem.

Previous studies have shown that just few papers on medico-legal aspect of scars and disfigurements in general, have been published [4]. Several scales for scar evaluation and evaluation of the effects of therapy are in use, but these scales do not contribute to the medico-legal evaluation of scars [Vancouver Scar Scale (VSS) and Manchester Scar Scale (MSS)] [5–8].

The aim of this study is to provide description and classification for all types of disfigurements according to their severity, all for the purpose of medico-legal expertise in disfigurements.

Methods

The research was conducted in 4 successive phases: the first one – drafting a list of disfigurements occurring as a result of injuries, surgery, illness and treatment; the second phase – evaluation of the draft of the disfigurement list provided by 11 experts (plastic surgery lecturers) using the Delphi method (in 3 iterations); the third one during which 51 medical doctors, members of Serbian Association of Plastic, Reconstructive and Aesthetic Surgery (SRBPRAS) participated in evaluating previously formed list of disfigurements; and the fourth phase during which the list, i.e. scale of disfigurements, formed in the previous, third phase, was applied to actual cases taken from the case-law.

In the process of preparing the questionnaire, 3 groups of disfigurements were hypothetically determined first, according to their exposure to view, i.e. according to the visibility of zones, regions or body parts where disfigurements could be identified. According to this criterion the first group includes disfigurements of body parts which are always exposed to view (disfigurements of the face, neck, scalp, and hands). The second group includes disfigurements identified on the body parts which are frequently exposed to view (forearms, lower legs, feet) and the third group includes disfigurements of the body parts which are rarely exposed to view (upper arms, thighs, torso, genitals).

After defining these groups, 176 individual disfigurements in total were hypothetically determined within each one of them according to the method of their quantitative and/or qualitative descriptions. Hence, 117 disfigurements were determined within the first group, 15 within the second one and 44 disfigurements within the third group. The following measurement units were used for the purpose of providing quantitative descriptions of disfigurements: 1) percentage (%) of the body surface area covered by disfigurement with respect to the total body surface area (TBSA); 2) the length of linear (expressed in cm) and surface spatial disfigurements (expressed in cm^2 or mm^2); 3) lack (amputation) of the whole organ or a part thereof, expressed in natural numbers (for example: one limb, 2 teeth, 3 fingers) or as a ratio (for example: 1/2 lip, 1/3 earlobe) according to the criterion of functional reconstruction of organs.

Specific characteristics of disfigurements were used for the purpose of providing their qualitative descriptions (for example: relief, elevation, depression etc.) [2].

The questionnaire was completed by determining 4 possible degrees for classifying each of 176 individually described disfigurements according to the severity criterion. Each of 176 individually determined disfigurements was classified under one of these degrees (degree I – small; degree II – medium; degree III – large and degree IV – very large disfigurements). The questionnaire designed in this way, represented the basis for the application of the Delphi method in the following phase of research. Within the received questionnaire, the experts were provided with the descriptions of individual disfigurements and 4 possible degrees for their classification according to the severity criterion, but they could not see their hypothetical classification under these degrees, nor did they know the individual opinions of other experts.

The first phase included using the method of content analysis and using personal expert experience of the candidates. The findings of research published in the available literature and electronic databases which defined the key concepts (scale, disfigurement, scar/scars, medico-legal evaluation, damage compensation) were used during the content analysis.

Delphi method implies that experts should provide their opinions, without any group discussions, by answering the questions given in the questionnaire in several phases. In each successive stage these experts again answer the questions on which no consensus was reached in the previous phase and they are able to confirm, modify or completely change their answers after analyzing answers of other experts given in the first phase. At the same time, none of the experts knows the identity of other participants nor their individual answers to the questions asked [9].

For the purpose of this research, the Delphi method was applied by obtaining expert opinions on each proposed disfigurement in the first iteration, whereby experts submitted their opinions individually and anonymously, which referred to the following: a) description, whereby the experts could accept, dismiss, correct or propose a completely new description; b) degree, whereby the experts could classify each proposed disfigurement under one of four offered degrees, according to their severity (I – small; II – medium; II – large and IV – very large disfigurements) provided that each of the proposed disfigurements was observed and evaluated from the distance of 50 cm from an unclothed patient [1].

All disfigurements that most of the experts (more than 50% or at least 6 out of 11) accepted in terms of their description and classified under the same degree, were considered accepted in terms of the description and degree and they were not included in the following iteration. Data on the expert opinions on disfigurements that, in terms of their descriptions, some of the experts dismissed or proposed their corrections, newly proposed disfigurements or disfigurements classified under different degrees, were presented in the following (second) iteration to all experts for another round of decision-making.

This procedure was repeated in the third iteration, during which the consensus was reached among the experts as regards the acceptance, dismissal or correction of each description and degree of disfigurement on which the consensus was not reached in the previous iteration. Up until this iteration, the experts did not know the identity of other participants in the survey, nor did they know who gave what answer in the previous iteration. A selection criterion for qualifying individual descriptions and degrees of disfigurements for the following iteration step was the consensus among the experts (at least 6 out of 11 experts, or more than 50%).

The first 3 phases of the research were conducted in the period between 2013 and 2015, at the Department of Surgery, i.e. the Department of Plastic Surgery, at the School of Medicine, the Universities of Belgrade, Novi Sad and Kosovska Mitrovica. Experts from these scientific areas, medical specialists in plastic and reconstructive surgery, engaged in teaching, participated in this research. The fourth phase research was conducted in April, 2016 in the Third Basic Court in Belgrade. We reviewed the total number of 75 different types of disfigurements that were subjected to forensic expertise in the period between 2005 and 2015. These cases included the evaluation of the severity of disfigurements by authorized forensic experts, who are specialists in forensic medicine, orthopaedics, physical medicine and plastic surgery. In providing their expertise on particular disfigurement, or very small forensic experts could declare that there was no disfigurement – 0, or that the disfigurement is small – 1, medium – 2, large – 3 or very large – 4.

The following statistical methods were used for processing these data: descriptive and inferential statistical analysis and non-parametric statistical method for testing the significance and strength of concordance (Kendall's W– coefficient of concordance). The first method was used to present individual descriptions of disfigurements in the form of a central tendency measure - the median, as well as the measure of dispersion, presented by the interquartile range. These parameters were complemented by the presentation of the number and percentage of experts who had a consistent position in determining the degree of individual disfigurements. The second method, i.e. testing the significance and strength of concordance was applied at the probability level of $p < 0.05$.

Results

The result of the first phase was a draft of a hypothetical model – a list of disfigurements occurring as a result of injuries, surgery, illness and treatment; the second phase – evaluation of the draft of the disfigurement list provided using the Delphi method. In the first iteration of the Delphi method experts accepted descriptions of all 176 proposed disfigurements. Ninety three out of 176 disfigurements were rated in the same manner as in the hypothetical model draft, whereas 49 disfigurements were rated differently, while consensus was not reached in the first iteration regarding 34 descriptions.

In the second iteration of the Delphi method, the consensus in terms of the evaluation was reached for 30 disfigurements. Kendall's coefficient of concordance (W) (Table 1) was obtained and it represented the level of consensus reached among experts in assessing the degree of disfigurement. In addition, majority of experts agreed that it was necessary to add a new group including 30 descriptions of very small disfigurements. Furthermore, a new description of a small disfigurement was added. The number of disfigurements in the end of this iteration reached 207.

In the third iteration, 4 disfigurements on which no consensus had been reached in two previous iterations, were evaluated. Based on the consensus of the experts involved, 12 descriptions of individual disfigurements were included, whereas 22 descriptions of disfigurements were rephrased and reevaluated in order to achieve greater accuracy of the disfigurement description. Therefore, the total number of descriptions of disfigurements increased from the initial 207 to 217 (Appendix).

The main characteristic of of the third phase was the process clustering disfigurements, after the members of SRBPRAS provided their opinions. In that phose the number of disfigurements classified as large and very large disfigurements slightly decreased [from 59 (27.2%) and 37 (17.1%) to 53 (24.4%) and 31 (14.3%) respectively], whereas a slight increase [from 30 (13.8%), 41 (18.9%) and 50 (23.0%) to 32 (14.8%), 45 (20.7%) and 56 (25.8%)] was identified in all other groups (very small, small and medium disfigurements, respectively). Thereby, the total number of disfigurements remained the same, i.e. 217.

Table 1

Statistical indicators of the Delphi method performance in the harmonization of disfigurement evaluations provided by 11 experts in three iterations

**Code disfigurements	Degreee as per hypothesis	First iteration		Second iteration		Third iteration	
		median (iq*)	approval of experts n (%)	median (iq*)	approval of experts n (%)	median (iq*)	approval of experts n (%)
33a	1	2 (2–3)	5 (45.5)	2 (2–3)	6 (54.5)	2 (2-3)	6 (54.5)
33d	1	3 (2–3)	4 (36.4)	2.5 (2–3)	4 (36.4)	3 (2-3)	6 (54.5)
33e	1	3 (2–4)	5 (45.5)	3 (2–4)	5 (45.5)	4 (2-4)	7 (63.6)
35c	1	3 (2–3)	5 (45.5)	3 (2–3)	5 (45.5)	3 (2-3)	6 (54.5)
1b	2	2 (2–3)	5 (45.5)	3 (2–3)	7 (63.6)	3 (2-3)	7 (63.6)
1c	2	3 (2–4)	4 (36.4)	4 (3–4)	7 (63.6)	4 (3-4)	7 (63.6)
2c	2	2 (2–3)	5 (45.5)	2.5 (2–3)	6 (54.5)	2 (2-3)	6 (54.5)
4b	2	2 (1–3)	5 (45.5)	2 (2–3)	7 (63.6)	2 (2-3)	7 (63.6)
4c	2	3 (2–3)	5 (45.5)	3 (3–3)	9 (81.8)	3 (3-3)	9 (81.8)
7b	2	2 (1–2)	5 (45.5)	2 (2–2)	9 (81.8)	2 (2-2)	9 (81.8)
13c	2	2 (2–3)	5 (45.5)	2 (2–3)	7 (63.6)	2 (2-3)	7 (63.6)
24d	2	3 (2–3)	5 (45.5)	3 (3–3)	9 (81.8)	3 (3-3)	9 (81.8)
25c	2	2 (2–3)	5 (45.5)	2 (2–3)	7 (63.6)	2 (2-3)	7 (63.6)
25d	2	3 (2–3)	5 (45.5)	3 (3–3)	9 (81.8)	3 (3-3)	9 (81.8)
27d	2	3 (2–4)	5 (45.5)	3 (3–4)	8 (72.7)	3 (3-4)	8 (72.7)
28d	2	3 (2–3)	5 (45.5)	3 (2–3)	7 (63.6)	3 (2-3)	7 (63.6)
29d	2	3 (2–3)	5 (45.5)	3 (2–3)	5 (45.5)	3 (2-3)	6 (54.5)
31d	2	3 (2–3)	5 (45.5)	2 (2–3)	7 (63.6)	2 (2-3)	7 (63.6)
32d	2	3 (3–4)	5 (45.5)	3 (3–3)	9 (81.8)	3 (3-3)	9 (81.8)
2d	3	3 (2–3)	5 (45.5)	3 (2–3)	8 (72.7)	3 (2-3)	8 (72.7)
3d	3	3 (3–4)	5 (45.5)	3 (3–4)	8 (72.7)	3 (3-4)	8 (72.7)
4d	3	3 (2–4)	5 (45.5)	4 (3–4)	7 (63.6)	4 (3-4)	7 (63.6)
6d	3	3 (2–3)	5 (45.5)	2 (2–3)	6 (54.5)	2 (2-3)	6 (54.5)
6e	3	3 (2–4)	4 (36.4)	4 (2–4)	6 (54.5)	4 (2-4)	6 (54.5)
15c	3	3 (2–3)	5 (45.5)	2 (2–3)	6 (54.5)		
15d	3	3 (3–4)	5 (45.5)	3 (3–4)	7 (63.6)		
20c	3	2 (2–3)	5 (45.5)	2 (2–2)	9 (81.8)		
24e	3	3 (3–4)	5 (45.5)	3 (3–4)	7 (63.6)	3 (3-4)	7 (63.6)
25e	3	3 (2–4)	5 (45.5)	3 (3–4)	7 (63.6)	3 (3-4)	7 (63.6)
26e	3	3 (3–4)	5 (45.5)	3 (3–4)	6 (54.5)	3 (3-4)	6 (54.5)
27e	3	3 (3–4)	5 (45.5)	3 (3–4)	6 (54.5)	3 (3-4)	6 (54.5)
28e	3	3 (3–4)	5 (45.5)	3 (3–4)	8 (72.7)	3 (3-4)	8 (72.7)
30b	3	2.5 (2–3)	5 (45.5)	2 (2–3)	8 (72.7)	2 (2-3)	8 (72.7)
1d	4	3 (2–4)	5 (45.5)	4 (3–4)	6 (54.5)	4 (3-4)	6 (54.5)
Kendall W signif-icance		0.277		0.517		0.562	
p		< 0.0001		< 0.0001		< 0.0001	

***Interquartile range of 25th and 75th percentile.**

****Code of disfigurement: eache disfigurement had a designed code which remained the same throughout the whole study in order to track accurately any potential change in the description of relevant disfigurement.**

Discussion

This final ordinal scale of disfigurements confirms that its draft established in the first phase of research, represented a good foundation for the implementation of the following 3 phases of research. In the second and the third phase, it was adjusted and qualitatively improved in a methodologically adequate scientific procedure, particularly with regard to.

The number of descriptions of individual disfigurements and the number of disfigurement severity degrees.

So far, just a few descriptions of disfigurement have appeared in relevant literature (elevated scar, depressed scar, hipertrophyc scar etc [7]. We provided disfigurement description and evaluation in terms of describing a linear scar, a relief scar, a scar with or without contracture, facial or body asymmetry, amputation, etc.

Several scales that provide rating from 1–13 or 1–100 are currently in use exclusively to evaluate scars as a disfigurement. Through a scientific procedure we obtained a scale which enables rating from 0–4 which is more practical.

Until now it was not possible evaluate disfigurements by using score of 0–4 according to the research that we reviewed.

Such results of the fourth phase of research confirmed the existence of good judicial practice, i.e. practice of forensic expert witnesses that turned out to be consistent with the findings of the conducted scientific research to a greater extent. In addition, results of the research may be of use in future forensic expertise, due to the possibility of using the scale of disfigurements as an instrument of expertise.

Conclusion

Through 3 phases of research and with the participation of 62 specialists in plastic and reconstructive surgery, a 5-degree ordinal scale was established, rating the total of 217 disfigurements according to the severity criterion as none/very small (0), small (1), medium (2), large (3) and very large (4) disfigurements.

Such ordinal scale of disfigurements is a result of a scientific process and may be used for uniform assessment of disfigurement severity in giving forensic expertise.

The results of this study indicate the need for their verification in practice and the need for possible adjustment of degrees of disfigurement, the number of individual descriptions of disfigurements, establishing new disfigurements or additionally, more precise definition of existing disfigurements.

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Appendix

VERY SMALL DISFUGUREMENT (0); n = 32	
Code	**Description of disfigurement**
6a	Absence or dental caries of on tooth (from one to four)
19a	Slight gait and posture deviation, barely visible (due to contractures in large joints, limping, involuntary movements or palsy)
32a	Asymmetry of male and female genital organs - hardly visible
35e	Amblyopia, poor eyesight
36a	Very small disfigurement of sclera or iris – barely visible
36b	Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length up to 1cm
36c	Multiple benign lesions present on the face and/or forehead (acne scars) up to 1cm^2
36d	Elevated/depressed and/or relief face and/or forehead scar (without contracture) up to 1cm^2
36e	Facial asymmetry, hardly visible at first sight
36f	Minimum eyelid deficiency, hardly visible at first sight
36i	Lack of any part of the earlobe up to 1cm^2
36j	Elevated and/or depressed and/or relief scalp scar up to 2cm^2
36k	Linear scalp scar (at the level of the surrounding skin or not), overall length up to 2cm
36l	Elevated and/or depressed and/or relief hand scar (with or without contracture) up to 2cm^2
36m	Multiple benign lesions present on a hand up to 2cm^2
36n	Elevated / depressed and/or relief neck scar without contracture, up to 2cm^2
36o	Coverage of neck benign lesions (acne scars) up to 2cm^2
36p	Linear neck scar (at the level of the surrounding skin or not), overall length up to 2cm
36q	Linear scar on both hands (at the level of the surrounding skin or not), overall length up to 2cm
36r	Loss of a part of a fingertip and/or finger nail plate
36s	Elevated/depressed/relief forearm and/or lower leg and/or foot scar with or without contracture, up to 8cm^2
36t	Multiple benign lesions present on a forearm and/or lower leg and/or foot (scars), up to 8cm^2
36u	Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length up to 4cm
36v	Elevated/depressed/relief torso and/or upper arm and/or upper leg scar, up to 2.5%
36w	Multiple benign lesions present on the torso and/or upper arm and/or upper leg (smallpox scars), up to 2.5%
36x	Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length up to 8 cm
36y	Hardly visible breast asymmetry, imperceivable at first sight
36z	Breast scar, imperceivable at first sight
37a	Asymmetry of genital organs, imperceivable at first sight
37b	Loss of up to ½ of glanspenis
37c	Lack of up to 2cm^{2}of vulva
37d	Relief scar of female or male genital organs, imperceivable at first sight, up to 2cm^2

SMALL DISFUGUREMENT (1); n = 45		
Code	**Degree**	**Description of disfigurement**
1a		Very mild strabismus, hardly visible
2a		Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length 1- 2cm
2b		Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length2-5 cm
3a		Multiple benign lesions present on the face and/or forehead (acne scars and/or smallpox scars and/or skin diseases and/or tumors) 1-2cm^2
4a		Elevated and/or depressed and/or relief face and/or forehead scar (without contracture)1-2cm^2
5a		Slightly visible facial asymmetry (due to scarring and/or desmogenous contracture and/or paralysis); facial expression of feelings is not altered
6b		Absence or dental caries of up to two teeth (from one to four)
7a		Deficiency of all or certain eyelid tissues, barely visible
10a		Deficiency of all or certain earlobe tissues from 1cm^{2}to 1/4 of helix
10b		Partial loss of the lobule
11a		Elevated and/or depressed and/or relief scalp scar (with or without alopecia or contracture) 2–4cm^2
12a		Linear scalp and/or ear scar (at the level of the surrounding skin or not), overall length 2–4cm
12b		Linear scalp and/or ear scar (at the level of the surrounding skin or not), overall length4cm-10cm
13a		Elevated and/or depressed and/or relief hand scar (with or without contracture) 1/4-1/2 TBSA
14a		Multiple benign lesions present on a hand (acne/smallpox scars, skin diseases and tumors) 2cm^2-1/4TBSA
15e		Elevated and/or depressed and/or relief neck scar without contracture, total coverage 2- 4cm^2
16a		Multiple benign lesions present on the neck (acne/smallpox scars, skin diseases and tumors) 2-4cm^2
17a		Linear neck scar (at the level of the surrounding skin or not), overall length 2- 4cm
18a		Linear hand scar (at the level of the surrounding skin or not), overall length 2-4 cm
18b		Linear scar on both hands (lateral, at the level of the surrounding skin or not), overall length4-10 cm

19b		**Slight gait and posture deviation due to contractures in large joints, limping, involuntary movements or palsy**
20f		**Amputation of one (any) hand phalanx, thumb excluded**
21a		**Amputation of one (any) foot phalanx**
21b		**Amputation of one toe, big toe excluded**
23a		**Elevated and/or depressed and/or relief forearm and/or lower leg and/or foot scar with or without contracture8-16 cm^2**
24a		**Multiple benign lesions present on a forearm and/or lower leg and/or foot (acne scars, smallpox scars, skin diseases and tumors)8- 16 cm^2**
25a		**Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length 4- 8 cm**
26a		**Elevated/depressed/relief torso and/or upper arm and/or upper leg scar (with or without contracture) 2.5-5 % TBSA**
27a		**Multiple benign lesions present on the torso and/or upper arm and/or upper leg (acne/smallpox scars, skin diseases and tumors) 2.5- 5 % TBSA**
28a		**Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length8- 16 cm**
29a		**Breast asymmetry,hardly visible**
29b		**Breast asymmetry, slightly visible**
29c		**Breast asymmetry, clearly visible**
31a		**Linear or relief breast scar, hardly visible**
31b		**Linear or relief breast scar, slightly visible**
32b		**Asymmetry of male and female genital organs, clearly visible**
32c		**Distinct asymmetry of male and female genital organs**
32d		**Striking asymmetry of male and female genital organs**
34a		**Lack, loss of 2cm^2 - ¼ of vulva**
35a		**Elevated and/or depressed, relief scar of female or male genital covering the surface area of 2 cm^2- ¼ of genital organs**
35b		**Elevated and/or depressed, relief scar of female or male genital covering the surface area of ¼ - ½ of genital organs**
35c		**Elevated and/or depressed, relief scar of female or male genital covering the surface area of more than ½ of genital organs**
35d		**Elevated and/or depressed and/or relief hand scar (with or without contracture)2 cm^2-¼TBSA**
37g		**Elevated and/or depressed scar of nasal dorsum and sidewalls covering the surface area of 0-3mm^2**
37e		**Lack of a part of the lip 0-3mm^2**

MEDIUM DISFUGUREMENT (2); n = 56	
Code	**Description of disfigurement**
1c	**Blindness (referring to the appearance of a blind person, to the eye function)**
2c	**Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length 5-10 cm**
3b	**Multiple benign lesions present on the face and/or forehead (acne/smallpox scars and/or skin diseases and/or tumors) 2-16 cm^2**
4b	**Elevated and/or depressed and/or relief face and/or forehead scar (without contracture) 1-16 cm^2**
5b	**Clearly visible facial asymmetry (due to scarring and/or desmogenous contracture and/or paralysis) and/or slightly distinct ectropion; facial expression of feelings is not altered**
6c	**Absence or dental caries of on up to three teeth (from one to four)**
6d	**Absence or dental caries of on six to eight front teeth**
6e	**Absence or dental caries of on more than eight front teeth**
7b	**Deficiency of all or certain eyelid tissues, up to 1/3 of a lid**
7c	**Deficiency of all or certain eyelid tissues, 1/3-1/2 of a lid**
8a	**Elevated and/or depressed nose scar, covering 0.5-1$cm^2$of nasal dorsum and sidewalls**
9a	**Lack of a part or the entire lip fullness 5 mm^2- 1/4usne**
9b	**Lack of a part or the entire lip fullness 1/4 -1/3**
10c	**Deficiency of all or certain earlobe tissues 1/4 - 1/3**
10d	**Loss of the lobule, more than½**
10e	**Deficiency of all or certain earlobe tissues 1/3-1/2**
11b	**Elevated and/or depressed and/or relief scalp and/or ear scar (with or without alopecia or contracture)od 4 cm^2 -1% TBSA**
12c	**Linear scalp scar (at the level of the surrounding skin or not), overall length 10-20cm**
13b	**Elevated and/or depressed and/or relief hand scar (with or without contracture) 1/2% -1% TBSA.**
14b	**Multiple benign lesions present on hands (acne/smallpox scars, skin diseases and tumors) 1/4-1/2 TBSA**
14c	**Multiple benign lesions present on hands (acne/smallpox scars, skin diseases and tumors) 1/2% -1% TBSA**
15f	**Elevated and/or depressed and/or relief neck scar without contracture, total coverage 4cm^2-0.5% TBSA**
15g	**Elevated and/or depressed and/or relief neck scar without contracture, total coverage 0.5-1% TBSA**
15i	**Elevated and/or depressed and/or relief neck scar with contracture, total coverage 2-4cm^2**
16b	**Multiple benign lesions present on the neck (e.g. acne/smallpox scars, skin diseases and tumors) 4cm^2 –0.5% TBSA**
17b	**Linear neck scar (at the level of the surrounding skin or not), overall length 4-10 cm**
17c	**Linear neck scar (at the level of the surrounding skin or not), overall length 10-20 cm**
18c	**Linear scar of both hands (lateral, at the level of the surrounding skin or not), overall length 10-20 cm**
19c	**Obvious gait and posture deviation due to contractures in large joints, limping, involuntary movements or palsy**
20g	**Amputation of two (any) hand phalanges, thumb excluded**
20h	**Loss of one finger, thumb excluded**
20k	**Lossof one thumb phalanx**

