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ORIGINAL ARTICLES / ORIGINALNI RADOVI

The International Migrants Day, and the International Day of Human Solidarity which are celebrated around the world on December 18 and 20, respectively, this year will be marked by a large migrant crisis, which has once again put to the test human solidarity and humanity. Serbia, as one of the countries through which the past few months passed and still runs a huge number of migrants, has been doing everything to provide them with the necessary assistance, mainly food, medicine, clothes, shoes and accommodations. The Government of the Republic of Serbia and its citizens showed once again how to help a man in trouble.

Međunarodni dan migranata i Međunarodni dan ljudske solidarnosti koji se obeležavaju širom sveta 18, odnosno 20. decembra, ove godine biće u znaku velike migrantske krize koja je još jednom stavila na probu ljudsku solidarnost i humanost. Srbija, kao jedna od zemalja kroz koju je proteklih meseci prošao i još uvek prolazi ogroman broj migranata, čini sve da im pruži neophodnu pomoć, u prvom redu hranu, lekove, odeću, obuću i smeštaj. Vlada Republike Srbije i njeni građani još jednom su delom pokazali kako treba pomoći čoveku u nevolji.

Poštovani autori, urednici, recenzenti i čitaoci Vojnosanitetskog pregleda,
Uz zahvalnost na saradnji i podršci u protekloj godini želim vam sve najbolje u nastupajućoj 2016!

SREĆNA NOVA GODINA GODINA I BOŽIĆNI PRAZNICI!

Srdačno,
prof. dr Silva Dobrić
glavni i odgovorni urednik

Dear authors, editors, peer reviewers and readers of the Vojnosanitetski Pregled,
I thank you for your cooperation and support in the last year and wish you all the best in the coming 2016!

MERRY CHRISTMAS AND A HAPPY NEW YEAR!

Cordially,
Prof. Silva Dobrić
Editor-in-Chief

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Poziv za reklamiranje u 2016. godini

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

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

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2016. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
2.	Oglas u c/b tehnici A4 formata za celu godinu (11-12 brojeva)	200 000,00 dinara
3.	Oglas u boji A4 formata za jedan broj	35 000,00 dinara
4.	Oglas u boji A4 formata za celu godinu (11-12 brojeva)	330 000,00 dinara
5.	Oglas u boji na koricama K3 za jedan broj	50 000,00 dinara
6.	Oglas u boji na koricama K3 za celu godinu (11-12 brojeva)	455 000,00 dinara
7.	Oglas u boji na koricama K2 i K4 za jedan broj	55 000,00 dinara
8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

Za sva obaveštenja, uputstva i ponude obratiti se redakciji časopisa „Vojnosanitetski pregled“. Sredstva se uplaćuju na žiro račun kod Uprave javnih plaćanja u Beogradu broj: 840-941621-02 **VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om) na adresu: Vojnosanitetski pregled, Crnotravska 17, 11000 Beograd; tel/faks: 011 2669 689, e-mail: vsp@vma.mod.gov.rs

ORIGINAL ARTICLES

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Introduction to verification of the GOHAI instrument for measuring the oral health-related quality of life in patients with dentures using the Serbian preliminary version – A pilot study

Uvod u verifikaciju instrumenta GOHAI za merenje oralnozdravstvenog kvaliteta života osoba sa zubnim nadoknadama primenom preliminarne srpske verzije – pilot studija

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Abstract

Background/Aim. Quality of life related to health should be seen as a multidimensional concept that, in addition to the physical symptoms associated with a disease and treatment, should include physical, psychological and social functioning of a person. The primary objective of this study was to use the Serbian preliminary version of the Geriatric Oral Health Assessment Index (GOHAI) questionnaire in oder to examine the consistency, reliability and stability, as well as an introduction to the verification tool. **Methods.** The GOHAI questionnaire with 6-level Likert scale, translated into Serbian, including the relevant oral health characteristics (oral hygiene, required dentures, number of teeth extracted), was filled by five specialists in prosthodontics for 30 randomly selected respondents, before and after the dental prosthetic treatment. Subsequently, in order to measure the reliability of the questionnaire, 27 patients were re-interviewed. **Results.** The value of Cronbach's Alpha Coefficient (Crα) before the treatment was 0.878, and after the treatment it was 0.788 confirming the internal consistency and stability of the questionnaire. The validity of discriminatory properties of the GOHAI was confirmed by the Spearman's correlation coefficient (r), which was highly significantly associated with oral health characteristics, confirming the high reliability of the measurement. The results of test-retest analysis measured by the individual Pearson's correlation coefficient (r) were in the range of 0.34–0.97, and for the total score r was 0.927, while the Kappa coefficient was 0.63. The correlation analysis of the GOHAI score before the treatment showed that for 10 questions there was a statistically significant correlation of the score with the answers to the questions, and for 6 questions Spearman's r was ≥ 0.7. After the treatment a highly significant correlation of the GOHAI was shown with the answers to 10 questions, while for 5 questions the Spearman's r was > 0.6. The GOHAI average score before the treatment was 19.44 ± 11.12, and after the treatment 2.77 ± 3.83, where the lower value indicates better quality of life. **Conclusion.** The results of this pilot study confirm internal consistency and stability of the Serbian preliminary version of the GOHAI questionnaire and the causal relation between the quality of life and the characteristics of oral health of the patients with dentures. Accordingly, instrument verification is recommended.