21c	**Loss of two toes**
21d	**Loss of two to five toes**
21f	**Amputation of a big toe**
23b	**Elevated and/or depressed and/or relief forearm and/or lower leg and/or foot scar with or without contracture, 16 cm^2- 5% TBSA**
24b	**Multiple benign lesions present on a forearm and/or lower leg and/or foot (acne scars, smallpox scars, skin diseases and tumors)16 cm^2- 5% TBSA**
24c	**Multiple benign lesions present on a forearm and/or lower leg and/or foot (acne scars, smallpox scars, skin diseases and tumors) 5% - 15% TBSA**
25b	**Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length 8-20 cm**
25c	**Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length 20-100 cm**
26b	**Elevated and/or depressed and/or relief torso and/or upper arm and/or upper leg scar (with or without contracture) 5-10 % TBSA**
27b	**Multiple benign lesions present on the torso and/or upper arm and/or upper leg (acne/smallpox scars, skin diseases and tumors) 5 -10 % TBSA**
27c	**Multiple benign lesions present on the torso and/or upper arm and/or upper leg (acne/smallpox scars, skin diseases and tumors) 10-40 % TBSA**
28b	**Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length 16- 40 cm**
28c	**Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length 40-100 cm**
29d	**Distinct breast asymmetry**
29e	**Striking breast asymmetry**
30a	**Lack of up to ¼ of a breast**
30b	**Lack of up to ¼ - ½ of a breast**
31c	**Linear or relief breast scar, overall length up to 10cm**
31d	**Linear or relief breast scar, overall length 10-30cm**
31e	**Linear or relief breast scar, overall length more than 30 cm**
33a	**Lack, loss of more than ½ or the whole of glans penis**
33d	**Lack, loss of one testicle**
37f	**Lack of a part of the lip 3-5 mm^2**
37h	**Elevated and/or depressed nose scar, covering 3-5 $mm^2$of nasal dorsum and sidewalls**

LARGE DISFUGUREMENT (3); n = 53

Code	Description of disfigurement
1b	**Clearly visible strabismus**
2d	**Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length 10-15 cm**
2e	**Linear face and/or forehead scar (at the level of the surrounding skin or not), overall length more than 15 cm**
3c	**Multiple benign lesions present on the face and/or forehead (acne scars and/or smallpox scars and/or skin diseases and/or tumors) 16 cm^2-2% TBSA**
3d	**Multiple benign lesions present on the face and/or forehead (acne scars and/or smallpox scars and/or skin diseases and/or tumors) 2% TBSA or more**
4c	**Elevated and/or depressed and/or relief face and/or forehead scar (without contracture) 16 cm^2-2% TBSA**
5c	**A distinct facial asymmetry (due to scarring and/or desmogenous contracture and/or paralysis, e.g. central facial palsy) and/or distinct ectropion; altered facial expression of feelings**
7d	**Deficiency of all or certain eyelid tissues, more than 1/2**
8b	**Elevated and/or depressed nose scar, covering up to ¼ of nasal dorsum and sidewalls**
8c	**Elevated and/or depressed nose scar, covering 1/4 -1/2 of nasal dorsum and sidewalls**
8d	**Elevated and/or depressed nose scar or amputation of the whole nasal dorsum and sidewalls**
8e	**Nasal tip amputation and/or ala of the nose and/or columella**
9c	**Lack of a part or the entire lip fullness 1/3 -1/2**
9d	**Lack of more than ½ of lip fullness**
11c	**Elevated and/or depressed and/or relief scalp and/or scar (with or without alopecia or contracture) 1 -2% TBSA**
11d	**Elevated and/or depressed and/or relief scalp and/or scar (with or without alopecia or contracture) 2% -3%**
12d	**Linear scalp scar (at the level of the surrounding skin or not), overall length 20-30cm**
12e	**Linear scalp and ear scar (at the level of the surrounding skin or not), overall length more than 30cm**
13c	**Elevated and/or depressed and/or relief hand scar (with or without contracture) 1% -2% TBSA.**
13d	**Elevated and/or depressed and/or relief hand scar (with or without contracture) more than 2% TBSA.**
14d	**Multiple benign lesions present on hands (acne/smallpox scars, skin diseases and tumors) 1% -2% TBSA**
14e	**Multiple benign lesions present on hands (acne/smallpox scars, skin diseases and tumors) more than 2% TBSA**
15h	**Elevated and/or depressed and/or relief neck scar without contracture, total coverage 1%-2% TBSA**
15j	**Elevated and/or depressed and/or relief neck scar with contracture, total coverage 4cm^2-0.5% TBSA**
15k	**Elevated and/or depressed and/or relief neck scar with contracture, total coverage 0.5-1% TBSA**
16c	**Multiple benign lesions present on the neck (acne/smallpox scars, skin diseases and tumors) 0.5%-1%TBSA**
16d	**Multiple benign lesions present on the neck (acne/smallpox scars, skin diseases and tumors) 1-2%TBSA**
17d	**Linear neck scar (at the level of the surrounding skin or not), overall length 20-30 cm**
17e	**Linear neck scar (at the level of the surrounding skin or not), overall length more than 30cm**
18d	**Linear scar of one or both hands (at the level of the surrounding skin or not), overall length 20-30cm**
18e	**Linear scar of both hands (at the level of the surrounding skin or not), overall length more than 30cm**
19d	**Asymmetry of the body, large gait and posture deviation due to contractures in large joints, limping, involuntary movements or palsy, aids mandatory**

20i	**Loss of two or more fingers, thumb excluded**
20j	**Loss of a part of a hand, thumb excluded**
20l	**Loss of two thumb phalanges**
20m	**Loss of the whole thumb (with the metacarpal bone)**
21e	**Aputation of a part of a foot**
23c	**Elevated and/or depressed and/or relief forearm and/or lower leg and/or foot scar with or without contracture 5% - 15% TBSA**
23d	**Elevated and/or depressed and/or relief forearm and/or lower leg and/or foot scar with or without contracture 15% - 25% TBSA**
24d	**Multiple benign lesions present on a forearm and/or lower leg and/or foot (acne scars, smallpox scars, skin diseases and tumors) 15%- 25% TBSA**
24e	**Multiple benign lesions present on a forearm and/or lower leg and/or foot (acne scars, smallpox scars, skin diseases and tumors) more than 25% TBSA**
25d	**Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length 100 cm-150cm**
25e	**Linear forearm and/or lower leg and/or foot scar (at the level of the surrounding skin or not), overall length more than 150cm**
26c	**Elevated and/or depressed and/or relief torso and/or upper arm and/or upper leg scar (with or without contracture)10-40 % TBSA**
26d	**Elevated and/or depressed and/or relief torso and/or upper arm and/or upper leg scar (with or without contracture)40-50 % TBSA**
26e	**Elevated and/or depressed and/or relief torso and/or upper arm and/or upper leg scar (with or without contracture) more than 50% TBSA**
27d	**Multiple benign lesions present on the torso and/or upper arm and/or upper leg (acne/smallpox scars, skin diseases and tumors) 40-50 % TBSA**
27e	**Multiple benign lesions present on the torso and/or upper arm and/or upper leg (acne/smallpox scars, skin diseases and tumors) more than 50 % TBSA**
28d	**Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length 100-200 cm**
28e	**Linear torso and/or upper arm and/or upper leg scar (at the level of the surrounding skin or not), overall length more than 200 cm**
33b	**Amputation of up to ½ of penis**
33e	**Loss of both testicles**
34b	**Amputation of ¼ - ½ of vulva**

VERY LARGE DISFUGUREMENT (4); n = 31	
Code	**Description of disfigurement**
1e	**Loss of an eye, eyelids preserved**
1f	**Loss of the eye socket contents with the eyelid and surrounding structure.**
4d	**Elevated and/or depressed and/or relief face and/or forehead scar (without contracture) more than 2% TBSA**
5d	**Striking facial asymmetry due to scarring and/or desmogenous contracture and/or paralysis, and/or distinct ectropion, highly altered facial expression of feelings, bizzare facial expression.**
8f	**Amputation ofmorethan one half ofthe nose or the whole nose**
8g	**Amputation, lackof the nosewith the surrounding structure**
9e	**Lack of the entire upper of lower lip**
9f	**Lack of the entire of major part of the upper jaw (maxilla)**
9g	**Lack of the entire of major part of the lower jaw (mandible)**
10f	**Deficiency of all or certain earlobe tissues more than 1/2**
10g	**Amputacija cele usneskoljkesaokolinom**
11e	**Elevated and/or depressed and/or relief scalp and/or scar (with or without alopecia or contracture) more than 3%**
15l	**Elevated and/or depressed and/or relief neck scar with contracture, total coverage 1-2% TBSA**
19e	**Asymmetry of the body, large gait and posture deviation due to contractures in large joints, limping, involuntary movements or palsy, bizzare body appearance**
20d	**Loss of two or more fingers with the thumb**
20n	**Amputation of a part of a hand with the thumb**
22a	**Lack (amputation) of one arm below the elbow**
22b	**Lack (amputation) of one armabove the elbow**
22c	**Lack (amputation) of one arm at the shoulder**
22d	**Lack (amputation) of one leg below the knee**
22e	**Lack (amputation) of one leg above the knee**
22f	**Lack (amputation) of one leg at the hip**
22g	**Lack (amputation) of both legs at any level**
22h	**Lack (amputation) of both arms at any level**
22i	**Lack (amputation) of any two limbs at any level**
22j	**Lack (amputation) of more than two limbs at any level**
23e	**Elevated and/or depressed and/or relief forearm and/or lower leg and/or foot scar with or without contracture more than 25% TBSA**
30c	**Amputation of the whole breast**
30d	**Amputation of both breasts**
33c	**Loss of more than ½ of penis or a complete amputation**
34c	**Lack of more than ½ of vulva or a complete loss.**

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Independent role of interleukin-6 and interleukin-8 in the etiology of transfusion reactions to platelet concentrates in children

Nezavisna uloga interleukina-6 i interleukina-8 u etiologioji transfuzijskih reakcija nakon primene koncentrovanih trombocita kod dece

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Abstract

Background/Aim. Transfusion reaction is an adverse event which manifests during or after administration of blood components to the patient. We aimed to show less known aspects of most common transfusion reactions (allergic and febrile non-hemolytic transfusion reactions – FNHTR) in the pediatric population at the platelet concentrates. The aim of this study was to determine the role of the accumulated cytokines interleukin-6 (IL-6), interleukin-8 (IL-8) and presence of anti-platelet antibodies in the etiology of transfusion reaction in children. **Methods.** The study included 239 pediatric patients, who received platelet concentrates. Data of reported transfusion reaction were collected and evaluated prospectively. The levels of IL-6 and IL-8 were determined using an immunoassay. Anti-human leukocyte antigen antibodies (anti-HLA) and anti-human platelet antigen antibodies (anti-HPA) were identified by Luminex flow cytometry. **Results.** Toral of 70 transfusion reactions were recorded 52 patients. Allergic reactions occurred in most of the cases (74.3%), followed by FNHTR (17.1%). Platelets derived from buffy coat caused the majority of reactions (73.5%). Patients with infection after platelet transfusion with FNHTR had the highest levels of IL-6, 483.30 ± 1,041.79 pg/mL (p = 0.020). Respectively, the febrile patients had IL-6, 302.52 ± 720.04 pg/mL (p = 0.004). The level of IL-8 in platelet units that caused transfusion reactions was 95.66 ± 319.10 pg/mL, which was significantly higher (p = 0.001) compared to the control platelet units. **Conclusion.** The predominant etiologic mechanism for FNHTR in our study was leukocyte derived cytokine accumulation during storage. Etiopathogenesis of FNHTR induced by IL-6 and IL-8 presented differently. We concluded that significant factors in the etiology of FNHTR by IL-6 were the factors related to the pediatric patient (infection, inflammation).

Key words:
platelet transfusion; transfusion reaction; interleukin-6; interleukin-8; child.

Apstrakt

Uvod/Cilj. Transfuzijske reakcije su neželjeni događaji koji se manifestuju tokom ili nakon primene krvnih komponenti. Prikazani su manje poznati aspekti najčešćih transfuzijskih reakcija (alergijske i febrilne nehemolizne transfuzijske reakcije – FNHTR) posle primene koncentrovanih trombocita u pedijatrijskoj populaciji. Cilj rada bio je da se ispita uloga akumuliranih citokina, interleukina-6 (IL-6) i interleukina-8 (IL-8) i prisustvo humanih anti-leukocitnih antitela (anti-HLA) i humanih anti-trombocitnih antitela (anti-HPA) kao etioloških faktora transfuzijskih reakcija kod dece. **Metode.** Studijom je bilo obuhvaćeno 239 pedijatrijskih bolesnika koji su dobili transfuziju koncentrovanih trombocita. Podaci o prijavljenim transfuzijskim reakcijama prikupljani su i obrađivani prospektivno. Nivoi IL-6 i IL-8 određivani su metodom indirektne hemiluminiscencije. Anti-HLA i anti-HPA identifikovana su Luminex protočnom citometrijom. **Rezultati.** Kod ukupno 52 bolesnika zabeleženo je 70 transfuzijskih reakcija. Najčešće su bile alergijske reakcije (74,3%), FNHTR (17,1%). Najveći broj reakcija izazvali su koncentrovani trombociti dobijeni iz "*buffy coat*" (73,5%). Bolesnici sa infekcijom su posle transfuzije trombocita na koje su ispoljili FNHTR imali najviši nivo IL-6 [483.30 ± 1 041.79 pg/mL (p = 0.020)], slede bolesnici sa febrilnošću [IL-6, 302.52 ± 720.04 pg/mL (p = 0.004)]. Nivo IL-8 u jedinicama trombocita koje su izazvale transfuzijsku reakciju

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bio je 95.66 ± 319.10 pg/mL, što je bilo značajno više (p = 0.001) u poređenju sa kontrolom. **Zaključak.** Dominantan etiološki mehanizam za FNHTR u ovoj studiji su citokini produkovani leukocitima akumulirani tokom skladištenja. Etiopatogeneza FNHTR izazvane IL-6 i IL 8 je različita. Zato smo zaključili da značajan faktor u nastanku FNHTR izazvane IL-6 u dečijoj populaciji predstavljaju faktori vezani za status bolesnika (prisustvo infekcije, inflamacije).

Ključne reči:
transfuzija trombocita; transfuzija, reakcija; interleukin-6; interleukin-8; deca.

Introduction

Platelet transfusions in the pediatric population are commonly used in hematology, oncology and hematopoietic stem transplantation. Acute reactions are defined as adverse events occurring during or within 4 to 6 hours of transfusion. The most common acute adverse events that occur to the transfusion of platelets in the pediatric population are allergic and febrile nonhemolytic transfusion reactions (FNHTR). If non-leukocyte-reduced platelets are used, FNHTR occurred in up to 37% of transfusions [1], whilst allergic reaction comprises 13–33% of all transfusion reactions [2]. The residual leukocyte content and storage time as well as patient factors are the determining factors for the occurrence of this type of reaction [2].

The pathogenesis of the FNHTR is multifactorial. The primary mechanism responsible for the FNHTR to platelets is leukocyte and platelet-derived biological response modifiers mechanism. Cytokine accumulation [regulated on activation, normal T cell expressed and secreted (RANTES), transforming growth factor beta (TGF-β), interferon gamma (IFN-γ), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8)] in platelet concentrates (PC) during storage contribute not only to the FNHTR, but also to allergic reactions associated with transfusions [3, 4]. Firstly, high levels of cytokines when infused to patient, cause fever by stimulation of the hypothalamus. Secondly, antigen-antibody hypothesis is believed that leukocyte antibody from patient's plasma reacts with leucocytes present in the PC. An antigen-antibody reaction occurs, resulting in the release of cytokines by the donor leucocytes. This mechanism accounts for less than 10% of the FNHTR to platelets [1].

This study aimed to present the frequency and incidence of transfusion reaction to platelets, to determine what type of PC and which the number of units of PC resulted in transfusion reactions and in which particular patient age.

The specific aims were: first, to determine the level of cytokines IL-6 and IL-8 in patients with transfusion reaction and in a unit of the platelets which caused transfusion reaction; second, to determine the presence of anti-human leukocyte antigen antibodies (anti-HLA) and anti-human platelet antigen antibodies (anti-HPA) in patients with transfusion reactions and afterwards, to investigate whether the cause of transfusion reaction is accumulation of cytokines or anti-HLA/anti-HPA; thirdly, to investigate whether there is an association of inflammatory states and fever in patients with higher levels of IL-6 and IL-8.

Methods

The study included patients (between one month and 18 years old) who received platelet transfusions between 2011 and 2014 at the Institute of Mother and Child Health Care of Serbia "Dr Vukan Čupić", a tertiary medical centre. Patients were treated at the Department of Hematooncology, the Department of Bone Marrow Transplantation as well as the Pediatric Intensive Care Unit.

Patients were stratified according to: age (infants - from 1 month to 1 year, children – 1 to 12 years and adolescents – 13 to 18 years), gender, diagnosis, and treatment of the underlying disease. The results were compared to levels of IL-6 and IL-8 in units of platelets which did not cause platelet transfusion reactions.

The patients received PC derived from the buffy coat (BC), single donor apheresis platelets and pooled platelets. The transplanted patients received the same platelet products but irradiated with 25 Gy per dose of platelets. The patients with aplastic anemia and the recurrent FNHTR received filtered platelets. The control group consisted of 156 patients who received a transfusion of platelets and did not have transfusion reactions.

Aliquots from transfused platelets were frozen and stored at -70 °C until the analysis. These samples were then thawed at room temperature and centrifuged at 3,000 rpm for 5 min. The supernatant plasma was used for the analysis.

The following laboratory tests were done: blood count (Beckman Coulter, USA), immune-serology tests for blood groups, direct and indirect antiglobulin test with gel ID-Card method (Bio-Rad, DiaMed GmbH, Switzerland), C-reactive protein (CRP) (Roche, Switzerland). IL-6 was quantitatively measured using the automatic analyzer Access Immunoassay Systems (Beckman Coulter, USA) and the IL-6 Immunoassay. IL-8 was determined by immunoassay on an automated immunochemical analyzer IMMULITE (Siemens, Germany).

The testing of anti-HLA was performed by lymphocytotoxic test dependent on complement (LCT) and Luminex flow cytometry. Anti-HPA were analyzed by Luminex flow cytometry. All analyzes were performed according to commercial instructions. The used reference values represented the standard normal age-specific values for the pediatric setting.

Statistical analysis was performed using the IBM SPSS software. Numerical data were presented as mean ± standard deviation (SD) or median, while categorical variables were presented as frequencies or percentages. Depending on the data types and the number of groups, differences between

independent samples were assessed using χ^2 test, Fisher test, Student's *t*-test, Kruskal-Wallis, Mann-Whitney *U* test and ANOVA, while differences between the related groups were examined by McNemar, Wilcoxon and Student's *t*-test for two related samples. Assessing the correlations, Pearson's and Spearman's tests were used. *p* values < 0.05 were considered to be significant.

Results

A total of 239 patients treated with platelet transfusion were included in this study. Complications during or after platelet transfusion were recorded in 52 (21.7%) patients. The characteristics of the study population are shown in Table 1.

A total of 70 transfusion reactions were recorded. Allergic reactions occurred in majority of cases, with 52 of 70 (74.3%) manifestations, followed by 12 (17.2%). FNHTR manifestations.

The first manifestation of the transfusion reaction included 39 allergy-type reactions; one of them was anaphylactic reaction, 8 FNHTR, transfusion-associated dyspnea (TAD), hypotensive reaction, 4 reactions with combined reaction type: 2 allergic reaction with the FNHTR and 2 allergic reactions with gastrointestinal symptoms with abdominal colic, with nausea and emesis as seen in Figure 1.

Repeated reactions had 11 (21.1%) of patients. The second manifestation included 11 reactions: 7 allergic, 3 FNHTR and one TAD (Table 2). The third manifestation included 4 allergic and one FNHTR reaction. One patient had the fourth and the fifth reaction to platelet transfusion, both allergic types. Secondary pulmonary transfusion reactions which occur in the wake of another transfusion reaction – secondary TAD, were present in 10 patients (19.2%).

According to the probability of adverse events associated with platelet transfusion, the association was the most probable in 76.6%, certain in 19.1%, and only possible in 4.3% of the patients.

According to the severity of clinical presentation, transfusion reactions were the most commonly mild in 51.1%, moderate in 36.2% and severe in only 12.8% of the patients. The most severe clinical picture of transfusion reaction had the patients with repeated transfusion reaction ($p = 0.001$). Greater number of the patients had the reactions while receiving the platelet transfusion 31 (59.6%).

Occurrence of unwanted reactions increased in the older age group of the patients. In the group aged 1 month – 1 year, reactions occurred in 25% patients; in the group aged 1–12 years, 56% of the patients had transfusion reaction, and in the group aged 13 and more years the transfusion reaction had 69% patients.

Table 1

Characteristics of the study population

Variable	Patients (n = 52)
Age (years), r ± SD	10.2 ± 5.6
Gender, n (%)	
boys	35 (67.3)
female	17 (32.7)
The group of aged, n (%)	
1 month-1 year	1 (1.9)
1–12 years	30 (18.8)
13 and more years	21 (40.4)

r – mean value; SD – standard deviation.

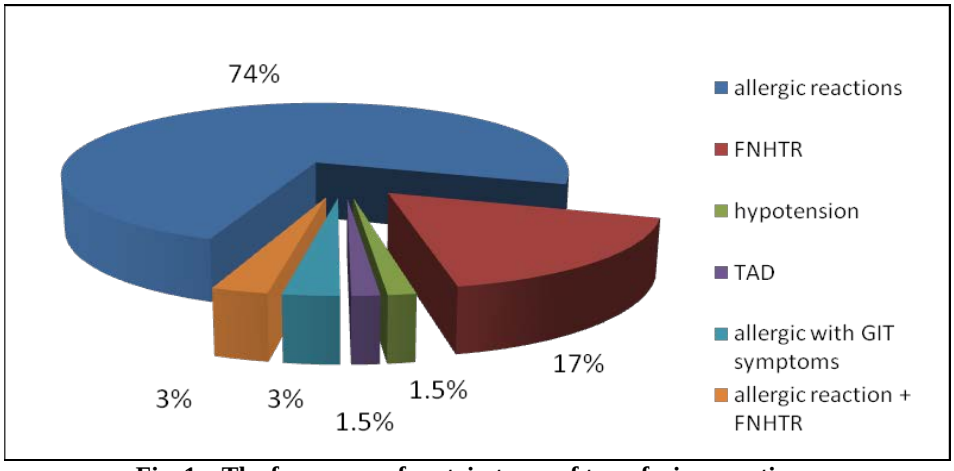

Fig. 1 – The frequency of certain types of transfusion reactions.
FNHTR – febrile non-hemolytic transfusion reaction; TAD – transfusion associated dyspnea; GIT – gastrointenstinal.

Table 2

Types of transfusion reaction regarding the diagnoses of the examined patients

Diagnosis	Total number	Types of transfusion reaction (n)						
		allergic	FNHTR	other	TAD sec.	Recurrent reactions		
						allergic	FNHTR	TAD
ALL	26	13	3	1	3	5	3	1
ALL rec	2	1	1					
AML	11	4	1	1	2	5		
Neuroblastoma	7	4		1	1	2		
NHL	4	3		1				
MH	1	anaphylaxis			1			
AA	4	4			1			
MDS	1	1			1			
SA Ewing	3	2		1				
JMML	1		1					
CGD	1	1						
AM sec ALL	1	1						
APL	1	1						
FA	2		1				1	
Rabdomio SA	2	1				1		
TU testis	1	1			1			
TU Wilms	1	1						
CA hepatocelul.	1		1					

FNHTR – febrile non-hemolytic transfusion reaction; TAD – transfusion associated dyspnea; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; AA – aplastic anaemia; MDS – myelodyslasia; JMML – juvenile myelomonocytic leukemia; CGD – chronic granulomatous disease; MH – Morbus Hodgkin; NHL – non-Hodgkin lymphoma; APL – acute promyelocytic leukemia; FA – Fanconi anemia; SA – sarcoma; CA – carcinoma hepatocelulare; TU – tumor.

Among the patients who received the medications affecting the platelets (cephalosporin antibiotics, antifungal medications), 58.8% did not expose any reaction to platelet transfusion, 23.5% had allergic reaction, but without the FNHTR. The rest of patients (41.2%) who did not receive therapy affecting platelets number or function had both, allergic reactions (55.6%) and FNHTR (14.8%) ($p = 0.015$).

PC-BC caused the majority of transfusion reactions (73.5%). Of those, 59.2% were non-irradiated, and 14.3% irradiated. Non-irradiated apheresis platelets counted for 12.2%, and irradiated apheresis platelets counted for 10.2% reactions. Non-irradiated platelet units (71.8%) more frequently provoked allergic transfusion reactions than irradiated ones ($p = 0.027$). The number of received units of PC in patients who had manifested the FNHTR was 25.63 ± 25.43 (3–84), $p = 0.853$, and in patients with manifested allergic reactions it was 14.53 ± 17.58 (1–73), $p = 0.384$. The patients without a transfusion reactions received 26.69 ± 28.38 (1–118) PC units. The most frequent adverse reactions occurred with platelets units after 3 days of storage (33.3%). The oldest (5 days) platelet units caused only 7.8% of adverse reactions.

Incidence of transfusion-associated adverse reactions

Cumulative incidence of all unwanted reactions, allergic reactions and the FNHTR related to number of units and quantity of PC in the 5-year period is shown in Table 3.

IL-6 and IL-8

Values of IL-6 and IL-8 were measured in the patients' samples before and after the transfusion reaction, as shown in Table 4. IL-8 did not differ significantly in patients with and without infection, before and after transfusion. The control group of healthy children had IL-6 1.91 ± 0.93 pg/mL (1.4–4.68 pg/mL), and IL-8 of all samples were < 5 pg/mL (below the measurement threshold analyzer).

There was a significant correlation between CRP and IL-6 values after platelet transfusion. Spearman's rho correlation coefficient was 0.453. Test of significance of the Spearman's rho correlation coefficient with selected two-tailed probabilities was $p = 0.023$, as seen in the Figure 2.

The number of neutrophils correlated with the IL-6 values after transfusion ($p = 0.019$), and the correlation itself was negative (Spearman's rho correlation coefficient – 0.484) as shown in Figure 3.

The mean IL-6 values in platelets units that caused the reactions were 6.06 ± 13.94 pg/mL (1.54–66.36 pg/mL), which was more than in control units 2.65 ± 1.62 pg/mL, (1.59–6.11 pg/mL), but it was not statistically significant ($p = 0.197$).

The IL-8 in platelets units that caused the reactions was 95.66 ± 319.10 pg/mL (4 to 2121.0 pg/mL), which was statistically significant ($p = 0.001$) comparing with the control units, 4.9 ± 1.34 pg/mL (4.0–5.9 pg/mL).

Table 3

Cumulative incidence of adverse transfusion reactions in a five-year period

Parameter	Transfusion reaction total	Allergic reaction	FNHTR
Per 1,000 units transfunded platelets	2.1	1.7	0.4
Per liter transfunded platelet product	0.03	0.02	0.005

FNHTR – febrile non-hemolytic transfusion reaction.

Table 4

Value of interleukin-6 (IL-6) and interleukin-8 (IL-8) in patients before and after febrile non-hemolytic transfusion reaction (FNHTR)

Patients	IL-6 (pg/mL) r ± SD	*p*	IL-8 (pg/mL) r ± SD	*p*
Before transfusion reaction	32.53 ± 43.47		55.67 ± 68.44	
After transfusion reaction (FNHTR)	94.17 ± 164.73	0.252	123.0–183.17	0.244
Without infection before platelet transfusion	26.45 ± 39.56		28.82 ± 24.20	
With infection before platelet transfusion	29.05 ± 43.24	0.732	83.97 ± 93.83	0.623
Without infection after platelet transfusion with FNHTR	63.33 ± 144.57		243.06 ± 380.47	
With infection after platelet transfusion with FNHTR	483.30 ± 1041.79	0.020	1223.61 ± 2775.03	0.491
Non-febrile, before platelet transfusion	3.27 ± 3.54		14.95 ± 5.73	
Febrile, before platelet transfusion	51.20 ±42.87	0.032	66.71 ± 66.86	0.073
Non-febrile, after platelet transfusion with FNHTR	10.28 ±13.79		137.67 ± 308.27	
Febrile, after platelet transfusion	302.52 ±720.04	0.003	762.78 ± 1958.32	0.070

r – mean value; SD – standard deviation.

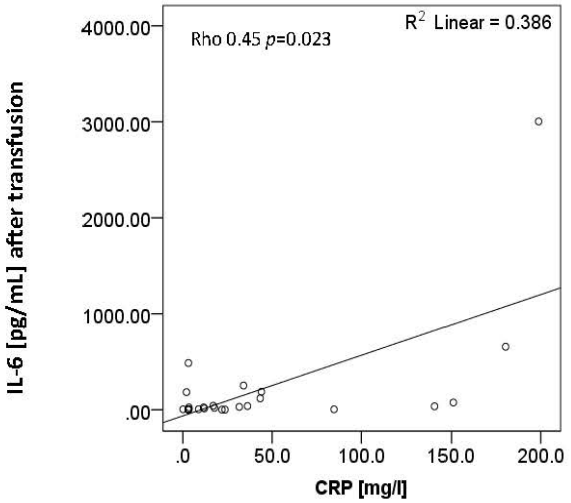

Fig. 2 – The correlation between values of interleukin-6 (IL-6) and C-reactive protein (CRP).

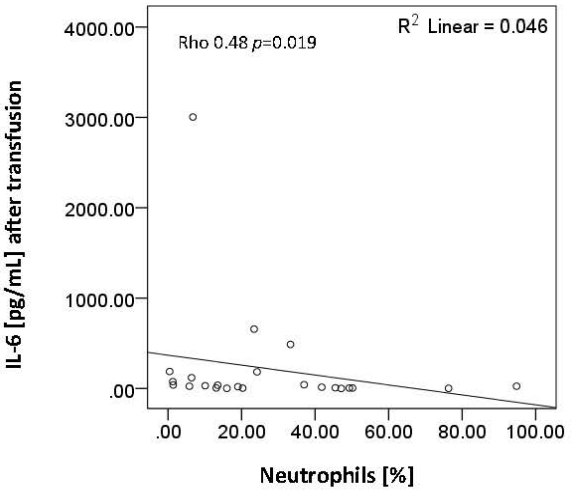

Fig. 3 – Correlation of interleukin-6 (IL-6) level and the number of neutrophils.

Anti-HLA, anti-HPA and transfusion reactions

Anti-HLA antibodies were present in 8 patients; 2 of them had anti-HLA and anti-HPA. Most frequent manifestation of presence of anti-HLA antibodies were allergic reactions, 5 of 8 (62.5%). The frequency of the FNHTR in the patient with anti-HLA antibodies was 12.5%. Connection of antibodies, anti-HLA and anti-HPA with occurrence of transfusion reaction was as follows: the patients with anti-HPA antibodies did not expose any reaction. Anti-HLA antibodies had 9.8% (5/51) of the patients with allergic reactions, and only one, 8.3% (1/12) (p = 0.007) of the patients with the FNHTR, (p = 0.004). The patients with antibodies, anti-HLA and anti-HPA, did not have the FNHTR, and 14.3% of the patients had allergic reaction (p = 0.004).

Discussion

Children represent a vulnerable population with more frequent transfusion-related reactions comparing to adults. Specifically, pediatric patients have a significantly higher

incidence of transfusion reactions associated with platelet transfusion (6.2 per 1,000 transfusions compared to 2.4 in adults, $p < 0.001$) [5]. According to Oakley et al. [5], the incidence of allergic reactions in pediatric transfusion is 2.7/1,000 in relation to the adults of 1.1/1,000 ($p < 0.001$), for the FNHTR is 1.9/1,000 in children, and 0.47 /1,000 ($p < 0.001$) in adults. Oakley et al. [5] also determined that male gender was predominant in the pediatric population with transfusion reactions (7.9/1,000 in boys and 4.3/1,000 in girls). The incidence of transfusion reactions in adults was not different based on gender.

Our 5-year cumulative incidence of allergic (1.7/1,000) and febrile reactions (0.4/1,000) is closer to the data for the adult population, which explains the high number of adverse reactions in adolescent patients aged 13 and over (40.1%). Transfusion reactions also occurred more frequently in boys (67.8%) in our study.

Clinical manifestations of acute transfusion reactions in pediatric patients differ significantly from study to study, depending on the department where the data were collected. The frequency of acute allergic reaction ranged from 6.7% to 57.8% and for the FNHTR from 12.5% to 60% [6–8]. Study from Pakistan [8] and our study present data from the Department of Haematooncology that has a higher incidence of the FNHTR and allergic reactions, while in intensive care wards were higher incidence of hypotension and transfusion reactions with dyspnea. Increased incidence of allergic reactions and the FNHTR in our study compared to previously published studies [6, 7] was most likely due to application the non-leucoreduced platelet products.

PLADO study came to the conclusion that the source of platelets (e.g. apheresis single donor or PC-BC), length of storage and ABO status were not significantly associated with the occurrence of any reaction. The number of transfused platelet units represented the most important characteristic that was associated with transfusion reactions [9].

Seghatchian et al. [10] in comparative analysis between PC-BC and apheresis platelets found the highest levels of IL-6 and IL-8 in the early stage of storage of platelets. An additional increase of interleukins was not found over the next 5 days. The level of interleukins present in units of platelets was affected by different process of collection, the degree of contamination of leukocytes and changes during storage. The use of filtration in line during the preparation of PC reduces the generation of cytokines at units of platelets during storage, and thus contributes to the reduction of transfusion reactions [10].

In our study there was no association between the transfusion reactions and age of the units of platelets, as well as no association with the number of units of platelets. The values of the cytokine IL-6 and IL-8 derived from platelets in units of different ages in our study did not differ significantly, which was in accordance with all the above-mentioned literature.

Muylle et al. [11, 12] showed that reaction could be caused by the administration of plasma containing large amounts of cytokines. The correlation found between the increased IL-6 plasma levels in PC and the transfusion reactions suggested that the cytokine was responsile for the reactions [11, 12]. The residual reactions that occurred with the plasma-removed product still correlated with IL-6, providing further support for the cytokine theory and suggesting that even low cytokine levels may cause reactions in some patients [13].

IL-6 serum levels are often elevated before onset of clinical symptoms and before routine laboratory test, such as measurement of high-sensitivity CRP, become positive. Owing to its short life, human seum IL-6 levels change rapidly. According to Schefold et al. [14], IL-6 may be used as a highly sensitive diagnostic tool for the early identification of sepsis in both newborns and adult. Also, IL-6 may be useful in the longitudinal monitoring of patients with sepsis. The bedside densitometric point-of-care IL-6 test (with a turn-around time of 20 min) may help to initiate early goal-directed therapy. The correlation between level of IL-6 after transfusion and the CRP test results of our patients was statistically significant indicating that both test measured the immunological host response consisted of pro-inflammatory cytokine release. The negative correlation between IL-6 and the neutrophil count showed that patients with a decrease in neutrophils had an increased level of IL-6 after transfusion reactions. A possible explanation is that the destruction of certain quantities of neutrophils during the transfusion reaction leads to their degranulation and the consequent release of cytokines.

IL-8 had a different impact on the occurrence of reaction; its increased concentration in a unit of platelets was sufficient to cause transfusion reactions in our patients. Increased amounts of IL-8 in the plasma of PC may be related to white blood cells (WBC) activation or its lysis [15]. Transfusion-related adverse reactions were attributed to the presence of substantially high levels of IL-8. Gamma irradiation could inhibit IL-6 accumulation, but did not prevent IL-8 production during storage in unfiltered irradiated units of PC [16].

The predominant etiologic mechanism for the FNHTR in our study was leukocyte derived cytokine accumulation during storage (96.2%). The FNHTR caused by the anti-HLA antibodies was only 3.8%. Also, the FNHTR occurred only if the applied units of PC contained increased concentrations of cytokines and the recipient had the disease or condition with the inflammatory process. According to the literature the additive effects of the cytokine IL-6 produced by the recipient along with donor's IL-6, represented the amount of the cytokine IL-6 sufficient to cause symptoms and signs of the FNHTR. Rate of metabolization of the cytokines and the presence of soluble IL-6 receptor in the recipient also influence on FNHTR incidence [14].

The presence of anti-HPA antibodies was associated only with allergic reactions in our patients. In literature anti-HPA-1a was shown to induce the release of the chemokine CCL5 RANTES from platelets, a proinflammatory chemokine that was implicated in allergic transfusion reactions [17]. Conceptual model of allergic transfusion reactions (ATRs) similar to the FNHTR was described by Savage et al. [18, 19]. ATRs may result from a combination of recipient atopic predisposition (chronic-genetic or subacute-

acquired) and a necessary plasma mediator in the blood component. The degree of recipient susceptibility at the time of transfusion and magnitude of the plasma mediators may determine the severity of an ATR [18, 19].

Application of premedication has not been proven effective in allergic reactions and in the FNHTR [2]. Significant proportions in the etiology of the FNHTR had patient-related factors. Possible prevention of the recurrent FNHTR is the treatment of local infection and inflammation in the patient's body. Reducing the concentration of cytokines in PC units, the risk for recurrent transfusion reaction also reduces. The cytokines produced during storage in platelet units cannot be removed by leukodepletion filtration. Additive solutions were developed to attenuate platelet activation. Adding magnesium and potassium ions to the commercial platelet additive solutions (PAS) II and PAS III completely prevented platelet activation [20]. A study that evaluated the cost-effectiveness of PAS to prevent allergic transfusion reactions concluded that using PAS may be financially and clinically beneficial when compared to current practice – washing platelets [21].

Our choice of cytokine reduction in PC units was to use plasma reduced platelets, resuspended in additive solution. We applied the platelets in additive solution to 2 children with allergic reactions. Evaluation of the effectiveness of this therapy is in progress.

Conclusion

In the study group of pediatric patients allergic transfusion reactions had the highest incidence and frequency. The greatest number of reactions was provoked by PC-BC units in the group of patients aged 13 to 18 years (69%). There was no difference between the number of received PC in the patients with transfusion reactions and without reactions. The predominant etiologic mechanism for the FNHTR in our study was leukocyte derived cytokine accumulation during storage (96.2%). The FNHTR incidence caused by the anti-HLA antibodies was only 3.8%. The mean IL-6 values in PC units that caused the transfusion reactions was not significantly different than those in the control PC units, but, IL-8 level was significantly higher in the PC units that caused the reactions. In addition, we determined that etiopathogenesis of FNHTR was different depending on whether it is induced by IL-6 or IL-8. The significant roles in the development of FNHTR induced by IL-6 have factors related to the status of a patient (presence of infection, inflammation, sensitivity).

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The frequency of metabolic syndrome in patients with the subclinical hypothyroidism

Učestalost metaboličkog sindroma kod bolesnika sa supkliničkom hipotireozom

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Abstract

Background/Aim. An increased cardiovascular risk of thyroid dysfunction is associated with the impairment of lipid and lipoprotein metabolism, endothelial dysfunction, metabolic, hormonal, hemodynamic changes and coagulation disorders. Subclinical hypothyroidism is characterized by supernormal thyroid-stimulating hormone (TSH) level along with normal values of thyroid hormones. The association of subclinical hypothyroidism with higher cardiovascular risk has not been fully clarified. The aim of the study was to determine the frequency of metabolic syndrome and the associated cardiovascular risk factors in patients with the subclinical hypothyroidism. **Method.** The study included 140 subjects aged from 18 to 65 years, out of which 105 subjects had subclinical hypothyroidism and 35 subjects were the euthyroid controls. The clinical trial program, completed in all subjects, included: detailed medical history and physical examination, waist circumference, and laboratory tests [fasting glycemia, lipid and lipoprotein status, free triiodothyronine (FT3) and free thyroxine (FT4) and TSH levels]. **Results.** Out of 105 patients with the subclinical hypothyroidism, mean age 44.15 ± 11.23 years, 77 (73.3%) patients had metabolic syndrome. In the control group consisting of 35 subjects, mean age 33.80 ± 10.60 years, only 3 (8.6%) subjects had metabolic syndrome. Mean values of the waist circumference, fasting glycemia, triglycerides, systolic and diastolic blood pressure were higher in subclinical hypothyroidism group in relation to the controls ($p < 0.0001$). Mean value of high-density lipoprotein (HDL) cholesterol was lower in subclinical hypothyroidism group as compared to the controls ($p < 0.002$). **Conclusion.** The frequency of metabolic syndrome was 9 times higher in subjects with the subclinical hypothyroidism in relation to subjects without any subclinical hypothyroidism.

Key words:
hypothyroidism; metabolic syndrome x; cardiovascular diseases; risk factors; risk assessment.

Apstrakt

Uvod/Cilj. Povećan kardiovaskularni rizik u disfunkciji štitaste žlezde u vezi je sa poremećajima metabolizma lipida i lipoproteina, endotelijalnom disfunkcijom, metaboličkim, hormonskim, hemodinamskim promenama i poremećajima koagulacije. Supklinička hipotireoza karakteriše se supranormalnim nivoom tireostimulišućeg hormona (TSH) uz normalne vrednosti tireoidnih hormona. Udruženost supkliničke hipotireoze sa povišenim rizikom od nastanka kardiovaskularnih bolesti još uvek nije u potpunosti razjašnjena. Cilj rada bio je određivanje učestalosti metaboličkog sindroma i pridruženih faktora rizika od nastanka kardiovaskularnih bolesti kod bolesnika sa supkliničkom hipotireozom. **Metode**. Istraživanjem je obuhvaćeno 140 ispitanika starosti 18–65 godina – 105 ispitanika sa supkliničkom hipotireozom i kontrolna grupa od 35 ispitanika bez nje. Kod svih ispitanika sproveden je program istraživanja koji je uključivao: detaljnu anamnezu i fizikalni pregled, merenje obima struka, laboratorijska ispitivanja [glikemija našte, lipidni i lipoproteinski status, slobodni trijodtrionin (fT3), slobodni tiroksin (fT4) i TSH]. **Rezultati.** Od 105 bolesnika sa subkliničkom hipotireozom, prosečne starosti 44,15 ± 11,23 godina, 77 (73.3%) bolesnika imala su metabolički sindrom. U kontrolnoj grupi od 35 ispitanika, prosečne starosti 33,80 ± 10,60 godina, samo 3 (8.6%) ispitanika imala su metabolički sindrom. Srednje vrednosti obima struka, našte glukoze u krvi, triglicerida, sistolnog i dijastolnog krvnog pritiska bile su više u grupi sa supkliničkom hipotireozom u odnosu na kontrolnu grupu ($p < 0.0001$). Srednja vrednost HDL-holesterola bila je niža u grupi sa supkliničkom hipotireozom u poređenju sa kontrolnom grupom ($p < 0.002$). **Zaključak.** Učestalost metaboličkog sindroma je oko 9 puta veća kod ispitanika sa supkliničkom hipotireozom u odnosu na eutireoidne ispitanike.

Ključne reči:
hipotireoidizam; metabolički sindrom x; kardiovaskularne bolesti; faktori rizika; rizik, procena.

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Introduction

Presently, atherosclerosis is the most significant factor of blood vessel diseases, causing the highest morbidity and mortality worldwide. The increased levels of a total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides as well as decreased values of high density lipoprotein (HDL) cholesterol have been also classified to risk factors of development of ischemic heart diseases.

Subclinical hypothyroidism (SH) attracts attention and the researchers expand the need for exploring the possible meaning of this condition.

SH is defined by finding the increased serum thyroid-stimulating hormone (TSH) concentration with concurrently normal thyroid hormone values [1]. The concept of SH appeared in 1980s when the sensitive tests for measurement of serum TSH were introduced. Classical population study from district of Whickham, England, found the prevalence of 75 and 28 per 1,000 women and 1,000 men, respectively [2]. Similar findings were reported in other studies [3]. According to the National Health and Nutrition Examination Survey (NHANES III) data, the frequency of SH in USA population was 4.3% [4]. On the other hand, some formerly published series of subjects established even higher SH frequency. Roterdam study revealed the frequency of 10.8% in older women [5], and Fremantle Diabetes Study, Fremantle, Western Australia, demonstrated the frequency of 8.6% in women with type 2 diabetes mellitus [6]. For example, a recent study using Korean population-based cohorts reported the SH prevalence of 11.3% [7].

The question whether the subclinical thyroid dysfunction may lead to fatal effects to cardiovascular system together with the increased risk of mortality still remains open.

The factors causing higher risk of cardiovascular diseases (CVD) in SH have not been fully clarified, but this association has been partially related to higher blood pressure, atherogenic lipid and lipoprotein status, proinflammatory condition, endothelial dysfunction and blood hypercoagulability [8–16].

The authors presume that benefit from l-thyroxine treatment of these patients will reflect in reduction of CD related mortality [13, 14, 17–19].

Metabolic syndrome (MetS) is a group of metabolic disorders which increases the risk of type 2 diabetes mellitus (T2DM) and CVD. MetS may be defined in different ways, but the central obesity, dyslipidemia, impaired glucose tolerance and hypertension represent its main characteristics [20–22]. The frequency of MetS in developed and developing countries is significant. It is estimated that about 20–25% of adult population is affected by MetS worldwide. The estimate is that MetS affects more than 34% of the USA population [23]. In Iran, 30% of men and 55% of women meet diagnostic criteria of MetS [24].

Coexistence of these disorders is more frequent than expected [25] and their associated occurrence is far more hazardous for developing CVD than the summation of their individual effects [22], which is not generally accepted viewpoint [26].

The association of SH with the increased cardiovascular risk has not been completely elucidated.

In this study, baseline hypothesis was that SH could have effect on MetS frequency**.** For this reason, we investigated the patients with and without SH as well as the resulting development of MetS.

The basic aim of the study was determination of frequency of MetS and associated cardiovascular risk factors in patients with the SH.

Methods

This clinical, non interventional cross-sectional study was approved by the Ethics Board of Medical Center in Novi Pazar and conducted in compliance with the Declaration of Helsinki. The study included 140 subjects aged 18-65 years, out of which 105 had SH and 35 were the euthyroid controls.

Before any study procedure, a patient was informed on study design and upon reading the informed consent, he/she signed it. History data were obtained by means of structured history questionnaire. Physical examination of each patient was performed. Anthropometric measurements of patients were taken in fasting state (not taking food 12 to 14 h prior testing). Biological samples were collected: 1 test-tube with citrate for erythrocyte sedimentation rate (ESR), 1 test-tube containing ethylenediamine tetracetic acid (EDTA) (5 mL) for complete blood count, 2 test tubes for serum separation (10 mL each) for biochemical analyses (glycemia, HDL, LDL) and immunometric analyses [free thyroxine (FT4), free triiodothyroxine (FT3), TSH].

The study included patients who met all inclusion and had none of exclusion criteria.

Inclusion criteria were: patients aged from 18 to 65 years and signed informed consent.

Exclusion criteria were: patients with diagnosed diabetes mellitus, evidence on acute infection in the last 2 weeks, positive biohumoral inflammatory syndrome and [accelerated ESR and leukocytosis with higher C-reactive protein (CRP) and fibrinogen level], use of medicaments that may interfere with studied parameters (glucocorticoids, iodine preparations, amiodarone, diuretics, lithium, cytostatics, antidepressants, estrogens, androgens), chronic diseases that may have effect on studied parameters (systemic autoimmune diseases, malignant diseases, chronic renal failure, liver insufficiency, acute coronary syndrome and stroke within the last 6 months), recent use of radioactive iodine, thyroid surgery and external neck radiation, pregnancy and breast feeding.