Key words:
dentures; aged; questionnaires; quality of life; serbia.

Apstrakt

Uvod/Cilj. Kvalitet života u vezi sa zdravljem treba posmatrati kao multidimenzioni koncept koji, pored fizičkih simptoma vezanih za bolest i lečenje, treba da obuhvati i fizičko, psihičko i društveno funkcionisanje osobe. Primarni cilj studije bio je da se korišćenjem preliminarne srpske verzije upitnika *Geriatric Oral Health Assessment Index* (GOHAI) ispita konzistentnost, pouzdanost i stabilnost, kao uvod u verifikaciju instrumenta. **Metode.** GOHAI upitnik sa 6-stepenom Likertovom skalom, preveden na srpski jezik, koji je uključivao i oralnozdravstvene karakteristike (oralna higijena, potrebne zubne nadoknade, broj izvađenih zuba), popunjavan je od strane petoro specijalista stomatološke protetike za 30 slučajno izabranih ispitanika, pre i posle stomatoprotetskog lečenja. Naknadno, radi merenja pouzdanosti upitnika, ponovo je intervjuisano 27 ispitanika. **Rezultati.** Vrednost *Cronbach's Alpha Coefficient* (Crα pre lečenja bila je 0,878, a posle lečenja 0,788, čime su potvrđene interna konzistentnost i stabilnost upitnika. Valjanost diskriminatornog

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svojstva GOHAI potvrđena je vrednostima Spearmanovog koeficijenta korelacije (r), koji je značajan kada su u pitanju oralnozdravstvene karakteristike, čime je potvrđena valjanost merenja. Rezultati test-retest analize merene pojedinačnim Pearsonovim koeficijentom korelacije (r) bili su u rasponu 0,34–0,97, a za ukupan skor r je bio 0,927, dok je Kappa koeficijent bio 0,63. Korelaciona analiza GOHAI pre lečenja ukazala je da je za 10 pitanja postojala značajna povezanost skora sa odgovorima na pitanja, a za 6 Spearmanov r bio je ≥ 0,7. Posle lečenja utvrđena je značajna veza GOHAI sa odgovorima na 10 pitanja, a za 5 Spearmanov r bio je > 0.6. Prosek GOHAI skora pre lečenja bio je 19,44 ± 11,12, a posle lečenja 2,77 ± 3,83, gde manja vrednost ukazuje na bolji kvalitet života. **Zaključak.** Rezultati ovog pilot istraživanja potvrdili su internu konzistentnost i stabilnost preliminarne srpske verzije GOHAI upitnika, kao i kauzalnu vezu kvaliteta života sa karakteristikama oralnog zdravlja ispitanika sa zubnim nadoknadama, te se može preporučiti sprovođenje verifikacije instrumenta.

Ključne reči:
proteze; stare osobe; upitnici; kvalitet života; srbija.

Introduction

In medical and health researches the concept of quality of life is the concept that spans the areas related to physical, functional, psychological and social health of a patient. The World Health Organisation Quality of life (WHOQOL) group gave the definition that "Quality of life is an individual's perception of his/her position in life, within the culture and value systems in which they live and in relation to the objectives, expectations, standards and concerns. It is a broad concept affected by a person's physical health, his/her mental state, level of independence, social relationships, and relations to the most important events in the environment" [1]. All the later definitions and researches of quality of life related to health, indicate that it should be seen as a multidimensional concept, which, in addition to physical symptoms related to the disease and treatment, should also include physical, psychological and social functioning of a person [2].

Although the objective dimension of health is extremely important in determining the health condition of individuals, subjective assessment and personal expectations transform the objective situation into the perceived quality of life. The reintegration of the patient to normal life is a reorganization of disturbed or lost functions of an individual (physical, psychological and social) into a harmonious whole, that is, a state of adaptation to good life after a disability due to illness or injury. Measuring quality of life in health-related entities should cover each objective and a subjective component (symptom status, social roles), which means that it should involve the measurement of all these functions. Physical and emotional function, considered together, constitute health related quality of life and social function is a very important aspect of the overall quality of life of people [3, 4].

Oral illnesses are progressive and cumulative and affect the quality of life of patients, especially the elderly. The loss of one or more teeth (partial edentulism), or all teeth (total edentulism) due to illness or injury represents a traumatic experience and a degree of disability. With aging, even when a person really cares about his oral health, there is a gradual tooth loss [5].