The initial study phase involved the collection of history data and thorough physical examination. Blood pressure was measured on both hands by indirect method over brachial artery in a patient assuming sitting position after 15 min of rest, using the mercury sphygmomanometer and listening to phases I and IV of Korotkoff sounds. The following measurement was done after 10 min on patient's hand showing the highest values. Mean values obtained from two measured systolic and diastolic pressure values were used. Waist circumference was measured with a patient

assuming standing position at the midline between costal arch and anterior superior iliac spine. Body mass index (BMI) was calculated as a quotient of body mass (kg) and body height (m^2) (Quetelet index). Blood samples collected after 12 hr fasting were used for the following measurements: fasting glycemia (adjustment method to dry chemistry principle), cholesterol, triglycerides, LDL, dHDL (colorimetric method based on end-point principle). TSH and FT4 were determined by immunochemical method – chemiluminescent procedure including chemiluminescent substrate. The method was automated (IMMULITE® DPC).

Reference values for: TSH were 0.27–4.20 mU/mL and FT4: 10–22 pmol/L, variation coefficient fT4: 6.20%, TSH: 5.50%. FT3 hormone was measured by enzyme-linked fluorescence assay (ELFA) using miniature VIDAS immunochemical analyzer. Reference values were 4.00–8.30 pmol/L, variation coefficient: 5.30%. Reference values for HDL cholesterolwere 40–50 mg/dL in men 50–60 mg/DL in women.

Forming study group: criterion to be enrolled in the SH group and in the controls was TSH > 4.2 IU/mL and TSH ≤ 4.2 UI/mL, respectively.

Diagnosis of MetS was based on the International Diabetes Federation (IDF) criteria. Central obesity was required condition (among European population, it is defined as waist circumference larger than 94 cm and 80 cm in males and females, respectively) plus any of two following factors: blood pressure higher than 130/85 mmHg or treated arterial hypertension which was previously diagnosed; triglycerides over 1.7 mmol/L or specific treatment of this lipid abnormality; HDL cholesterol lower than 1.03 mmol/L and 1.29 mmol/L in males and females, respectively, or specific treatment of this lipid abnormality; morning fasting plasma glucose (FPG) > 5.6 mmol/L or previously diagnosed T2DM.

Before starting statistical analysis, laboratory reports with the patients' results of analyses were anonymized and they all were granted identification numbers (for protection of patient privacy, while patient data were known only to investigators). Electronic database was created using the program SPSS version 20.0. Mean, standard deviation (SD), median, minimum and maximum as well as normal distribution of all studied continuous variables (normal distribution of values within the group was analyzed by Kolmogorov-Smirnov test) were determined. To compare the mean values of continuous variables, we used repeated-measures unifactorial ANOVA (non-parametric Kruskal Wallis test, alternative to Variance analysis F test) and dependent sample *t*-test in normal distribution, or alternatively, Mann-Whitney *U*-test (non-parametric test, alternative to *t*-test), and Wilcoxon matched pairs test for outcomes not following normal distribution, as well as χ^2 test for comparison of frequency of categorical (dichotomous) variables. $p < 0.05$ was statistically significant, with 95% confidence interval.

Results

Out of 105 subjects with SH, mean age 44.15 ± 11.23 years, 77 (73.3%) patients had MetS. In the control group consisting of 35 subjects, mean age 33.80 ± 10.60 years, only 3(8.6%) subjects had MetS. Accordingly, the frequency of MetS was nine times higher in the SH subjects.

Out of 77 subjects with SH and MetS, there were 72 (93.5%) females and 5 (6.5%) males. The number of women was significantly higher than men ($\chi^2 = 58.299$, $p < 0.0001$). Out of 97 females with SH, 72 (74.2%) had SH and MetS, and among a total of 8 males with SH there were 5 (62.5%) with SH and MetS. Testing of distribution of female subjects according to groups formed in relation to presence of SH and MetS confirmed a significant difference ($p < 0.0001$) while no significant difference was found between males.

Table 1 shows mean values ± standard deviation, and the results of analysis of variance (ANOVA test), as well as the result of testing the differences of mean values by Student's *t*-test of studied parameters both in the experimental and the control group.

Mean values of the waist circumference, morning fasting plasma glucose, parameters of lipidogrom, systolic and diastolic blood pressure were higher in the SH group in comparison to the controls ($p < 0.0001$). Mean HDL-cholesterol value was significantly lower in the SH group as compared to the controls ($p < 0.05$).

Discussion

The study found that the frequency of MetS was nine times higher in the SH subjects in relation to the subjects without SH. Our results indicate that MetS should be actively searched for in the SH patients.

The patients with the SH might not be identified by symptoms and signs even if they were discreetly present. Higher frequency of SH in females than in males and older people in relation to younger age group was corresponding to higher incidence of thyroglobulin and thyroperoxidase antibodies in women and elderly people [27, 28]. In our study of 105 subjects with SH, 97 (92.4%) were women.

The relationship between SH and CVD has been examined in many studies. A more recent review of the clinical consequences of thyroid function variations within the normal reference range documented that even modest elevations of TSH might have substantial health outcomes, including cardiovascular mortality [16]. Ten-year follow-up of Korean cohort revealed that elevated serum TSH levels significantly increased the risk of CVD by approximately 20% per one standard deviation in males [7].

Studies of euthyroid individuals found positive association between the TSH levels and coronary heart disease (CHD)-related mortality [29]. The TSH levels within the normal range are inversely associated with all-cause mortality [18, 30–33]. Adult Taiwanese subjects with SH were reported to have an increased risk of all-cause mortality and CVD death over a 10-year period [34]. However, some other studies failed to establish any association with all-cause or cardiovascular mortality rates [35–39]. Such discrepancy of findings could result from different sample size and power, the inconsistent age and sex ratios of study population or varied iodine intake in different regions.

Table 1

Descriptive statistics of selected parameters in relation to groups with and without hypoparathyreoidism (SH)

Parameters	Experimental with SH	Controls without SH		*p*	Test
	mean ± stand. deviation	median	mean ± stand. deviation	median		
Age (years)	44.15 ± 11.23	43.00	33.8 ± 10.6	33.00	0.0001	2
Waist circumference (cm)	90.88 ± 11.53	90.00	77.66 ± 8.24	77.00	0.0001	1
Body height (cm)	166.47 ± 7.74	166.00	171.14 ± 8.99	170.00	0.004	1
Body mass (kg)	80.18 ± 14.26	80.60	65.81 ± 10.42	63.80	0.0001	1
Body mass index	28.89 ± 5.02	28.41	22.35 ± 2.11	22.97	0.0001	1
FT3 (pmol/L)	4.98 ± 0.86	4.96	4.71 ± 0.64	4.77	ns	1
FT4 (pmol/L)	14.45 ± 2.37	14.32	14.94 ± 2.38	14.90	ns	1
TSH (mIU/ml)	6.87 ± 1.34	6.67	1.9 ± 0.88	1.62	0.0001	2
Morning fasting glycemia (mmol/L)	5.42 ± 0.74	5.30	4.98 ± 0.47	4.95	0.001	1
Total cholesterol: mmol/L	6.06 ± 0.88	6.00	4.98 ± 1.06	4.83	0.0001	1
Triglycerides (mmol/L)	2.17 ± 0.84	2.15	1.22 ± 0.51	1.14	0.0001	2
HDL cholesterol (mmol/L)	1.23 ± 0.33	1.15	1.39 ± 0.29	1.38	0.013	1
LDL cholesterol (mmol/L)	3.98 ± 0.79	3.94	3.04 ± 0.97	3.01	0.0001	2
Arteriosclerotic index: LDL-C/HDL-C	3.43 ± 1.21	3.27	2.33 ± 1.01	2.26	0.0001	2
Relation: TC/HDL-C	5.25 ± 1.6	5.00	3.75 ± 1.19	3.60	0.0001	2
Systolic BP (mmHg)	138.48 ± 16.96	120.00	119 ± 11.49	140.00	0.0001	1
Diastolic BP (mmHg)	92.38 ± 10.61	80.00	78.29 ± 7.47	100.00	0.0001	2

SH– subclinical hypothyreoidism; FT3 – free triiodothyronine; FT4 – free thyroxine; TSH – thyroid stimulating hormone; LDL-C – low density lipoprotein-cholesterol; HDL-C – high density lipoprotein-cholesterol; TC – total cholesterol; BP – blood pressure; 1 – ANOVA F-test; 2 - Kruskal Wallis test, ns – not statistically significant.

In the study, SH was associated with the increased cardiovascular risk. Mean values of the waist circumference, morning fasting plasma glucose, triglycerides, systolic and diastolic blood pressure were higher in the SH group than in the controls ($p < 0.0001$). Mean values of HDL-cholesterol were lower in the SH group in comparison to the controls ($p < 0.002$).

Cardiometabolic risk is a comprehensive risk of T2DM and CVDs, as a result of co-effect of multiple risk factors such as atherogenic dyslipidemia, impaired glucose tolerance, hypertension, augmented intra-abdominal fat tissue as well as the presence of prothrombotic and proinflammatory conditions. This human population is exposed to two times higher risk of death as well as to acute myocardial infarction, that is, the frequency of having acute myocardial infarction and stroke is as high as three times. The frequency of T2DM development is five times higher in people with MetS [22, 40].

There is currently controversial data regarding the prevalence of MetS among the SH patients. The geographic location, age, gender, diet, intake of iodine and other genetic and environmental factors might possibly account for these discrepancies in patterns and relationships.

Our study shows a high prevalence of MetS in patients with SH. The results of meta-analyses of Yang et al. [41] demonstrated that SH was significantly associated with a higher risk of MetS. Lai et al. [42] found the subclinical thyroid dysfunction was present in about 8% of Taiwanese elderly population, one-third of whom had MetS. According to Pangaluri et al. [43], 43.3% of the SH patients were found to satisfy the criteria for MeTS. In a cross-sectional study, Choudhary and Iani [44] found that the overall prevalence of thyroid dysfunction in patients with MetS was 41.5% with a high prevalence of SH (27%). In a Nigerian study, Udenze et al. [45] found that a third of patients with MetS had SH. Similar results were published by Gyawali et al. [46] in their Nepalese study. In a study conducted in South India, Agarwal et al. [47] found that 76 (53%) women with MetS had SH.

However, in a study in Turkey, TSH was not related to any MetS [48].

Moreover, cross-sectional and longitudinal follow-up studies from Japan noted high associations between SH and MetS [49]. One cross-sectional analysis of cohort studies emphasized that the probability of having MetS was positively associated with TSH levels within the reference range [16].

Upon their systemic review, Iwen et al. [50] documented convincing evidence supporting the major impact of SH on all MetS components. In addition, another study of 2,760 young Korean female volunteers with normal TSH levels established that the high-TSH group had two-fold higher risk of MetS compared to subjects in the low-TSH group [51].

Conclusion

The results of our study suggest that patients with the subclinical hypothyroidism, although having a moderate

form of thyroid dysfunction, represent a whole category of people with the increased cardiovascular risk, and, consequently, metabolic syndrome should be searched for in any of these patients. Proper timing and precise identification of cardiometabolic risk in patients with the subclinical hypothyroidism opens up the opportunities for specific therapeutic interventions directed against individual atherogenic risk factors.

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Protein-energy wasting in maintenance hemodialysis patients – etiology and diagnosis

Proteinsko-energetski gubitak kod bolesnika na hroničnoj hemodijalizi – etiologija i dijagnostički kriterijumi

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Ključne reči:
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Introduction

In patients with chronic kidney disease (CKD), especially those on hemodialysis (HD), nutritional status plays an important role. It is directly connected with longevity, life quality, rate of hospitalization and mortality [1–5]. Despite the fact that dialysis procedure is constantly improving, common complication in HD patients is still protein-energy wasting (PEW). Although PEW is a very common problem, it often remains undiagnosed and untreated. Also, PEW is the strongest risk factor for poor clinical outcomes, including death, especially in the elderly patients [4, 6].

Considering that CKD has a high prevalence of 13.4%, and the number of patients with diagnosed CKD is growing worldwide, including the fact that annual mortality of dialysis patients was 20% higher compared to the mortality from cancer disease, the seriousness and the magnitude of the problem in patients with PEW seems to be much higher [2, 7]. Several studies related to the nutritional status in the HD patients have shown that the prevalence of PEW varies from less than 20% to almost 80% depending on markers used for assessment of the nutrition status [4, 8, 9]. Many different terms, such as protein-energy malnutrition, uremic malnutrition, uremic cachexia, malnutrition-inflammation syndrome, are used for a condition associated with loss of muscle and fat, malnutrition, and inflammation in patients with CKD, and may lead to confusion and misinterpretation of clinical data.

The existence of different criteria can produce the problems in diagnostic procedures, comparison of results and establishing recommendations for the prevention and therapy. Therefore, in order to avoid confusion in the nomenclature, the International Society of Renal Nutrition and Metabolism (ISRNM) expert panel has recommended the term "protein-energy wasting" to describe a "state of decreased body stores of protein and energy fuels" that usually results from a complex interplay of reduced nutrient intake and/or increasing catabolism [10]. Since protein and energy wasting sometimes occur as separate entities, terms "protein wasting" or "energy wasting" can be used to refer to the occurrence of only one of the mentioned [10].

Etiology of PEW

Dialysis patients often develop malnutrition at a very early stage of dialysis [9, 11, 12]. PEW in patients with CKD, particularly those who are on HD, has its own specificity as compared to patients who suffer from other chronic diseases or those in which it is only the result of insufficient food intake or restrictive diets. Many factors that contribute to PEW are directly associated with chronic renal failure [2, 13, 14].

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The possible causes of PEW in CKD patients are listed in Table 1, created and presented from the ISRNM as a consensus review of current knowledge [13].

A long list of causes and variety of their combination can lead to PEW in different ways in each HD patient. Although the etiology of PEW is undoubtedly multifactorial, inadequate dietary nutritional intake is likely to play an important role. Predialysis patients are mostly on low-protein diets. Sometimes these types of diets are hypocaloric and as a consequence of predialysis restrictive diets, patients start HD in poor nutritional conditions [9, 11, 12, 15]. Hemodialysis procedure itself can directly affect energy balance, i.e. energy intake and energy expenditure. Several studies indicated that dietary energy and protein intakes were below recommended levels or the actual needs in the HD patients [9, 16–18]. In addition, the progressive reduction in spontaneous food intake occurs with a decrease in kidney function [18]. Factors that affect food intake including not only dietary restrictions, but also anorexia, taste changes, depression, social behavior, customs, low social status, solitude, and inability to obtain or prepare food [2, 16, 19].

Anorexia in the HD patients may develop as a result of nitrogen-based uremic toxin retention as well as dysregulation in some of appetite mediators – from gastric, cytokines and adipokines to hypothalamic amino acid sensing [2, 13, 15, 20, 21]. Malnutrition in the HD patients is also often caused by too many restrictive or monotony diets, sometimes due to unnecessary prohibition of various foods without considering the preferences, needs and possibilities of patients [15]. Furthermore, the imbalance between dietary intake and actual needs can be increased by additional loss of nutrients during dialysis, such as amino acids, certain peptides, glucose, vitamins, trace elements, leading to further increased risk of PEW [13, 15]. Although the reduced food intake or poor absorption of nutrients play a key role in most cases of PEW, other factors in addition to starvation (especially hypermetabolism, inflammation, metabolic acidosis, comorbidities and dialysis) are required for the occurrence of PEW [13, 14].

It was postulated that inflammation can cause PEW. Many factors, such as increased production and decreased elimination of proinflammatory cytokines, acidosis, oxidative stress, altered metabolism of adipose tissue, and other intracorporeal factors, contribute to persistent inflammation in CKD, especially in the HD patients. In addition to these, many extracorporeal factors mainly related to dialysis itself, such as chemical and microbiological contaminants in dialysis water, or bioincompatible in dialysis circuit, cannot be neglected [13, 20, 22]. Kidney has a role of a modulator of endocrine function, whilst kidney disease

Table 1

Causes of PEW in CKD patients [13]

1. Decreased protein and energy intake
   - a) Anorexia
     - i. dysregulation in circulating appetite mediators
     - ii. hypothalamic amino acid sensing
     - iii. nitrogen-based uremic toxins
   - b) Dietary restrictions
   - c) Alterations in organs involved in nutrient intake
   - d) Depression
   - e) Inability to obtain or prepare food
2. Hypermetabolism
   - a) Increased energy expenditure
     - i. inflammation
     - ii. increased circulating proinflammatory cytokines
     - iii. insulin resistance secondary to obesity
     - iv. altered adiponectin and resistin metabolism
   - b) Hormonal disorders
     - i. insulin resistance of CKD
     - ii. increased glucocorticoid activity
3. Metabolic acidosis
4. Decreased physical activity
5. Decreased anabolism
   - a) decreased nutrient intake
   - b) resistance to GH/IGF-1
   - c) testosterone deficiency
   - d) low thyroid hormone levels
6. Comorbidities and lifestyle
   - a) comorbidities (diabetes mellitus, CHF, depression, coronary artery disease, peripheral vascular disease)
7. Dialysis
   - a) nutrient losses into dialysate
   - b) dialysis-related inflammation
   - c) dialysis-related hypermetabolism
   - d) loss of residual renal function

PEW – protein-energy wasting; CKD – chronic kidney disease; GH – growth hormone; IGF-1 – insulin-like growth factor 1; CHF – chronic heart failure.

causes abnormalities in the synthesis, excretion, and action of many hormones. Insulin resistance, growth hormone (GH), insulin-like growth factor (IGF)-1, and elevated glukucocorticoides levels are causative of an increase in protein and amino acid catabolism and suppression of protein synthesis. Thus, these are implicated in the loss of muscle mass in the CKD patients [2, 13, 20]. Inflammation is associated with increased resting energy expenditure (REE), oxidative stress, protein catabolism, loss of muscle mass, high C-reactive protein and proinflammatory cytokine levels, hypoalbuminemia and the presence of comorbid conditions as well as with suppression of hormones such as anabolic hormones, IGF-1, and

Table 2

Readily utilizable criteria proposed by International Society of Renal Nutrition and Metabolism (ISRNM) expert panel for the clinical diagnosis of PEW in CKD [10]

Criteria
Serum chemistry
serum albumin < 3.8 g/dL (method: Bromcresol Green)[a]
serum prealbumin (transthyretin) < 30mg/dL (for maintenance dialysis patients only; levels may vary according to GFR level for patients with CKD stages 2–5)[a]
serum cholesterol < 100 mg/ dL[a]
Body mass
BMI < 23 kg/m^2 [b]
unintentional weight loss over time: 5% over 3 months or 10% over 6 months
total body fat percentage < 10%
Muscle mass
muscle wasting: reduced muscle mass 5% over 3 months or 10% over 6 months
reduced mid-arm muscle circumference area[c] (reduction > 10% in relation to 50th percentile of reference population)
creatinine appearance[d]
Dietary intake[e]
unintentional low DPI < 0.80 g/kg/day- for at least 2 months for dialysis patients or < 0.6 g /kg/day for patients with CKD stages 2–5
unintentional low DEI < 25 kcal/kg/day for at least 2 months

At least three out of the four listed categories (and at least one test in each of the selected category) must be satisfied for the diagnosis of kidney disease-related PEW.
Optimally, each criterion should be documented on at least three occasions, preferably 2–4 weeks apart.
[a]Not valid if low concentrations are due to abnormally great urinary or gastrointestinal protein losses, liver disease, or cholesterol-lowering medicines.
[b]A lower BMI might be desirable for certain Asian populations; weight must be edema-free mass, for example, post-dialysis dry weight.
[c]Measurement must be performed by a trained anthropometrist.
[d]Creatinine appearance is influenced by both muscle mass and meat intake.
[e]Can be assessed by dietary diaries and interviews, or for protein intake by calculation of normalized protein equivalent of total nitrogen appearance (nPNA or nPCR) as determined by urea kinetic measurements.
BMI – body mass index; CKD – chronic kidney disease; DEI – dietary energy intake; DPI – dietary protein intake; GFR – glomerular filtration rate; nPCR – normalized protein catabolic rate; nPNA – normalized protein nitrogen appearance; PEW – protein–energy wasting.

Table 3

Other potential tools (including those still in development) for assessment of PEW in individuals with CKD stages 3–5 [10]

Appetite, food intake, and energy expenditure
appetite assessment questionnaires
population-based dietary assessments: food frequency questionnaires
measuring energy expenditure by indirect or direct calorimetry
Body mass and composition
weight-based measures: weight-for-height
total body nitrogen
total body potassium
energy-beam-based methods: DEXA, NIR, BIA, and vector bioimpedance analysis
underwater weighing and air displacement weighing
14 kDa fragment of actomyosin
microarrays
muscle fiber size
relative proportions of muscle fiber types
muscle alkaline soluble protein
CT and/or MRI of muscle mass
Laboratory markers
serum biochemistry: transferrin, urea, triglyceride, bicarbonate
hormones: leptin, ghrelin, growth hormones
inflammatory markers: CRP, IL-6, TNF-α, IL-1, SAA
peripheral blood cell count: lymphocyte count or percentage
Nutritional scoring systems
SGA and its modifications.

PEW – protein-energy wasting; CKD – chronic kidney disease.
BIA – bioelectrical impedance analysis; CRP – C-reactive protein; CT – computed tomography; DEXA – dualenergy X-ray absorptiometry; IL – interleukin (e.g., IL-1 and IL-6); MRI – magnetic resonance imaging; NIR – near infrared interactance; SAA – serum amyloid A; SGA – subjective global assessment of nutritional status; TNF-α – tumor necrosis factor-α.

testosterone. Pro-inflammatory cytokines may directly decrease appetite and increase REE via influence on the central nervous system. They can also indirectly reduce the appetite in the CKD patients, through depression and consequently reduced dietary nutrients intake [13, 22, 23].

Metabolic acidosis, often in the HD patients, is also a potential cause of PEW. Studies have shown that acidosis increases degradation of the whole-body protein, breakdown of the skeletal muscle protein and oxidation of branched chain amino acids decreases albumin synthesis and determines nutritional abnormalities. It also causes insulin resistance that leads to loss of muscle mass [13, 20]. Some of comorbidities associated with CKD such as diabetes and metabolic syndrome, cardiovascular disease, hyperparathyroidism, anemia, gastrointestinal disorders, autoimmune and rheumatologic disorders, chronic lung diseases, liver disease, psychiatric and neurologic disorders and, malignant diseases, contribute to PEW [13].

Although inadequate dialysis can lead to PEW itself, some factors associated with dialysis may contribute to PEW through nutrient losses into dialysate, infectious, inflammation, hypermetabolism and loss of residual renal function. Amino acid and protein losses during the dialysis session, together with low nutrients intake, promote low availability of nutrients for muscle synthesis. Dialysate loss of proteins, including amino acids, peptides and whole proteins, are estimated to be approximately 10–12 g per session. Dialyses procedure is a catabolic event, that accelerates the rate of whole-body and muscle proteolysis and stimulates active muscles on the release of amino acids, raising net whole-body and muscle protein loss. These undesirable effects of net skeletal muscle protein breakdown persisted for at least 2 hours after the completion of the HD procedure. Simultaneous amino acid supplementation can prevent or reverse these adverse effects in the HD patients which can be an opportunity for the treatment of PEW [2, 13, 20, 24, 25].

Diagnostic criteria for PEW

Clinical guidelines recommend routine assessment of nutritional status in the CKD and HD patients. There is not a single specific measurement that provides complete assessment of the nutritional status of the HD patients, although many different parameters for assessing the PEW are used in clinical practice and research [5, 15, 26, 27]. Therefore, the ISRNM established a set of criteria for identifying PEW in the CKD patients. According to the ISRNM expert panel diagnostic criteria, PEW has 4 categories in assessment: biochemical indicators, body mass, muscle mass and dietary intake. The ISRNM recommended that the diagnosis of PEW at least 3 out of 4 criteria categories, and that at least one test from each of the selected categories must be abnormal, on at least 3 examinations 2–4 weeks apart. Then, as a potential indicator of the PEW additional measures of nutrition and inflammation are recommended (Table 2) [10].

Biochemical indicators

Among biochemical indices, serum albumin concentration has been used in detection of malnutrition in the HD patients for a long time. Low serum albumin level is a relatively late manifestation of malnutrition, since albumin has a long half-life and hepatic synthetic reserve is very large [10, 15, 26]. The association between serum albumin levels and mortality is highly gradual and linear. A decrease of 0.3 g/dL in serum albumin levels is associated with an increased risk of mortality by 20% in the HD patients [3]. However, serum albumin is also influenced by several non-nutritional factors which are often present in the HD patients, including infection, inflammation, comorbidity and hydration status. Serum albumin should always be taken into account when assessing malnutrition in the HD patients. Thus, low albumin level in the HD patients may not always result from PEW, and albumin alone is not a clinically useful marker for PEW. The serum albumin concentration should be measured monthly [5, 10, 26, 28]. Prealbumin, or transthyretin has a shorter half-life than albumin, a close relationship with nutritional status, and acts as a good predictor of clinical outcome in the HD patients [5, 10, 26, 28]. The serum total cholesterol concentration is reduced in PEW. It is a less sensitive nutritional marker, cheap, and more accessible [5, 10, 26].

Although, the expert panel did not recommend numerous laboratory markers as part of criteria for the diagnosis of PEW, such as serum transferrin, bicarbonate, urea, triglyceride, hormones (leptin, ghrelin, growth hormones) concertation, inflammatory markers (CRP, IL-6, TNF-α, IL-1, SAA) levels and lymphocyte count or percentage, they could be useful indicators of protein-energy nutritional status in maintenance dialysis patients and potential tools for assessing PEW (Table 3) [10].

Body mass index (BMI)

Even though BMI is one of the most used indicators of body mass, it has some limitations [29]. In general population normal range of BMI is between 18.5 and 24.9 kg/m^2, while according to the ISRNM criteria, BMI less than 23 kg/m^2 is a marker of PEW in the HD patients [10, 26, 30]. A higher BMI is associated with increased cardiovascular mortality in the general population. It is quite the opposite situation in the HD patients where higher BMI is associated with lower death risk ("obesity paradox") [31]. While obesity is a long-term risk for cardiovascular diseases in general population, in the HD patients, the risk mentioned above is not crucial. In the CKD patients risk of short-term consequences related to PEW is much greater than the risk of obesity, thus mortality depends on short-term risk meaning malnutrition. Therefore, in the CKD patients, especially in the HD patients, it is crucial to improve their nutritional status and prevent malnutrition and PEW [1]. All anthropometric measurements should be performed after a routine HD session and must be performed by trained personnel [5, 10, 26]. Since BMI is influenced by hydration status, it is recommended to use an edema-free mass to calculate BMI. Unintentional edema-free weight loss of 5% over 3 months or 10% during 6 months indicate risk of PEW [10].

Muscle mass

Reduced muscle mass is one of the most important criteria for the presence of PEW [10]. Mid-arm muscle circumference (MAMC) is useful in assessment of muscle mass. MAMC depends upon lean body mass and body water. It is thus expected that MAMC could increase when body water increases, although the lean body mass and somatic protein can be kept stable. It is necessary to bear this in mind when using MAMC in the HD patients as an index of somatic proteins status [5, 26].

Dietary intake

To evaluate the nutrition status, it is important to provide an adequate assessment of the food intake by some of the available methods like dietary diaries or interviews [5, 10, 26]. In metabolic stable patients, it may be useful to indirectly assess dietary intake of protein by counting normalized protein equivalent of total nitrogen appearance (nPNA) [5, 10, 26]. As food data collection, the following tools can be used: 24-hour recall, food diaries, and food frequency questionnaire (FFQ), but each of these tools has some limitations. 24-hour dietary recall covers a short period of time and it does not represent a typical food intake. Food diary covers a longer time period (3–7 days) and it is recommended to patients to include dialysis and non-dialysis days. It is a more useful tool, if the patient weighs the portions of food [32]. FFQ can estimate the long term dietary intake. A diet that contains a daily energy intake of 35 kcal/kg body weight (bw) (30–35 kcal/kg bw for those aged 60 years and older) and 1.2 g protein/kg bw (at least 50% is of high biological value), is usually prescribed for the HD patients [1, 5]. According to the ISRNM expert panel, unintentional low dietary protein intake less than 0.80 g/kg bw/day and unintentional low dietary energy intake less than 25 kcal/kg bw/day for at least 2 months can be associated with PEW [10].

According to the ISRNM, nutritional scoring systems were not included in diagnostic criteria of PEW (only in potential tools), but others advised subjective global assessment (SGA) and its modifications for dialysis malnutrition score (DMS), and malnutrition inflammation score (MIS) for the diagnosis of malnutrition in the HD patients [5, 8, 10, 26, 33]. SGA is based on history and physical examination, and gives a global score of protein-energy nutritional status. SGA is a practical, inexpensive, easily doable clinical tool, with one potential problem – its subjective nature which may reduce its reproducibility. SGA should be used in combination with other measures in diagnosis of PEW [10, 33] Body composition, except by anthropometry, can be assessed by more sophisticated methods such as bioelectrical impedance analysis (BIA) or dual energy X-ray absorptiometry (DEXA). There is a good correlation between anthropometry and BIA and DEXA but they are expensive and not widely available [5, 10].

Monitoring

Nutritional status is considered an important prognostic factor for the risk of mortality, so early diagnosis and nutritional interventions could improve clinical outcome in the HD patients. Therefore, regular screening of the HD patients on PEW is extremely important. Nutritional status of the HD patients should be assessed at the start of HD and reassessed every 3–6 months. Malnourished and unstable patients may require monitoring at shorter intervals. Individual parameters such as serum albumin, BMI, nPNA, midweek predialysis creatinine should be evaluated monthly, but body weight should be measured on each dialysis [2, 5, 26].

Conclusion

The available evidence suggests that nutritional status in the HD patients is an extremely important predictive and causative factor for the good clinical outcome. Nutritional deficits and PEW are frequent problems in the dialysis population and implies an increased risk of negative health outcomes such as mortality risk and quality of life deterioration. There is no single specific measurement that provides complete assessment of the nutritional status in the HD patients, and the results should be analysed in the clinical context of each individual patients. As malnutrition is potentially reversible with appropriate nutritional support, early identification of patients at high nutritional risk may facilitate effective treatment and improve prognosis in the HD patients.

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Health risks from recent migrations to Europe

Zdravstveni rizici od nedavnih migracija ka Evropi

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Key words:
prolaznici i migranti; zdravstvena zaštita; rizik, procena; preventivno-medicinska zaštita; srbija; evropa.

Ključne reči:
transients and migrants; primary health care; risk assessment; preventive health services; serbia; europe.

Introduction

Throughout the history, as well as today, migrations appear as health problem if they are massive, sudden and with an independent flow [1]. In the last several years Europe met a large influx of migrants. Even before recent wave of mass migrations, 3.8 million migrants entered European Union (EU) in 2014 and 1.9 million from them was not from European countries. In 2015, however, 1.5 million more migrants arrived [2], and during the first nine months of 2015 over 600,000 applications for asylum were filed in the EU [3].

The main causes of modern massive migrations to Europe are: demographic explosion in Africa and in the Middle East, long-lasting wars and growing possibility of social engineering. At the same time, birth rates and population in Europe – the wealthiest and best governed continent – are in decline. Above all, migrations to Europe are highly facilitated by the fact that Europe shares the sea with Africa and the Middle East, while the European countries share common borders. One of the effects of the increase and escalation of international migrations is that it certainly intensifies the process of globalization [1, 2].

Despite the fact that birth rates in Africa is the highest on the world, most migrants come from the Middle East because wars were always more important reason for migrations than demographic explosion, and the number of interstate and internal wars and activities of terrorist organizations in the same area is increasing in the last several decades. They come mostly from Syria, but also from Afghanistan, Eritrea, Iraq, Pakistan, Somalia and other war-turned countries from this parts of the world.

With the first wave of migrants emerged also the concern in Europe whether they will bring disease and death to the domestic population. This concern created various fears in Europe and opened numerous questions which, if answered, could help to determine health condition of the migrants and the health risk they pose for domestic population of the countries through which they are travelling or where they are staying. Key questions, under the conditions of increased panic in some, above all Central European states, were mostly related to concerns about European population health, but not about health of the migrants: Are refugees carriers of contagious diseases and what is the percentage of those with infectious diseases in the migrant population? Do migrants pose a health risk for domestic population in terms of possible epidemic outbreaks? To which extent? Which new diseases do they bring? Are migrants carriers of microbes (viruses and bacteria) causing diseases typical (or atypical) for the region where they come from?

In this article both the level of health risk presented by the migrants to domestic populations of the EU and Serbia as well as the level of risks presented to the various migrant groups as they mingle together are discussed.

Migrations as the health risk for Europe

The first reaction of the EU institutions was slow and not very effective. It showed that, in the last decade, Europe was not well prepared to meet the huge challenge brought by uncontrolled migrations. As a result, many political and other anomalies arose in many European countries regarding the issue of mass migrations [4]. Aside from the late reactions of the European health institutions, they ultimately paid special attention to security challenges posed by migrants [5]. Therefore, the European countries mostly paid attention to the increased danger of terrorism, rape and other crimes [6]. They

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paid little attention to unsuccessful integration of migrants and even less attention to screening the migrants and identifying those aspects which might present possible public health risks for Europe.

Health risks related to sudden mass migrations, which are still going on, albeit with less intensity, can be divided into two periods: intensive fluctuations of migrants throughout a series of European countries including Serbia, and the current period when migrants, willingly or unwillingly, stay in one of the European countries for longer periods.

Thus, during the first period from 2015 to 2016, is characterized by the most intense migration movements, the health risk consisted not only of the possibility of epidemic outbreaks and spreading of contagions among domestic population, but also of the lack of complete and reliable insight into the health conditions in migrant groups. Reasons for this lie in enormous numbers of migrants and the speed which they proceeded with across Europe without being restricted by many states.

In the beginning, the screening process showed that migrants reported their health condition as being good. One can logically assume that if they were ill they would not undertake such a long and perilous journey. But, the fact is that the migrants came from countries plagued by war, disease and poverty and where health practicies are low, vaccinations and other forms of immunological protection are irregular and infrequent even in times of peace. It is also the fact that they traveled to their destinations staying in unsanitary, overcrowded accommodations, often without access to fresh water or health care. In the beginning of these migrations many countries did not provide sanitary or safe health accomodation, nor sufficient health services. Inadequate sanitation, suboptimal hygiene, and unsafe water and food can increase the risks of outbreaks of water and foodborne diseases such as salmonellosis, hepatitis A virus infection and cholera. The highest percentage of foreigners in need of medical help came from Africa or the Middle East. Many did not report their health issues from the fear of being deported. However, due to the harsh conditions and the very long travel, there were significant chance that they would become ill, or that some hidden disease would manifest itself and become intense. Additionally, migration itself can impair both mental and physical health [5].

Health risk was additionally increased by the fact that there were numerous illegal crossings over the borders. It is impossible to control the health conditions of those migrants who entered the country illegally and avoided registration and accommodations in the Migrant Centers, often living in unsanitary conditions.

During the second period, which still lasts, health risk stemming from unsanitary living conditions and lack of health care among migrants is significantly reduced, while levels of risk due to their more extensive contact with local population increase.

By examining health condition of migrants during both periods carried out by European states, affected by migrations, the World Health Organization (WHO) and other international organizations, shown that the fear of epidemic outbreaks, mass contagion of domestic population with infectious diseases or its exposure to rare and endemic diseases was unfounded. Migrants were mostly ill with seasonal respiratory infections and digestion problems, as well as with dermatological diseases (scabies) and feet injuries to the degree consistent with the conditions of their long travel and poor accommodation. Relatively high levels of post-traumatic stress diagnosis and depression [6] were also found as expected.

Migration has been mostly talked about as a driver of infectious diseases to European countries, particularly in those which receive migrants from places with high prevalence of infectious diseases [7]. National Surveillance Systems of European countries demonstrated higher incidence and prevalence rates of certain infectious diseases among migrants, such as human immunodeficiency virus (HIV) infections, tuberculosis and hepatitis. Pulmonary tuberculosis was one of the main foci for migrant health assessment (MHA) process. But the evidence from epidemiological studies indicates that the risk of transmission from migrants to the general population was relatively low, approximately as one decade before [8]. Possibility of contagion with new strains of viruses and bacteria dangerous for European population, has not been confirmed so far. Just the opposite, there is a growing evidence that migrants from countries with high prevalence of HIV are at much higher risk of acquiring HIV after arrival in the EU [9]. More than one-third of all newly diagnosed HIV cases in 2015 in the EU were among migrants [10]. Migrants account for more than half of all newly diagnosed HIV cases in 10 EU countries and in some of them (Sweden, Luxembourg) the rate is over 70% (Figure 1). Higher rates of acquired immunodeficiency syndrome (AIDS) deaths in migrants can, at least in part, be attributed to the high frequency of late diagnosis (e.g. diagnosis with an AIDS defining illness) [11]. HIV testing rates among migrants are low. This reflects the existence of many different barriers, not only the lack of knowledge of other cultures and backgrounds, but, also, the lack of testing and cure, fear of the disease and death, the fear of discrimination in the community as well as fear of deportation [12].

Lately, health and safety/security experts in the EU are more interested in mental health of the migrants considering that certain percentage of terrorist and extremist attacks in the EU and the USA over the last few years were perpetrated by persons of migrant origin having mental health issues. Some of data suggest that almost two-third of asylum seekers may meet the diagnostic criteria for post traumatic stress disorder (PTSD) [13].

Migrations as the health risk for Serbia

According to the Commissariat for Refugees, 556,393 migrants passed through Serbia in 2015 alone and according to the data provided by UNHCR, total number for 2015 and 2016 indicate that 800,000 migrants crossed Serbia. Another data point provided by United Nations High Commissioner for Refugees (UNHCR) shows that by the end of June 2015,

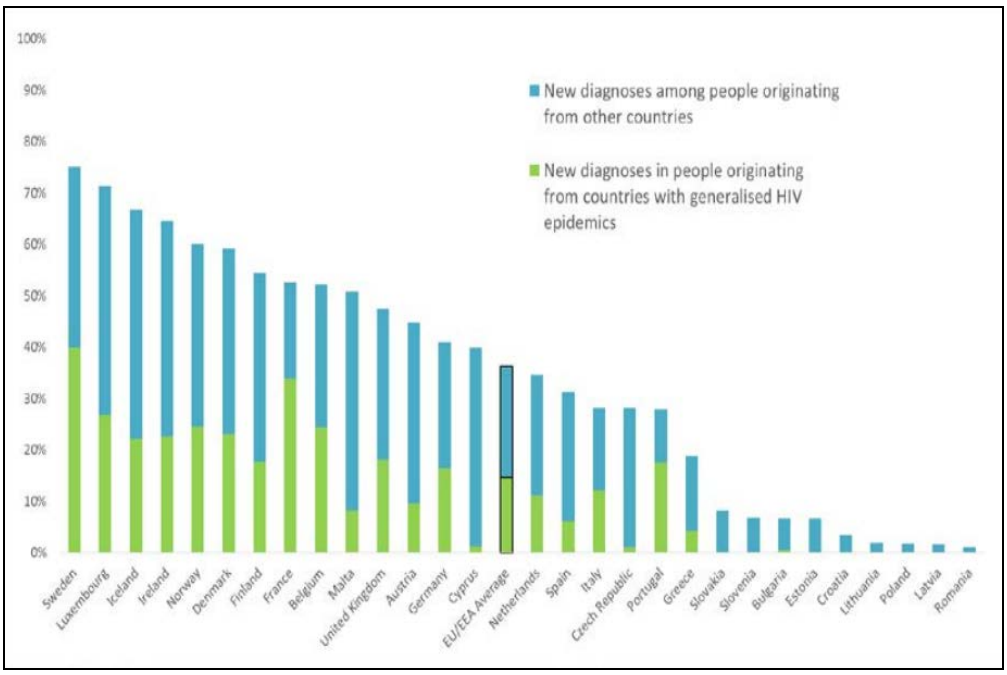

Fig. 1 – Newly diagnosed HIVcases in migrants in 2015 in the European Union.
Source: European Centre for Disease Prevention & Control/WHO Regional Office for Europe. HIV/AIDS surveillance in Europe 2015. Stockholm: ECDC; 2016.

600–1,000 migrants entered Serbia daily. The number of sylum seekers, originally small, increased each yearand it stood at 30,000 in 2015 [14].

Medical triage is availabe at the point of entry to Serbia. There, the primary concern dealt with is the health status of the asylum seekers, as such. However, despite the fact that medical staff in some asylum centers in Serbia is available 24h per day, migrants do not ltake enough advantage of this opportunity.

Data obtained from systematic health monitoring of asylum centers show that 68,802 health hazards were observed during the period from June 1, 2015 to January 10, 2016. Respiratory diseases dominated among migrants with increasing incidence in cold months, and there were 45% respiratory infections, 8% injuries, 5% intestinal infections with diarrhea, and 4% without diarrhea. They also reported 15 births, 6 abortions and 2 sudden deaths. Thus far there have not been any reports of unexpected communicative diseases, but they are always possible [14]. The number of health issues showing growing incidence peeked in late October and early November but stabilized in the winter. During the analyzed period, 71,327 medical examinations were conducted on migrant patients with predominance of curative treatments (94%) [15]. So far, no cases of plague were reported nor there were any reports on the Middle East respiratory syndrome coronavirus, both represented serious concern [16]. In this respect, situation in Serbia is almost completely consistent with the findings in other Europeans countries along the migration route.

The asylum centers in Serbia are unrestricted centers – migrants can freely move not only from centers to town but also from town to town as well.

During the measles epidemic this winter (2018) in Serbia there were 12 deaths. It represents 1/3 of the number of deaths from smallpox (36) in the year 1972 in former Yugoslavia, which at that time had population three times larger than the population of Serbia today [16]. There were rumors connecting the last outbreak of measles in Serbia with the migrants, but there is no evidence confirming this claim.

Among the many problems facing Serbia today, there is also difficulty with vaccines and related supplies arriving slowly and often times late. For example, during the recent flood crisis, vaccines for hepatitis A were requested, but they arrived one year later [14].

In response to the recent large influxes of migrants arriving in Serbia, the Ministry of Health requested that the WHO conduct a joint assessment and review of the Serbian health system's capability to manage large and sudden groups of migrants [17]. To that end, in April 2015, the WHO Regional Office for Europe started the project Public Health Aspects of Migration in Europe (PHAME), aiming to strengthen capability and adequate management of the public health challenges (including the health of migrants) related to the issue of large influx of migrants [14].

The migrant population coming in Europe is relatively healthy and doesn't present a significant health hazard for locals. There is no evidence or expert opinion that suggests that migrants increase the risk of epidemics caused by infectious disease in the host population of Europe [18].

Conclusion

Health risk from migration in Europe was not excessive in any period. Nevertheless, certain health risk still exist for domestic population as well as for the migrants themselves. That is primarily due to the fact that migrants legally residing in a country routinely avoid medical examinations and poor

personal hygiene among some migrant, even when they are provided with accommodations that meet the necessary sanitary requirements. Therefore, today, health risks related to the migrants in Europe are higher for the migrants themselves than for the domestic populations.

Although health risk related to migrants as a threat to public health, it is not high in Europe, it is still listed in official European documents as an important security challenge due to constant illegal influx of new migrants. There is also an ever present chance of revived and increased migrant flow caused by the real possibility of revival of old war conflicts as well as the eruption of the new ones in the Middle East and in Africa.

Thus, preventing measures remain mandatory in all EU countries, as well as in other countries along the migrations` route including Serbia.

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CASE REPORTS

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Synchronous malignant multicentric cerebral glioma with atypical neuroradiological presentation and comparatively long survival: Case report and literature review

Sinhroni maligni multicentrični gliom mozga sa atipičnom neuroradiološkom prezentacijom i komparativno dugim preživljavanjem: prikaz bolesnika i pregled literature

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Abstract

Introduction. Synchronous multicentric cerebral gliomas are uncommon brain tumors, mostly malignant, with unknown pathogenesis, unfavorable prognosis and still controversial management. Preoperative differentiation from other multiple brain pathologies by conventional magnetic resonance imaging (MRI) is often difficult, but supplemental use of advanced magnetic resonance techniques should allow the tumor biology to be predicted and an appropriate treatment strategy planned. **Case report.** We reported a 59-year-old man with double synchronous multicentric cerebral lesions, which had initial MRI and diffusion-weighted imaging presentation as left parietal metastasis and ipsilateral amygdalo-hippocampal low-grade glioma. However, magnetic resonance spectroscopy (MRS) of both lesions showed different metabolite profiles of malignant glioma. *En bloc* resection of the easily accessible parietal lesion revealed glioblastoma with methylated O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter. Subsequently, the patient was treated with temozolomide (TMZ)-based chemoradiation according to Stupp's protocol, with continuous standard (5/28) adjuvant TMZ in 12 courses. Despite prolonged stabilization of the disease with good life-quality during treatment, the patient died 19 months after diagnosis. The time to tumor progression estimated by MRI was 17 months. **Conclusion.** MRS significantly improved the differential diagnostic accuracy of conventional MRI in our patient. In accordance with reviewed literature data, the younger age, good initial performance status and methylated MGMT gene promoter were all favorable predictors of longer survival in the reported case. Resection of at least one easily accessible tumor lesion, followed by TMZ-based chemoradiation, with continuous adjuvant TMZ in more than 6 standard courses, seems currently to be the most beneficial therapeutic option for such cases.

Key words:
glioma; glioblastoma; diagnosis; magnetic resonance imaging; magnetic resonance spectroscopy; mgmt protein, human; temozolomide; prognosis; treatment outcome.

Apstrakt

Uvod. Sinhroni multicentrični maligni gliomi mozga su retki moždani tumori koji su uglavnom maligni, sa nepoznatom patogenezom, nepovoljnom prognozom i još uvek spornim dijagnostičko-terapijskim pristupom. Preoperativna diferencijacija u odnosu na druge multiple moždane lezije uz pomoć konvencionalne magnetne rezonance (MR) je često teška, tako da bi dodatna primena naprednih MR tehnika trebala da omogući predikciju biologije tumora i planiranje odgovarajuće strategije lečenja. **Prikaz bolesnika.** Prikazali smo muškarca starosti 59 godine, sa dve sinhrone multicentrične moždane lezije, koje su na inicijalnoj MR mozga sa difuzionom sekvencom izgledale kao parijetalna cerebralna metastaza sa leve strane i ipsilsteralni amigdalo-hipokampalni niskogradusni gliom. Medjutim, magnetno-rezonantna spektroskopija (MRS) obe lezije pokazala je prisustvo metaboličkih profila malignog glioma. Lako

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dostupna parijetalna lezija koja je resecirana u potpunosti, potvrdjena je kao glioblastom, sa metilisanim promotorskim genom za O^6-metilguanin-DNK-metiltransferazu (MGMT). Postoperativno, bolesnik je lečen hemioradioterapijom baziranoj na temozolomidu (TMZ) prema *Stupp*-ovom protokolu, uz kontinuiranu standardnu (5/28) adjuvantnu monohemioterapiju TMZ u 12 ciklusa. Uprkos prolongiranoj stabilizaciji bolesti sa dobrim kvalitetom života tokom lečenja, bolesnik je preminuo 19 meseci nakon dijagnostikovanja bolesti. Vreme do progresije bolesti koje je procenjivano MR pregledom iznosilo je 17 meseci. **Zaključak.** Tehnika MRS je značajno poboljšala diferencijalno-dijagnostičku preciznost konvencionalne MR kod našeg bolesnika. U odnosu na prikazane literaturne podatke, mlado životno doba, inicijalno dobro opšte stanje i metilisani promotorski gen za MGMT bili su povoljni prediktori dužeg preživljavanja kod prikazanog bolesnika. Resekcija bar jedne lako dostupne tumorske lezije, uz postoperativnu hemioradioterapiju baziranu na TMZ i kontinuiranu adjuvantnu hemioterapiju TMZ sa više od 6 standardnih ciklusa, čini se kao trenutno najpovoljnija terapijska opcija kod ovakvih bolesnika.

Ključne reči:
gliom; glioblastom; dijagnoza; magnetna rezonanca snimanje; magnetna rezonanca, spektroskopija; mgmt protein, humani; temozolomid; prognoza; lečenje, ishod.

Introduction

Multicentric glioma (McG) is a well-recognized but uncommon clinical entity, with reported incidence ranging from 0.4–16.2% of all gliomas [1–6]. Glioblastoma (GB) is the most frequent histological pattern of McG [4–6], but extremely rarely multicentric low-grade glioma (LGG) may also be present, though only in 0.4–1.4% of all gliomas diagnosed [3]. Depending on the timing of initial presentation, McG may be synchronous or metachronous, whereby synchronous multicentricity is more frequent one [3–6]. According to Batzdorf and Malamud's [7] widely accepted criteria, McG is characterized by distant, widely separated tumor *foci* localized in different lobes or hemispheres, with no continuity between them and with no apparent dissemination route. Their simultaneous presence at the time of disease detection, or metachronous appearance during disease progression, cannot be attributed to any of the established spreading pathways. In contrast, multifocal glioma (MfG) grows and disseminates along established routes, including white matter tracts, cerebrospinal-fluid channels, blood or local extension by satellite formations [7].

The pathogenesis of McGs is unknown, prognosis is unfavorable and management still remains controversial [4–6, 8]. Due to the limited capacity of conventional (structural) magnetic resonance imaging (MRI) to differentiate McG from others multiple brain pathologies, supplemental use of advanced magnetic resonance techniques such as diffusion-weighted imaging (DWI), perfusion-weighted imaging (PWI) and magnetic resonance spectroscopy (MRS), should allow more accurate preoperative diagnosis and more precise planning of an appropriate treatment strategy [9]. Here, we reported on a patient with ipsilateral, double, synchronous, multicentric cerebral lesions, who had initial MRI and DWI presentation as parietal metastasis and ipsilateral amygdalo-hippocampal LGG. However, MRS of both lesions showed different metabolite profiles of malignant, high-grade glioma (HGG). This was confirmed by the histopathology of the resected parietal lesion, which proved to be GB with methylated O^6-methylguanine-DNA methyltransferase (MGMT) gene promoter. We discussed the diagnostic and therapeutic modalities for patients with malignant McGs, with regard to those used with the reported patient who experienced a comparatively long survival of 19 months, with time to progression (TTP) of 17 months.

Case report

A 59-year-old right-handed Caucasian man presented with an episode of generalized convulsive seizure. On initial neurological examination the patient was disoriented with transient right hemiparesis and sensomotor dysphasia. Physical examination revealed no abnormal findings. Past medical history was unremarkable. A contrast-enhanced brain 3T MRI revealed two different and independent supratentorial ipsilateral cerebral lesions: a solid, nonenhancing left amygdalo-hippocampal lesion with no surrounding edema or mass effect and without restriction of diffusion (Figure 1 A-D), and a parietal, subcortical, regular ring-enhancing necrotic lesion, with recent hemorrhage into the tumor core surrounded by mild peritumoral edema, with a slight mass effect and only marginal restriction of diffusion (Figure 2 A-D). The amygdalo-hippocampal lesion had the MRI- and DWI-characteristics of LGG, while the parietal lesion had the appearance of solitary hemorrhagic metastasis. DWI quantification by calculating the apparent diffusion coefficient and PWI was not performed for technical reasons. However, a single-voxel MRS of both lesions showed metabolite characteristics highly suggestive of HGG: absence of N-acetylaspartate (NAA) with a high myo-inositol (mI) to creatine (Cr) ratio (mI/Cr = 1.8) in the amygdalo-hippocampal lesion (Figure 1 E, F); an increased choline (Cho) to NAA ratio (Cho/NAA = 2.36) and decreased NAA/Cr (NAA/Cr = 0.67) ratio with low mI and the presence of lipids (Lip) in the peritumoral area of the parietal lesion on T2-weighted (T2W) image (Figure 2 E, F). Consequently, the parietal lesion was totally resected through a left parietal craniotomy. The histopathology revealed GB (Figure 3), with a cellular proliferation Ki67 (MIB-1) labeling index of 20%. Nested methylation-specific polymerase chain reaction analysis showed that the tumor contained methylated MGMT gene promoter (Figure 4). In view of these findings, as well as the result of MRS of the amygdalo-hippocampal lesion, no attempt was made to

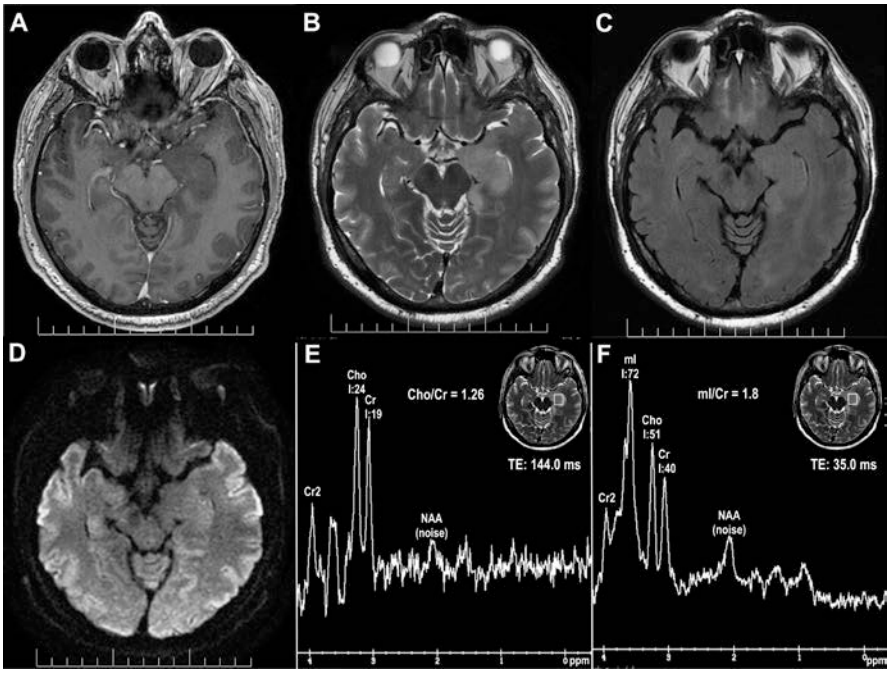


Fig. 1 – Post-contrast axial 3T magnetic resonance imaging: (A) a nonenhancing, isointense left amygdalo-hippocampal lesion compared to cortex in T1-weighted; (B) T2-weighted and (C) fluid attenuated inversion recovery sequences, with no surrounding edema, and (D) with no restriction of diffusion on diffusion-weighted imaging. Single voxel magnetic resonance spectroscopy with the voxel located within the lesion (inserts in E and F): tumor spectra (E) at long echo-time TE (TE = 144 ms) and (F) at short TE (TE = 35 ms) sequences shows absence of N-acetylaspartate (NAA) on both TEs, with (E) normal choline (Cho) to creatine (Cr) ratio (Cho/Cr = 1.26) at long TE, and with (F) high myo-inositol (mI) to Cr ratio (mI/Cr = 1.8) at short TE. Cr2 is the second creatine peak at 3.9 ppm, integral (I) with numbers indicates the relative signal intensity of the corresponding metabolite.

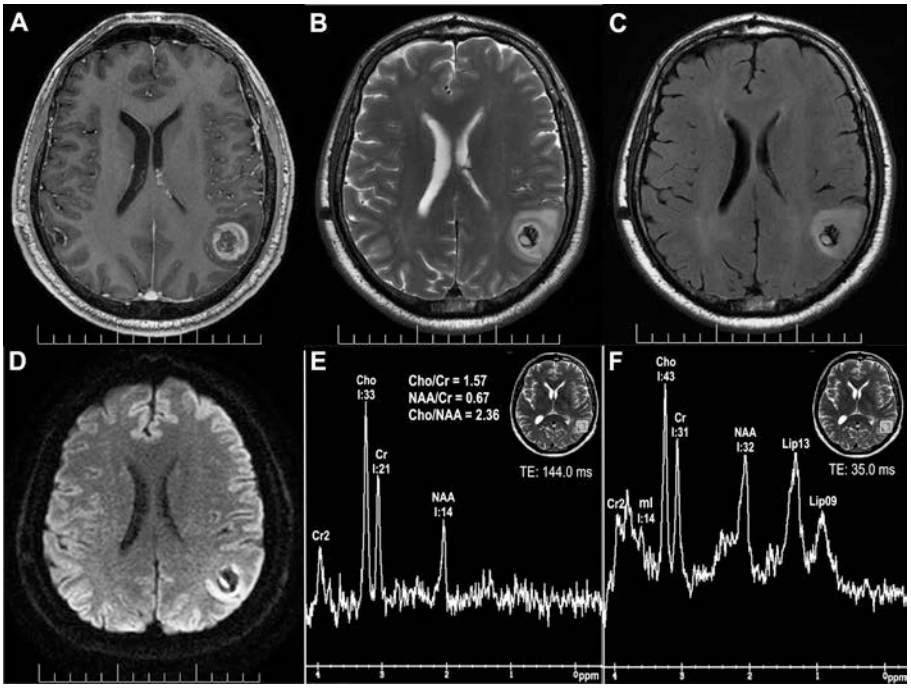


Fig. 2 – Post-contrast axial 3T magnetic resonance imaging: (A) a left parietal, subcortical, necrotic, ring-enhancing lesion, with recent hemorrhage into the tumor core in T1-weighted sequence, surrounded by mild peritumoral edema in (B) T2-weighted and (C) fluid attenuated inversion recovery sequences, and (D) with only marginal restriction of diffusion on diffusion-weighted imaging. Single voxel magnetic resonance spectroscopy with the voxel located within the lower part of the peritumoral vasogenic edema (inserts in E and F), positioned so as to avoid the cystic-hemorrhagic core of the lesion and thus partial volume effect: tumor spectra (E) at long echo-time TE (TE = 144 ms) and (F) at short TE (TE = 35 ms) sequences shows (E) increased choline (Cho) to N-acetylaspartate (NAA) and Cho to creatine (Cr) ratios (Cho/NAA = 2.36, Cho/Cr = 1.57, respectively) and decreased NAA/Cr ratio (NAA/Cr = 0.67) at long TE, and (F) low myo-inositol (mI) with presence of lipid (Lip) peaks at 1.3 and 0.9 ppm at short TE. Cr2 is the second creatine peak at 3.9 ppm, integral (I) with numbers indicates the relative signal intensity of the corresponding metabolite.

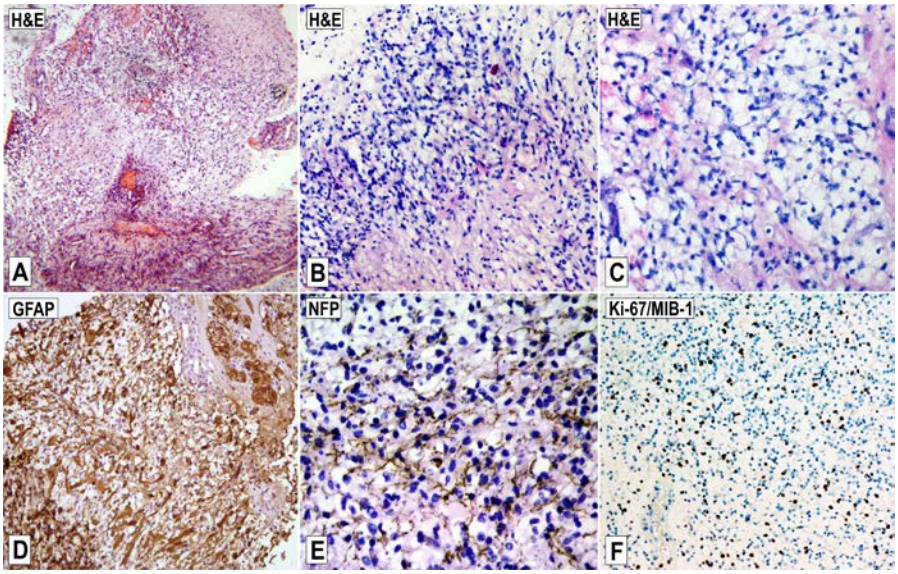
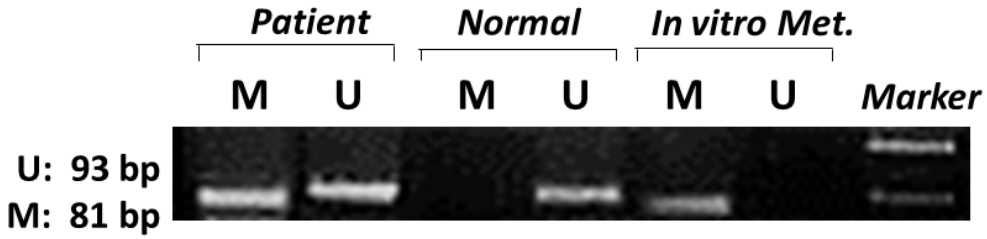


Fig. 3 – Photomicrographs of the surgical specimen from the left subcortical, parietal, ring-like tumor lesion reveals the typical features of glioblastoma: (A) endothelial proliferation with microvascular bleeding into the tumor tissue, (B) pseudopalisading necrosis and (C) hypercellularity with marked cellular atypia and frequent atypical mitosis. (D) Glial fibrillary acidic protein (GFAP) immunoreactivity is strongly and diffusely present in neoplastic glial cells, with only weak (E) neuronal neurofilament protein (NFP) immunoreactivity between them. (F) The Ki-67(MIB-1) nuclear immunoreactivity with labeling index of 20%. Hematoxylin and eosin (H&E) staining, (GFAP, NFP, Ki-67) immunohistochemical staining with DAB as a chromogen contrasted with hematoxylin. Original magnification (A) × 40, (B, D, F) × 100, (C) × 200, (E) × 400.



remove it or performe biopsy. The postoperative course was uneventful. Four weeks after surgery, the patient was well recovered, and his performance status (PS) according to the World Health Organization (WHO) scoring system was 1. He started the standard Stupp's et al. [10] chemoradiation protocol: conformal radiotherapy (CRT) delivered to both lesions, daily fractions of 2 Gy for a total of 60 Gy, with concomitant temozolomide (TMZ) at a dose of 75 mg/m^2 per day over 42 days, followed by 6 standard cycles of adjuvant TMZ 150 – 200 mg/m^2 per day, 5 consecutive days every 28 days (5/28). Since the patient's tolerance to the applied therapy was good, and periodic MRI follow-ups confirmed stabilization of the disease, TMZ was continued with the same dosing schedule up to tumor progression or development of unacceptable toxicity. The patient received a total of 12 standard adjuvant TMZ courses. Despite prolonged stabilization of the disease with good life-quality during extended TMZ treatment, the patient died 19 months after the diagnosis. The TTP estimated by MRI was 17 months: tumor progression was observed 2 months before death as a centrifugal bilateral GB spreading along the white matter tracts. The patient rapidly deteriorated during the final 2 weeks before death, due to a massive parietal intratumoral hemorrhage. In accordance with the wishes of the patient's family an autopsy was not performed.

Discusion

Even though the concept of McG was proposed more than 50 years ago [7], the true origin and nature of this pathology are still moot. Is it a separate nosological entity, a biologically different subtype of glioma with as yet unknown genomic specifics, or just an unpredictable variation in the development of "ordinary" gliomas, mostly malignant, with specific molecular pathways, which we discover in the multicentric phase of their expansion? Several theories have been proposed to explain the synchronous coexistence or metachronous development of glioma multicentricity, such as tumorogenesis at multiple sites or active glioma cell migration invisible to MRI [5, 8, 11–13], but still without conclusive answers. Akimoto et al. [14] recently reported a case of radiologically multicentric, but genetically identical synchronous GB in opposite cerebral hemispheres, which indicates a common monoclonal origin. Schroeder et al. [15] also reported a case of multicentric GB in which all 4 tumor foci shared a common genetic origin, although each had its

own unique set of genetic aberrations. Liu et al. [16] identified overexpression of the *CYB5R2* gene in multicentric and multifocal GB, suggesting that the methylation status of its promoter may serve as a new epigenetic biomarker of multiple GB.

Malignant McGs are mostly localized supratentorially [4–6], but combined supra- and infratentorial localization [4–6, 17–20], and cerebral GB multicentricity with metachronous spinal seeding have also been reported [5, 20, 21]. Ipsilateral multicentricity is more frequent in synchronous than in metachronous malignant gliomas [5]. The number of distant tumor foci may range from 2 to 5 or more, but in most cases there are 2 or 3 [4-6, 18, 22]. Usually, they are of the same histological appearance, but different histotypes or grades of the same histotype are also possible [6, 12, 17, 18, 22]. In three recent clinical studies [1, 2, 23], the frequency of synchronous multicentric GBs among all newly diagnosed multiple (multicentric and multifocal) GBs was between 13% and 24.2%, and in a subanalysis, multicentric GBs ranged from 1.9% to 5% in regard to all newly diagnosed GBs.

Despite the undoubted advantages of conventional contrast-enhanced MRI in diagnosing McGs, their differentiation from other multiple cerebral pathologies such as metastases, lymphoma, infections and vascular or demyelinating diseases may prove difficult or even impossible [8, 9, 24]. Conventional MRI has a limited capacity to differentiate McGs from multiple brain metastases, because their neuroimaging appearance is often similar, equivocal, or indistinguishable [8, 24, 25], as was the case with the parietal lesion in our patient. An additional difficulty in distinguishing these intracerebral lesions is posed by the possibility of the simultaneous presence of brain metastasis in patients affected by glioma, even multicentric [6, 18]. All these facts indicate the need for the use of the advanced MRI techniques such as DWI, PWI and MRS, in order to increase the diagnostic accuracy of conventional MRI, even though none of them are lesion-specific [9].

In contrast to most solid or necrotic metastases, HGGs infiltrate diffusely into the peritumoral area and form neoplastic neovascularization. Consequently, there is a different degree of hyperperfusion on PWI, a restriction of diffusion on DWI and pathologic metabolite ratios of elevated Cho, reduced NAA and mI as well as the appearance of Lip on MRS in the region of the vasogenic edema that surrounds the contrast-enhancing part of HGG on T2W images [9, 26]. Therefore, a metabolic profile consisting of increased Cho/NAA and decreased NAA/Cr ratios at long echo-time (TE = 144 ms), together with low mI and the presence of Lip peaks at short TE (TE = 35 ms), as in the perienhancing region of the parietal lesion in our patient (subsequently confirmed as GB by histopathology), indicated HGG rather than metastasis [9, 27]. Elevated Cho was reported in the peritumoral region of gliomas but not in metastases [26]. The mI signal is absent in metastases, and decreased, but nonetheless present in HGGs [28]. The Lip peaks as a hallmark of necrosis, even if microscopic, in both cases confirm malignancy, whereas in contrast to GB, the prominent Lip signals of metastases are often still seen at long TE [9].

Unlike the parietal lesion, the synchronously present amygdalo-hippocampal lesion in our patient had the MRI- and DWI-characteristics of LGG. Myo-inositol has been shown to be the MRS marker of the astrogliosis and glioma invasion [9, 27, 29]. A high level of mI is present mainly in LGGs, even with no elevation of the Cho/Cr ratio [30]. However, their aggressive continuous infiltrative growth and invasion along the white matter tracts and basement membrane-like structures, leads to further displacement, deviation, and destruction of both neurons and their axons [29]. Consequently, along with a mI increase, NAA as a marker of neuronal density and viability will decline markedly, indicating the more aggressive growth of LGG and its early transformation to a higher grade of malignancy [26, 29]. Therefore, we interpreted the amygdalo-hippocampal lesion in our patient as an "early" or secondary HGG, rather than LGG, given that the high mI/Cr ratio at short TE and absence of NAA on both TEs, were the key characteristics of its spectra. Since the mentioned lesion with “early” malignant glioma characteristics on MRS was not confirmed by histopathology, but was synchronously present with the histopathologically proven parietal GB and the criteria for multicentricity were clear, it was reasonable for us to qualify the reported case as a synchronous malignant McG.

Treatment of patients with malignant McGs is still controversial, and the prognosis remains unfavorable. Reported treatment options range from a biopsy alone, to resection of one or all tumor lesions followed by TMZ chemoradiation [4–6, 8, 17–24, 31]. On one hand, extensive resection increases the risk of hemorrhage and further neurological deterioration. On the other hand, adjuvant chemoradiation will be more effective when the tumor bulk has already been reduced [8, 11]. With regard to these endpoints, it remains debatable how aggressively patients with synchronous or metachronous multicentric disease should be treated [4].

Reportedly, the median survival time (MST) of patients with malignant McGs, regardless of treatment, was between 7.6 and 11 months [4, 6]. Hefti et al. [5] reported that patients with synchronous malignant McG showed a similar MST (110 days) to patients with metachronous disease (72 days), once they developed multicentricity. All the patients in their synchronous cohort underwent surgical resection (gross total or partial), but only 17% of them completed radiotherapy and one third received TMZ chemotherapy with a mean duration of 3.9 cycles [5]. Regarding the treatment applied by Salvati et al. [6] in the pre-TMZ era (before 2005) and di Russo et al. [4] during the TMZ era, longer MST was observed in patients who underwent surgical resection of at least one tumor focus followed by adjuvant therapy, than in those who were treated with a stereotactic biopsy followed by radio- and/or chemotherapy: 9.5 and 12 months *vs* 2.8 and 4 months, respectively. Di Russo et al. [4] stated that MST results similar to theirs were also obtained from a literature review. Median progression free survival (PFS) after surgical resection in their study was 8.5 months with no differences between patients who underwent single or multiple resections. The authors concluded that surgical resection of at least one

easily accessible lesion seems to have a beneficial effect on the survival of selected patients with malignant McGs [4]. We applied the same surgical strategy with our patient by resecting only the easily accessible parietal lesion. However, a recent report by Hassaneen et al. [31] suggests that aggressive resection of all tumor lesions via two separate craniotomies in the same session, in selected patients with synchronous multicentric GB, resulted in a survival duration comparable to that of patients undergoing single-lesion surgery (12.9 *vs* 14.6 months, respectively), without a significant increase in postoperative morbidity. In the cases where surgical resection is not feasible, mainly due to older age and/or poor PS, stereotactic biopsy is recommended as the solution of choice for obtaining the histopathology and planning palliative treatment [8, 31].

In the study by Thomas et al. [1], patients with multicentric GB had worse MST of 3 months compared to those with multifocal and single (unifocal) GBs (10 and 18 months, respectively). However, differences in survival between single and multifocal or multicentric GBs ceased to be significant when taking into consideration independent predictors of outcome such as age, initial PS score, extent of resection, and MGMT gene promoter methylation status. The authors concluded that neither multifocal nor multicentric GB independently predicted worse outcome for patients, but rather were associated with a lower PS score at the time of diagnosis, and the impossibility of performing a gross total resection. Therefore, according to Thomas et al. [1], these findings suggest that unifocal, multifocal and multicentric GB are in fact a spectrum of presentation of a single disease. Paulsson et al. [23] showed that PS remains a dominant prognostic factor in the GB patients, which is particularly relevant in multifocal and multicentric GBs. The authors concluded that response to the standard therapies and overall survival do not differ significantly between multiple GBs and their single focus counterparts, while the worse PFS in multiple GB may be due to increased likelihood of gross total resection in unifocal GB [23].

Since 2005, Stupp's chemoradiation protocol [10] consisting of concurrent CRT with TMZ followed by 6 adjuvant 5/28 cycles of TMZ chemotherapy became a standard of care for patients with newly diagnosed solitary GB after maximal safe tumor resection [32–34]. The reported MST for such patients with methylated MGMT gene promoter was 21.7 months [35]. However, in multicentric disease considerable variations in postoperative treatment protocols were reported [1, 2, 4–6, 8, 16, 17, 19, 20, 22, 23].

Regarding radiotherapy (RT) in patients with synchronous multicentric and multifocal GBs, Showalter et al. [36] found no significant differences in the median TTP or MST between CRT and the whole-brain RT (WBRT), while clinical PS was a consistent and independent predictor of both TTP and MST. On the basis of the progression pattern, but with no clear evidence of its superiority, CRT was recommended as a preferable RT approach, while WBRT should be reserved mainly for patients with poor PS who are unable to complete a prolonged course of CRT [36, 37]. Our patient was treated in accordance with this recommendation, with the addition of concomitant TMZ to CRT according to Stupp's chemoradiation protocol [10] and with good tolerance to the applied therapy. However, significant worsening of PS during postoperative RT in malignant McG patients was reported relatively often in relevant literature as a reason for refusal or termination of further treatment [4, 5, 20].

The exact impact of concomitant and adjuvant TMZ chemotherapy on the MST of patients with malignant McGs is still unknown, mainly due to the rarity of the disease and thus the lack of controlled clinical trials. In a recent report by Paulsson et al. [23], on 8 patients with multicentric GB, various degrees of surgical resection and CRT were followed by TMZ chemotherapy in 6 patients, but without data on their MST or PFS. Moreover, the impact of TMZ chemotherapy cannot be estimated even from the major published studies of Salvati et al. [6] and Hefti et al. [5], because they included patients treated in the pre-TMZ era when TMZ did not become the standard of care in the GB treatment [32, 33]. Only in di Russo et al. [4] study, in all patients except one (17/18), there was resection followed by TMZ chemotherapy and by radiotherapy in 7 of the 18 patients, which might explain, at least in part, the longest MST reported in their study. Moreover, methylation of MGMT gene promoter was recently identified as an independent favorable predictor of outcome in patients with newly diagnosed multicentric and multifocal GBs [1]. Accordingly, as in methylated patients with solitary GB [35, 38], a longer MST could be expected irrespective of a treatment, as well as better response to TMZ in the multicentric GB patients with methylated MGMT gene promoter. Furthermore, this is also a basic argument for continuing adjuvant TMZ beyond 6 standard cycles [39]. Longer adjuvant TMZ in the methylated GB patients should additionally improve tumor control due to their higher sensitivity to the therapeutic TMZ cytotoxicity [34, 39–41]. Keeping this in mind, we extended adjuvant TMZ treatment in our methylated patient to 12 standard cycles while maintaining stable disease and good life-quality all the time during adjuvant therapy and without the appearance of significant additional toxicity. However, in unmethylated patients who are much less responsive to TMZ, there is the option of time- and dose-intensified TMZ regimens which theoretically could reduce the level of MGMT through the enzyme depletion/consumption mechanism. This should lead to the increased and protracted MGMT inactivation, and thus more effective triggering of therapeutic TMZ cytotoxicity [34, 41]. Although, time- and/or dose-modified TMZ regimens have not been shown to be superior to the standard TMZ regimen for newly diagnosed solitary GBs so far [34], their potential role (including the standard one) for malignant McGs still needs to be determined.

Taking all aspects of the applied therapy into account, we believe that our patient received clear benefits from the applied treatment, accomplishing comparatively long survival period of 19 months with a TTP of 17 months. This is considerably longer compared to the reported data for such pathology [1, 2, 4–6, 19, 20, 23], and closer to the MST for newly diagnosed methylated solitary GBs [33, 35]. The main parameters that guided us in choosing the therapeutic

approach were the patient's age and PS and MGMT methylation status, with the basic goal of increasing survival while maintaining good life-quality for as long as possible during the therapy. In addition to the applied therapy, an important factor which probably contributed to longer survival and therefore certainly cannot be ignored, was an "early" malignant glioma lesion synchronously present with a methylated GB lesion, which remained morphologically stabile all through the treatment.

Conclusion

Despite significant improvements in the diagnosis and treatment of malignant gliomas during the last decade, management of synchronous multicentric disease still remains controversial. MRS significantly improved the differential diagnostic accuracy of conventional MRI in our patient and should be included in the initial evaluation of such cases. In accordance with reviewed literature, the younger age, good initial performance status and methylated MGMT gene promoter were all favorable predictors of longer survival in the reported case. Resection of at least one easily accessible tumor lesion, followed by TMZ-based chemoradiation, with continuous adjuvant TMZ in more than 6 standard courses, seems to be currently the most beneficial therapeutic option for such cases. As with all gliomas, an individualized approach based on the structural and metabolic MRI characteristics of the tumor lesions and their particular genomic features, should form the base for the future treatment strategy of synchronous malignant McGs.

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CASE REPORT

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Surgical treatment of the lower leg open fracture with lesions of the main blood vessels. A case report

Hirurško lečenje otvorenog preloma potkolenice sa lezijom magistralnih krvnih sudova

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Abstract

Introduction. Open fractures of the lower leg degree IIIC by Gustilo belong to the most serious open fractures of the lower leg segment.These fractures are, along with the main blood vessels damage, often followed by a severe soft-tissue damage of the lower leg. **Case report.** Patient 20 years old, sustained a severe open fracture of the left lower leg in a car accident, with the disruption of the continuity of the main left lower leg arteries. After clinical examination and radiography (X-ray) diagnosis, the primary treatment of the open fracture fragment wound, reposition of the left lower leg and stabilization of the open fractures by the external skeletal fixator were performed. In the postoperative period, there was a manifestation of acute ischemia of the left foot. Arteriography verified discontinuity of all three crural arteries at the level of the fracture line. The patient was immediately surgically treated. Revascularization of the extremities was performed by reconstruction of the anterior tibial artery by 15 centimeters long saphenous graft interposition with creation of distal anastomosis at the level of the dorsal artery of the foot. Large soft tissue defect and reconstructed vessels were covered with transpositional fasciocutaneous flap. The postoperative course was accompanied by proper graft flow. Control X-ray examination verified fracture of the distal pin of external skeletal fixator with the healing of fractures of the tibia with angular deformity. The external fixator was removed, except for the residual part of the pin in the distal part of the lower leg. During the control examination after completion of the treatment, the patient walked without mobility aid. **Conclusion.** External skeletal fracture fixation, damaged blood vessels reconstruction and early covering of the soft-tissue shin defect are basic elements in saving the injured limb. The long term goal of treatment of open lower leg fractures with an injury of the main blood vessels is to allow patients return to usual daily activities and professional work.

Key words:
lower extremity; fractures, bone; fractures, open; diagnostic techniques and procedures; surgical procedures, operative; transplants; surgical flaps; recovery of function.

Apstrakt

Uvod. Otvoreni prelomi potkolenice III C stepena po Gustilu spadaju u najteže otvorene prelome potkolenog segmenta. Često su praćeni, pored povreda krvnih sudova, i velikim oštećenjem mekotkivnog omotača potkolenice. **Prikaz bolesnika.** Bolesnik, star 20 godina, zadobio je težak otvoreni prelom leve potkolenice u saobraćajnoj nesreći, sa prekidom kontinuiteta magistralnih arterija leve potkolenice. Nakon kliničkog pregleda i radiološke dijagnostike, urađena je primarna obrada rane otvorenog preloma, repozicija fragmenata leve potkolenice i stabilizacija otvorenog preloma spoljnim skeletnim fiksatorom. U postoperativnom toku došlo je do manifestacije akutne ishemije levog stopala. Arteriografski je bio verifikovan diskontinuitet sve tri kruralne arterije u nivou linije preloma. Bolesnik je hitno operisan. Revaskularizacija ekstremiteta izvedena je rekonstrukcijom prednje tibijalne arterije interpozicionim safenskim graftom, dužine 15 cm, uz kreiranje distalne anastomoze u nivou dorzalne arterije stopala. Veliki mekotkivni defekt i rekonstruisani krvni sudovi prekriveni su fasciokutanim transpozicionim režnjem. Postoperativni tok praćen je urednom prohodnošću grafta. Na kontrolnom rentgenskom snimku verifikovan je lom distalnog klina spoljnog skeletnog fiksatora, uz zarastanje preloma tibije

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sa angularnim deformitetom. Spoljni skeletni fiksator je odstranjen, osim zaostalog dela klina u distalnom delu potkolenice. Na kontrolnom pregledu po završenom lečenju, bolesnik je hodao bez pomagala. **Zaključak.** Spoljna skeletna fiksacija preloma, rekonstrukcija povređenih krvnih sudova i rano pokrivanje mekotkivnog defekta potkolenice su osnovni elementi u spašavanju povređenog ekstremiteta. Dugoročni cilj lečenja je potpuni funkcionalni oporavak povređenog ekstremiteta i pun povratak bolesnika životnim i radnim aktivnostima.

Ključne reči:
potkolenica, prelomi; povrede, otvorene; dijagnostičke tehnike i procedure; hirurgija, operativne procedure; graftovi; režnjevi, hirurški; funkcija, povratak.

Introduction

Gustilo grade IIIC open fractures belong to the most serious open fractures of the lower leg segment. Gustilo's classification system defines IIIC fractures as open fractures associated with the arterial injury requiring the treatment [1, 2]. The degree of soft tissue damage is not significant although it is usually extensive. There is also a lesion of the main blood vessels that threaten the vitality of the limb. These injuries usually occur in traffic accidents under the influence of extensive mechanical force, where, in addition to the lesion of the main blood vessel, there may appear the extensive damage of soft tissue of the lower leg and periosteum removing of the injured bone in the fracture segment.

When treating these injuries, it is very important to have a multidisciplinary approach, both in diagnostics and in surgical treatment.

External skeletal fracture fixation, damaged blood vessels reconstruction and early covering of the soft-tissue shin defect are basic elements in saving the injured limb [3].

In a case of severe soft-tissue lower leg damage, sometimes a multidisciplinary decision making (an orthopedist, a vascular surgeon and a plastic surgeon) about the possible primal amputation is necessary. In the last two decades, the published studies suggest that the rescued limb gives a better quality of life and lower cost of treatment, despite the subsequent reconstructive surgery treatments [4–6].

The aim of this case report was to present the treatment of the patient with severe open fracture of the left lower leg and the injury of the main blood vessels (grade IIIC open fractures by Gustilo) that was treated at the Department of Orthopedics and Traumatology and Vascular Surgery Department, Clinical Center in Niš, Serbia.

Case report

The patient, 20 years old, sustained a severe open fracture of the left lower leg (IIIC), in a car accident, when a thick tin of the damaged fence along the road broke into the door and the bottom of the passenger car and seriously injured the left lower leg of the patient. He was taken by the ambulance to the Department of Orthopedics and Traumatology, Clinical Center in Niš.

Immediately after the admission and clinical examination, X-ray examination was done, showing an open fracture of the left lower leg in the middle third part. After preoperative preparation, the surgical treatment was performed with primary treatment of the open fracture wound, reposition of the left lower leg fragments and stabilization of the fracture with an external skeletal fixator (Figure 1).

Due to the technical reasons, an arteriography of the injured limb has been done right after the external fixation of the fracture. Arteriography verified discontinuity of all three lower leg arteries at the level of the fracture line (Figure 2). The patient, after consulting a vascular surgeon, was instantly taken into the Department of Vascular Surgery.

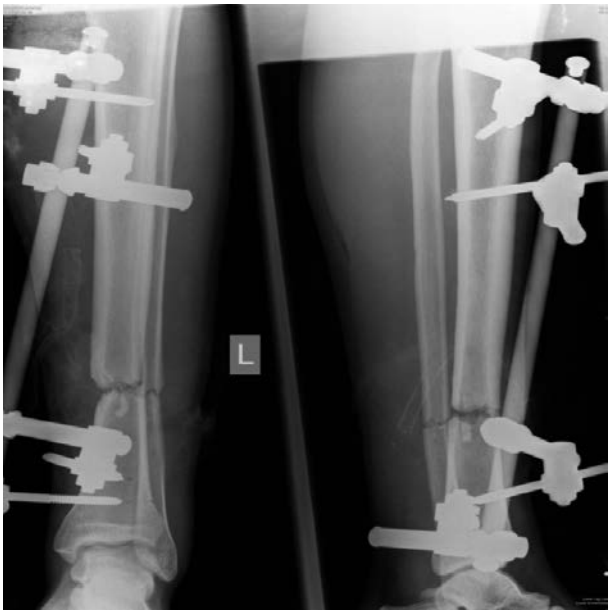

Fig. 1 – Open fracture of the left lower leg after reposition of the fragments of the tibia and external skeletal fixation.

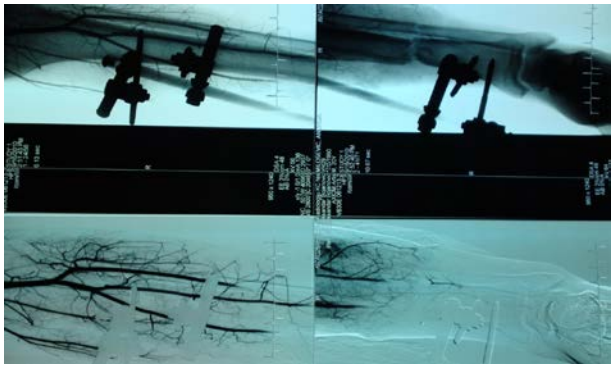

Fig. 2 – Arteriography of the left lower leg shows the break in the continuity of all three main lower leg arteries.

The patient was immediately surgically treated. Intraoperatively, crushing lesion was confirmed with defect of all three arteries of the lower leg, especially the posterior tibial artery, where hardest lesions throughout its length were verified including the most distal segments. Extremity revascularization was performed by reconstruction of the anterior tibial artery by using 15 centimeters long interpositional saphenous graft, with the creation of the distal anastomosis at the level of the dorsal artery of the foot. Anastomoses were created by continuous circular everting suture, by prolene 7-0 (Figure 3).

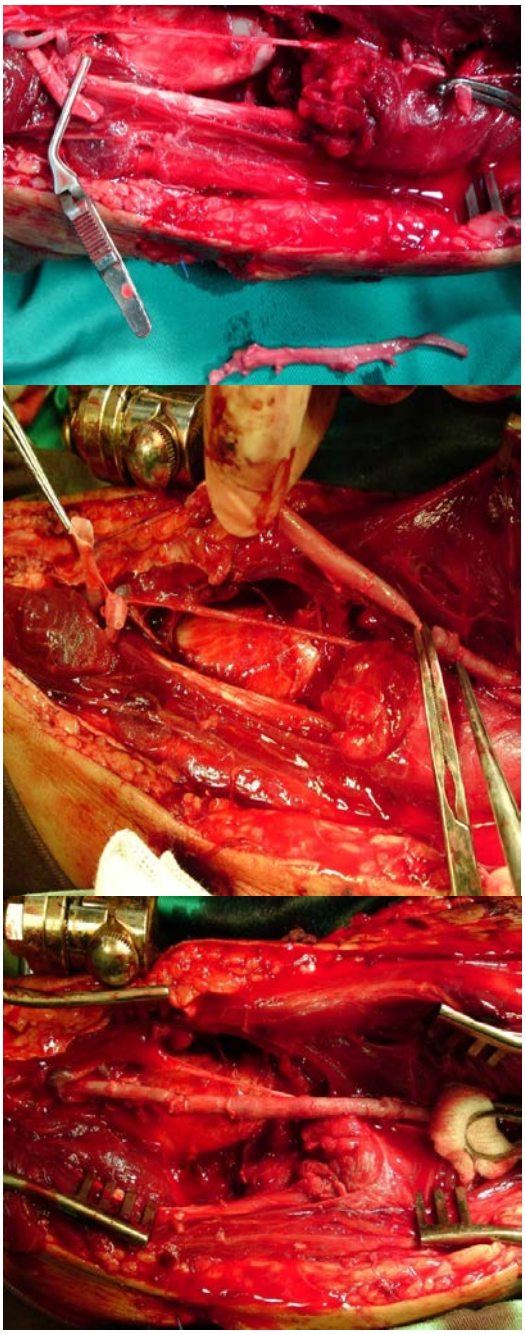

Fig. 3 – The reconstruction of the anterior tibial artery.

Because of the extensive soft tissue injuries and prolonged posttraumatic acute ischemia, in the same act we performed open anterolateral and posteromedial fasciotomy. Large soft tissue defect and reconstructed vessels were covered with transpositional fasciocutaneous flap. The postoperative course was accompanied by proper graft flow with palpable, well-filled pulse of *a. dorsalis pedis*.

In the postoperative period, there was a very slow secondary recovery of the soft tissue defects of the lower leg, which were at the stage of mature granulation and absent infection, covered by skin autograft (Figures 4 and 5).

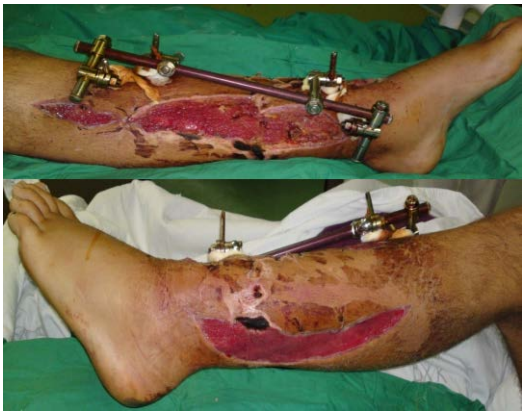

Fig. 4 – Soft tissue defects of the lower leg and the presence of the wound caused by fasciotomy.

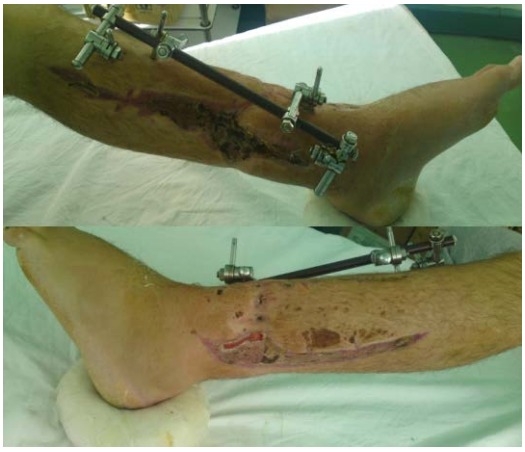

Fig. 5 – The state of the lower leg after soft tissue defect covering.

Due to the difficulty in walking, limited mobility of the ankle and the toes of the left foot, swelling of the foot and weakness of the muscles of the left lower leg, the patient was taken into the Department of Physical Medicine and Rehabilitation.

During his stay in the Clinic, kinesis therapy, magnetic therapy and work therapy were applied. The patient was activated with the help of axilla crutches with relieving support of the injured leg. Besides, a correction of the bar position of the external skeletal fixator was made by an orthopedic surgeon and increased reliance on the injured leg up to 50% of the weight. During the therapy, there was a reduction of the swelling of the left leg and increase of the range of motion in the left ankle. The patient was discharged from the Clinic on December 28, 2010 with a recommendation to continue with previously used exercises

at home. He was physically treated as an outpatient at the Health Center in Kuršumlija.

In the Clinic, the patient was regularly bandaged around the pins of the external skeletal fixator. Because of the occurrence of infection around the pins of the external skeletal fixator, the patient recieved an antibiotic therapy with continued daily bandaging around the pins. After the therapy, there was a decrease of infection around the pins of the external skeletal fixation, and the patient continued with physical therapy in an outpatient settings. Due to the intensifying of infection around the pins of the fixator, the patient was treated in hospital at the Health Center in Kuršumlija, with parenteral application of antibiotics and daily bandaging. The therapy initiated decrease of infection and the outpatient physical therapy was continued.

On the control X-ray examination of the left lower leg with ankle, which was made on May 25, 2011, the fracture of the most distal pin of external skeletal fixator was visible, besides the healing of fractures of the tibia with angular deformity – valgus of 10 degrees. Valgus deformity was the result of loosening pins of the external skeletal fixator and fracture of the distal pin of the external skeletal fixator (Figure 6).

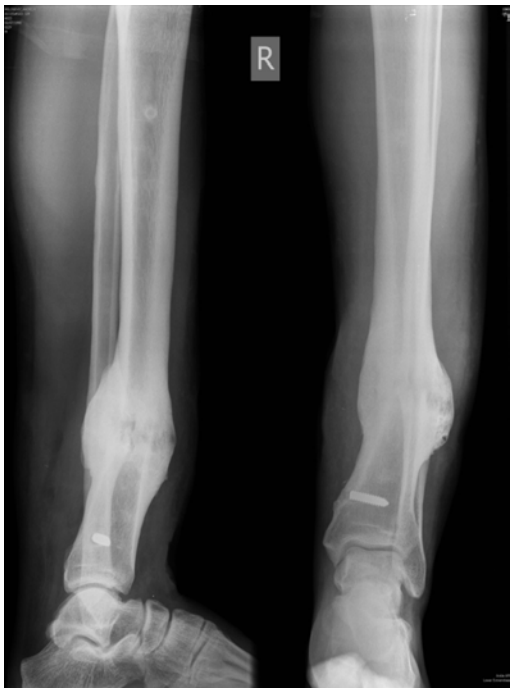

Fig. 6 – The X-rays of the left lower leg present the healed fracture of the left tibia with valgus deformity and partly broken pin of the external skeletal fixator, which was not removed from the the tibial bone.

The external skeletal fixator pins were removed on June 24, 2011. After healing the wounds caused by pins, patient was sent to physical therapy to the Ribarska spa, where he was admitted on July 19, 2011 for further treatment. After the treatment was completed, the patient returned to his life, work and sport activities – the patient was the national competitor in taekwondo. During the control examination performed on March 4, 2013, the patient was walking without mobility aid. Movements in the left ankle were limited at the medium level. At the site of the residual part of the pin of the external skeletal fixator there was no secretion. Figure 7 present the condition of the lower leg after the completion of treatment.

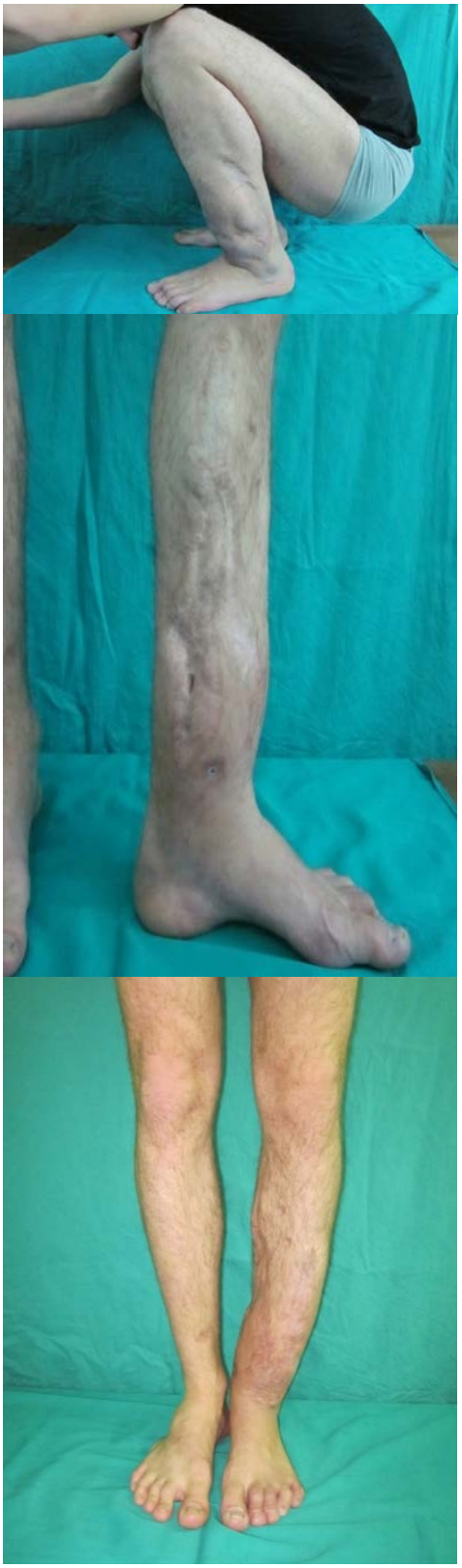

Fig. 7 – The condition of the lower leg after the completion of treatment.

Discussion

Open fractures of the lower leg grade IIIC by Gustilo belong to the most serious open fractures of the lower leg segment, because beside the severe damage of soft and bone tissues, there is a violation of the main blood vessel that threatens the vitality of the extremities [7]. Injuries of the main blood vessels of the extremities are one of the most urgent medical conditions that require immediate surgical treatment. Blood vessels, which are located along the bone, are most commonly exposed to injuries, where injuries can be caused by ends of the broken bone. During the injury of the blood vessels, the opening of the lumen can happen (laceration, transection) with resultant bleeding, or obstruction of the flow (thrombosis) and the interruption of circulation. These injuries usually occur in traffic accidents, under the high intensity force, whereas beside lesions of the main blood vessel, there is a great damage of the soft tissues of the lower leg and periosteum removal from the injured bone in the fracture segment [3, 8].

Open lower leg fractures with arterial vascular injury require urgent surgical treatment, as soon as the patient's condition allows it [9]. Treatment of open lower leg fractures includes copious rinsing of the wound with removal of foreign bodies from the wound, the primary treatment of open fracture wound, stabilization of the fractures with external fixator, reconstruction of the injured arterial blood vessels, antibiotics, anti tetanus protection and delay of the wound closure [3]. In order to do an adequate reconstruction of the main blood vessels, a stable fixation of fragments of the open lower leg fractures is required because any movement of bone fragments can harm the reconstructed vessels. If ischemia lasts more than 6 hours, the prophylactic fasciotomy should be performed. Postoperatively, the vascular status of extremities should be estimated. If there is some doubt, intraoperative or postoperative arteriography should be applied [3, 8, 9].

Reconstruction of the injured blood vessels aims to restore flow and preservation of vital functions in the tissues which are on the distal position from the injury of the blood vessel. Revascularization can be successfully performed even after 6 hours starting from the injury of the blood vessels and blood flow stoppage. To assess the appropriateness of the reconstruction, besides the duration of the ischemic period, the degree of the ischemic changes in the distal tissues should be taken into account. Murmuration and muscle rigor appearance, with spastic paralysis or limp legs mark the beginning of irreversible tissue ischemia. When beside the arteries, larger deep vein is injured, it is necessary to carry out the reconstruction of the vein. Vein ligation produces venous stasis, swelling and exacerbates tissue ischemia. Good venous circulation gives significant contribution to the reconstruction of injured arteries [9].

Davidovic et al. [10] presented the treatment of 44 patients with popliteal artery injuries during Yugoslavian civil war. Autors recommended *in situ* or lateral subcutaneous reconstruction in cases of complicated popliteal artery injuries, such as concomitant bone fractures accompanied by a massive soft tissue damage.

The treatment of the open lower leg fractures with injury to the main blood vessels (grade IIIC by Gustilo) is accompanied by significant morbidity and additional psychosocial effects. The treatment of these injuries often requires the prompt decision regarding salvage or a limb amputation. Previously, it was thought that the absence of plantar sensation is the most important variable in making decision whether to amputate limbs in severely injured leg. Bosse et al. [11] changed this oppinion and found that in more than a half of the patients who were subjected to an early reconstruction of injured blood vessels of the legs, the sensitivity was renewed within two years. They concluded that the initial sensitivity of the foot was not a prognostic sign for future foot sensitivity and functional outcome. Primary amputation was an option for patients with avascular extremities and the cold foot, after 4–6 hours from injury, because of the loss of muscles in more than two compartments and the loss of bone tissue over a third of the length of the tibia. Violation of the arteries below the knee had a higher risk for amputation in relation to violations of the artery above the knee [12, 13].

Davidovic et al. [14] analyzed the early results of civil and war peripheral arterial injury treatment on 413 patients (with 448 arterial injury) and tried to identify risk factors associated with limb loss. Significant risk factors for amputation were found to be failed revascularization, associated injuries, secondary operation, explosive injury, war injury ($p < 0.01$) and arterial contusion with consecutive thrombosis, popliteal artery injury and late surgery ($p < 0.05$). The most significant independent risk factor for limb loss was failed revascularization.

The percentage of secondary amputation, after revascularization, ranges from 5.5% to 28% [15]. McNutt et al. [16] reported that the main reasons for the secondary amputation of extremities were the following ones: extensive muscle necrosis, infection, delayed revascularization, thrombosis of the distal vascular tree and poor collateral blood flow. Chung et al. [5] followed the cost of treatment by comparing amputation with limb salvage. They showed that amputation was more expensive than limbs salvage, taking into consideration the quality of life after completion of the treatment. They concluded that the limb salvage was cost-effective strategy if the injury was not so severe that amputation was necessary. Alexander et al. [15] and Lin et al. [17] reported that 80% of patients with open fractures and arterial blood vessels (grade IIIC by Gustilo) require secondary reconstructive procedures. Brinker et al. [18] reported that tibial fractures with injury to *a. tibialis posterior* had a high risk of delayed healing and non-healing, because the main nutritional tibial artery was the branch of this blood vessel which also supplies the periosteum.

The infection was common in open lower leg fractures with injury to the main blood vessels of the lower leg (grade IIIC by Gustilo). The incidence of infection was higher in open lower leg fractures grade IIIC versus open fractures of grades IIIA and IIIB by Gustilo [1, 7, 19, 20].

Sony et al. [7] presented the results of the treatment of open fractures grade IIIC by Gustilo. In the fifteen-year

period, 18 patients with open fracture grade IIIC were followed. In the analyzed group there were 15 men and 3 women. The average age was 30.7 years and the average Mangled Extremity Severity Score (MESS) was 6.9 (3–10). In total, 15 limbs were rescued and 3 underwent amputation (2 primary and one delayed). In 4 patients, the fracture was stabilized by the external skeletal fixator and in 12 patients with the internal fixation. In 7 patients, wound infection was noticed, and in 4 patients there was a non-healing of the fracture which required further surgery treatment. Fractures of the distal tibia frequently had delayed healing when they are associated with lesion of *a. tibialis posterior*. Upon completion of the treatment, 39% of patients were not able to return to a previous occupation.

Conclusion

Open fractures of the lower leg grade IIIC by Gustilo, mostly caused by traffic accidents, under the force of high-intensity, involve the lesions of the main blood vessels, a great damage to the soft tissues of the lower leg and periosteum removing of the injured bone in the fracture segment. Due to the injuries of main blood vessels, the vitality of the extremities is threatened. After primary wound treatment of the open fracture and stable external fixation of the fracture, it is necessary to do the reconstruction of main blood vessels, and thus enable revascularization of the part of the lower leg below the injury site. Neurovascular structures after reconstruction, are necessary to be covered by the vital soft tissue structures. The long-term goal of treatment of open lower leg fractures with injury of the main blood vessels is to allow patients to return to usual daily activities and professional work.

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CASE REPORT

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Churg-Strauss vasculitis in patient who received montelukast

Churg-Strauss vaskulitis kod bolesnice lečene montelukastom

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Abstract

Introduction. Montelukast is a selective leukotriene receptor antagonist. One of side effects of this drug class is the Churg-Strauss syndrome (CSS). There is still no reliable evidence whether the expression of this syndrome could be masked by high doses of corticosteroids and become expressed by termination of corticosteroid use, or whether it could be a consequence of leukotriene receptor antagonists use. **Case report.** Female patient, aged 49 years, was hospitalized with symptoms of fever, dyspnea, cough and increased sputum production with occasional hemoptysis. She was treated for asthma during the previous year. Leukocyte differential formula registered 44% of eosinophils. IgE value was extremely elevated, with value measured to 580 kU/L and eosinophile cation protein value was 15.1 μg/L. Computed tomography of the chest described changes in the form of ground glass located in all lobes of the right lung and in the upper lobe of the left lung. Computed tomography of paranasal sinuses described changes that could resemble to polyposis, chronic sinusitis, and possible granulomatosis. Mononeuritis of peroneal nerve of the right leg was proven by electromyographic examination. Bone marrow biopsy indicated hypercellularity with domination of eosinophilic granulocytes (30%). Five out of six criteria were noted in patient's clinical presentation, after which the diagnosis of CSS was set. The patient began treatment with high doses of corticosteroids while montelukast was discontinued which resulted in disease remission. **Conclusion.** Although there is no evidence that leukotriene modifiers cause the CSS in all patients with asthma, in case of frequent exacerbations with the appearance of pulmonary infiltrates, eosinophilia and paranasal sinus abnormalities make one think of this form of vasculitis.

Key words:
churg-strauss syndrome; leukotriene antagonists; diagnosis; drug therapy; treatment outcome; asthma.

Apstrakt

Uvod. Montelukast je selektivni antagonista leukotrienskih receptora. Jedno od neželjenih dejstava ove klase lekova je Churg-Strauss sindrom (CSS). Još uvek nema pouzdanih dokaza da li ekspresija ovog sindroma može biti maskirana visokim dozama kortikosteroida i postati manifestna sa prestankom primene kortikosteroida ili bi mogla da bude neposredna posledica primene antagonista leukotrienskih receptora. **Prikaz bolesnika.** Bolesnica, stara 49 godina, hospitalizovana je sa simptomima groznice, dispneje, kašlja i povećane produkcije sputuma sa povremenim hemoptizijama. Lečila se od astme u toku prethodnih godinu dana. Leukocitnom diferencijalnom formulom registrovano je 44% eozinofila. Vrednost IgE je bila izrazito povišena, 580 kU/L, kao i vrednost eozinofilnog katjonskog proteina – 15.1 mg/L. Kompjuterizovana tomografija grudnog koša ukazala je na promene po tipu "mlečnog stakla" u svim režnjevima desnog plućnog krila i u gornjem režnju levog plućnog krila. Kompjuterizovana tomografija paranazalnih sinusa ukazala je na promene koje mogu odgovarati polipozi, hroničnom sinusitisu i mogućoj granulomatozi. Mononeuritis peronealnog nerva desne noge je potvrđen elektromiografskim pregledom. Biopsija kostne srži je ukazala na hipercelularnost sa dominacijom eozinofilnih granulocita (30%). Kod bolesnika je na osnovu pet pozitivnih od šest kriterijuma potvrđeno postojanje CSS. Terapija montelukastom je prekinuta i započet je tretman visokim dozama kortikosteroida što je rezultiralo povlačenjem znakova i simptoma bolesti. **Zaključak.** Iako ne postoje dokazi da modifikatori leukotriena izazivaju CSS kod svih bolesnika sa astmom, u slučaju čestih pogoršanja sa pojavom plućnih infiltrata, eozinofilije i abnormalnosti paranazalnih šupljina treba imati na umu i ovu formu vaskulitisa.

Ključne reči:
angiitis, alergijski, granulomatozni; leukotrieni, antagonisti; dijagnoza; lečenje lekovima; lečenje, ishod; astma.

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Introduction

Montelukast is a selective leukotriene receptor antagonist which became available in 1998. Specifically, it binds to and blocks cysteinyl-leukotriene type 1 receptors which are located in the airways and involved in inflammation in patients with bronchial asthma and allergic rhinitis.

One of side effects of this drug class is the Churg-Strauss syndrome (CSS). There is still no reliable evidence whether the expression of this syndrome could be masked by high doses of corticosteroids and become expressed by termination of corticosteroid use, or whether it could be a consequence of leukotriene receptor antagonists. We presented the case of a patient who was hospitalized on three occasions over 3 months due to asthma exacerbations. During these hospitalizations she was treated with systemic corticosteroids, while in regular therapy she was taking high doses of inhaled corticosteroids (ICS) and leukotriene receptor antagonist in the past year.

Case report

Female patient, aged 49 years, came to pulmonologist's office for examination with symptoms of fever (38°C), dyspnea, cough and increased sputum production with occasional hemoptysis. It was the third time in 3 months that she came to pulmonologist with the same symptoms, and she was hospitalized for the third time at the Clinic for Pulmonology, Clinical Center Kragujevac. The patient's first hospital stay was 3 months before, when she was diagnosed with asthma exacerbation and interstitial pneumonia. One month later, the patient was admitted to the hospital again with signs of acute respiratory failure and radiographically detected bilateral lung infiltrates. She was treated with antibiotics, corticosteroids and oxygen therapy until notable clinical and radiological improvement. Third hospitalization occurred 10 days after the second one with the identical symptoms.

She was treated for asthma during the previous year and her current therapy at that moment was: salmeterol/fluticasone propionate, 50/500 μ 2 times a day (BID), montelukast 10 mg once of day (QD), theophylline 250 mg BID, fenoterol/ipratropium bromide 0.05/0.021 mg/mL, as needed. She was former smoker with 10 pack/years.

Auscultatory findings of the lungs showed impaired breathing sound, prolonged expiratory flow, wheezing and inspiratory crackles bilaterally in basal areas. Results of laboratory analyses showed positive biohumoral inflammatory syndrome: Sedimentation rate – 30 (1st hour), White blood cell count (WBC) –10.6 x 10^9/L, C-reactive protein (CRP) – 182.6 mg/mL. Leukocyte differential formula registered 44% of eosinophils (absolute number of 4.4 x 10^9/L). Other hematology parameters were within normal ranges. Immunological analyses (immunoglobulines, C3, C4, antinuclear antibody – ANA, anti-neutrophil cytoplasmic antibody – ANCA) were within normal ranges, except for immunoglobulin E (IgE) value which was extremely elevated, with value measured to 580 kU/L (reference range: up to 113 kU/L) and eosinophile cation protein (ECP) value was 15.1 mcg/L (reference range: up to 11.3 mcg/L). The analysis of respiratory gases on admission to the hospital showed hypoxemia with normal CO_2 partial pressure (pO2–7.6 kPa; pCO2–5.9 kPa; oxygen saturation was 91%). Chest radiography showed accentuated interstitia bilaterally in basal areas with inhomogeneous opacities and infiltrates in the upper lobe of the left lung and along the right lateral wall (Figure 1). Pulmonary function tests on admission showed predominantly obstructive disorder of moderate/severe degree [FVC = 2.24 L (72.6%), FEV_1 = 0.90 L (34.2%), FEV_1/FVC = 40.17%]. Multislice computed tomography (MSCT) of the chest described changes in the form of ground glass located in all lobes of the right lung and in the upper lobe of the left lung (Figures 2 and 3).

Shortly afterwards, the patient developed a vesicular rash on nose.

Computed tomography (CT) of paranasal sinuses was also performed during the hospitalization. Changes were observed in the left maxillary and sphenoid sinuses that could resemble polyposis, chronic sinusitis, and possible granulomatosis.

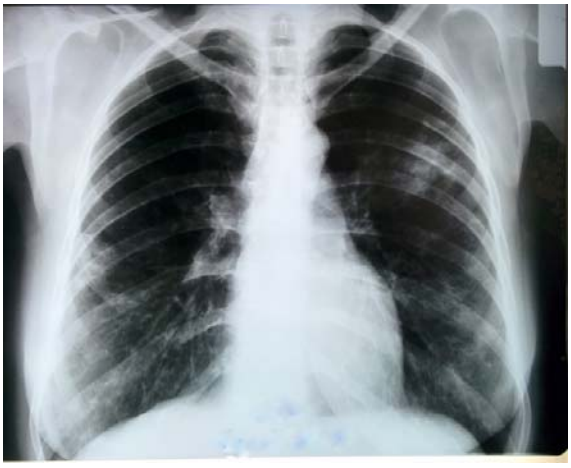

Fig. 1 – Chest radiography showing accentuated interstitia bilaterally in basal areas, inhomogeneous opacities and infiltrates in the upper lobe of the left lung and along the right lateral wall.

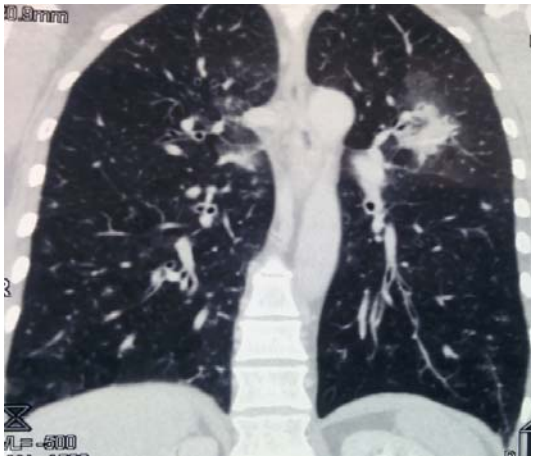

Fig. 2 – Multislice computed tomography (MSCT) of the chest showing changes in the form of ground glass located in all lobes of the right lung and an infiltrate in the upper lobe of the left lung.

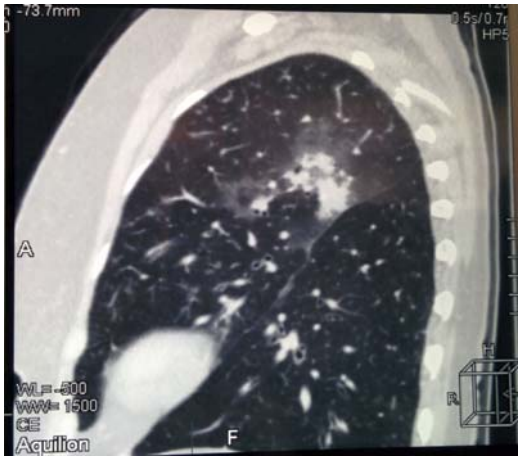

Fig. 3 – Multislice computed tomography (MSCT) of the chest, lateral view, showing changes in the form of ground glass and an infiltrate in the upper lobe of the left lung.

Because of the small pericardial effusion which was noted on MSCT of the chest, the patient underwent echocardiographic examination and the finding was normal. After consultation with the neurologist, the patient underwent electromyographic examination where neurogenic lesions in the tested muscles were found with findings characteristic of mononeuritis of peroneal nerve of the right leg.

Bone marrow biopsy was performed and pathohystological finding indicated hypercellularity, with domination of eosinophilic granulocytes (30%).

According to the results of all examinations which were performed, the diagnosis of the CSS was set, and the patient began treatment with high doses of corticosteroids, while montelukast was discontinued from further asthma treatment. Soon after the systemic corticosteroid therapy was introduced, regression of all signs and symptoms, and radiographic changes were notable (Figure 4). During the following year, she was weaned off oral corticosteroids and now she has intermittent asthma that is controlled by inhaled corticosteroids/long-acting beta-2-agonists ICS/LABA combination (salmeterol/fluticasone 50/500 μ) twice a day therapy.

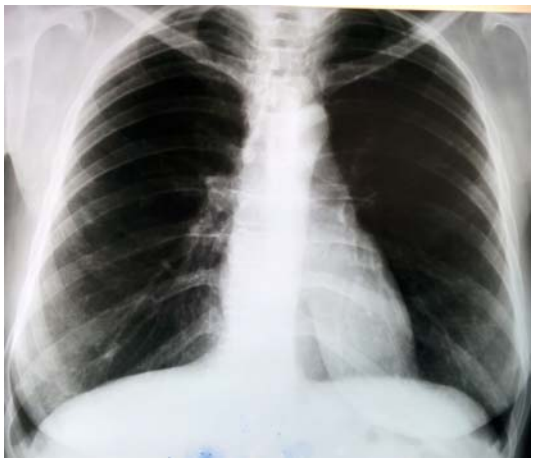

Fig. 4 – Chest radiography on patient's discharge from hospital treatment, showing complete regression of all previously described changes.

Discussion

The Churg-Strauss syndrome is a systemic form of vasculitis associated with the presence of positive ANCA antibodies. The syndrome is characterized by asthma and strictly uniform clinical presentation, whose main features are fever, eosinophilia, heart failure, renal impairment and peripheral neuropathy. The first case of CSS was described in 1951.

According to the classification made by the American College of Rheumatology, this syndrome is characterized by the following six criteria: eosinophilia, asthma, neuropathy, lung infiltration, paranasal sinus abnormalities and accumulation of eosinophiles in tissues [1].

Natural course of the disease usually evolves through three stages. Prodromal phase is also referred to as allergic phase and is characterized by asthma and rhino-sinusitis. Eosinophilic phase is manifested by the presence of peripheral eosinophilia and the involvement of internal organs (lungs, heart and/or gastrointestinal tract). Active form of the disease is characterized by peripheral eosinophilia > 10% or 1500 cells/μ. Cytotoxic proteins released by activated eosinophils could play a role in the development of the CSS. Chest X-ray is usually registering peripheral, uneven and migrating shadows or infiltrates. The third (the last) phase is the vascular phase with clinical manifestations in the form of small vessel vasculitis. In this stage pulmonary hemorrhage and glomerulonephritis usually appear.

In patients with the CSS, indicators of poor prognosis include cardiac and gastrointestinal involvement. The study of Guillevin et al. [2], showed 39% of mortality due to myocardial disease, and most of these patients died during the acute phase of their illness. Corticosteroids are the first-line treatment for the CSS, and remission rates are 80% to 90% when applying this therapy.

Five out of six criteria were noted in the described patient's clinical presentation. There were no parameters that would indicate the organ damage such as impaired kidney function, damage to the heart muscle or the gastrointestinal tract.

In recent years, new articles have been published linking the emergence of the CSS with the implementation of therapy with leukotriene modifiers such as montelukast [3–7]. The authors suggested that vascular component of the CSS was suppressed with administration of corticosteroids and the add-on therapy of montelukast usually reduced doses of corticosteroids which exposed vasculitis.

However, several patients who were not in the midst of a steroid taper developed the Churg-Strauss disease after the administration of leukotriene-receptor antagonists. Villena et al. [8] described a patient who developed rash, eosinophilia, and bilateral pulmonary infiltrates 4 months after beginning montelukast treatment while taking inhaled corticosteroids and bronchodilators only. Solans et al. [9] described an asthmatic patient who had never received oral corticosteroids and developed the CSS 4 months after initiating montelukast treatment.

It is difficult to establish the exact incidence of the CSS in patients taking leukotriene-receptor antagonists, since the available literature data are rare and variable. Keogh [10] reported that the incidence of the CSS in general population is 1 to 4 cases per million. In patients with asthma it is 20–60 cases per million patient-years, which is similar to that seen in the population receiving leukotriene receptor antagonists. A literature review from Jamaleddine et al. [11], using Medical Literature Analysis and Retrieval System Online (MEDLINE) from February 1966 to October 2000, states that 22 case reports of patients receiving leukotriene receptor antagonists who developed the CSS were identified. On the other hand, Wechsler et al. [12] reported that within 6 months of zafirlukast being made available on the market, 8 patients who received this agent for moderate to severe asthma treatment, developed the CSS. All of the patients had discontinued systemic corticosteroid use within 3 months of presentation and all developed the syndrome within 4 months of zafirlukast initiation.

Even though relationship between the CSS and montelukast treatment still remains unclear, if this case is analyzed through Naranjo algorithm[13] in order to determinate the likelihood of whether this suspected adverse drug reaction (ADR) is actually due to the drug that was administered rather than the result of other factors, the scoring result of 4 rates it as "possible ADR". Probability is assigned via score termed definite (≥ 9), probable (5-8), possible (1-4) or doubtful (0).

Regardless of this estimated probability, according to most authors [3–12], the occurrence of the CSS in the asthmatic patients receiving leukotriene receptor antagonists appears to be related to unmasking of an underlying vasculitic syndrome that is treated with corticosteroids and montelukast does not appear to directly cause the syndrome in these patients.

Conclusion

This female patient was treated with high doses of inhaled corticosteroids and leukotriene modifiers when symptoms of the CSS appeared. After the diagnosis was set, the patient was treated with high doses of corticosteroids and montelukast was discontinued. This therapy resulted in notable regression of signs and symptoms of the CSS as well as radiographic changes. In the following year, she was weaned off oral corticosteroids and now her asthma is characterized as intermittent and controlled by ICS/LABA, with no signs or CSS symptoms relapse.

In all patients with asthma and frequent exacerbations with the appearance of pulmonary infiltrates, eosinophilia and paranasal sinus abnormalities, this form of vasculitis undoubtly should be taken into consideration.

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

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

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

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

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

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

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

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Literatura

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

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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