Longer life expectancy and preservation of teeth due to timely dental treatment, lead to the fact that more patients at the oldest age require dental prosthetic care and tooth loss and their restoration is one of the major dental problems faced by the elderly [6]. According to the data of the Gerontology Center of Public Health in Zagreb on the use of medical aids of persons aged between 65 and 94, dentures (fixed and mobile) were in the second place, just behind the glasses [7]. For these reason it is necessary to pay special attention to measuring oral health-related quality of life in patients with dentures [8].

During examination of the impact of oral health on quality of life, a large number of instruments was built in order to find adequate and reliable questionnaire that can be used in everyday practice. The literature states that among the most appropriate indices for testing the quality of life of the elderly population are the Geriatric Oral Health Assessment Index (GOHAI), the Oral Health Impact Profile-49 (OHIP-49) and its shortened version of the OHIP-14 [9].

The GOHAI [2] has been adapted for general use, translated and tested on the samples of adults in many countries, independently [6, 10–15] or compared to other indices [16, 17] .

In our environment, researches in this area are rare and more recent. In the conclusion of a study conducted in order to translate the index OHIP-14 into Serbian language, assessing its validity in practice, it is stated that "The Serbian version of shortened form of OHIP index can be used in dental prosthetic work with patients of older age. The information obtained from the questionnaire can be used as an aid in setting the indication, determining the need for treatment, assessing the state of oral health and the conducted prosthetic treatment. It is also desirable to translate and use another index and check its applicability in practice. In this way it will be possible to make a comparison with the OHIP index and perhaps give way to a specific index for Serbian speaking area, which will suit our mentality and cultural characteristics" [18].

Linguistic and cultural norms, as well as the health care system in Serbia are different from other countries, which imposes the need to confirm the validity of the GOHAI questionnaire in our country and to carry out the verification, before it's recommendation for mass use [14, 18].

Methods

The Ethics Committee of the Faculty of Dental Medicine, University of Belgrade, gave approval no. 36/18 to conduct the survey. The study was conducted on a group of

30 patients, randomly selected from the patients who contacted the Department of Prosthodontics at the Military Medical Center of New Belgrade, in a period of 3 months. Five dentists took part in the pilot study, and their selection was random.

This pilot study was designed as a cross sectional study evaluation using a questionairre before and after the treatment. The instrument of the study was an individual questionnaire with questions relatied to sociodemographics of the respondents (gender, age and education level), and the oral health condition established by oral examination (oral hygiene, the number of extracted teeth and the dentures requirement). In addition, the questionnaire contained questions about oral health-related quality of life of the patients before the treatment and three months afterwards.

A specialist in prosthodontics, while examining the respondents, identified condition of oral health and the treatment requirements of the respondents and their sociodemographic status and entered the data in a predefined questionnaire. At the same time he interviewed the patients about the state of their oral health quality of life and marked one of the options for each question in the questionnaire. During the check-ups, three months after the completion of dental prosthetic treatment, the same dentist asked the same respondents the same questions about the quality of their lives and marked the answers in the questionnaire.

Subsequently, in order to measure the reliability of the questionnaire [19], two months after the check-ups, 27 patients were re-interviewed, and the questions were related to the current rating of the quality of their lives.

For testing oral health-related quality of life of patients the GOHAI was used. The GOHAI was consisted of 12 questions, and the answers to them were graded by a scale of Liket form (0 = never, 1 = almost never, 2 = occasionally, 3 = often, 4 = very often, 5 = always). The values of GOHAI score ranged from 0 to 60, where higher scores indicate more problems [9, 20, 21].

The questions were translated into Serbian language by a professional translator of English, in cooperation with the authors of the study, in accordance with the recommendations from the references [6, 22, 23] and adapted to our patients. In the Serbian version, the questions were: 1) Is there any kind of food you cannot eat or cannot eat the desired amount? 2) Do you have difficulty eating certain food, such as meat or a hard apple? 3) Do you have difficulty swallowing certain food? 4) Does the condition of your teeth and mouth prevent you from speaking as clearly as you wish? 5) Are you, due to a feeling of discomfort, unable to eat all kinds of food? 6) Do you avoid contact with other people because of the condition of your mouth and teeth? 7) Are you unhappy with the condition of your mouth and teeth? 8) Do you use any medications to reduce pain or discomfort caused by the condition of your teeth and mouth? 9) Are you worried about the problems with your mouth and teeth? 10) Do you feel uncomfortable or stressed because of the condition of your mouth and teeth? 11) Are you embarrassed to eat in front of others because of the condition of your mouth and teeth? 12) Are your teeth sensitive to hot, cold or sweet food?

Based on the collected data, the base of respondents was created in the standard software package SPSS for Windows version 17.0, USA, which was used for statistical analysis. Statistical analysis was primarily related to the establishment of stability and internal consistency of the questionnaire, as well as discriminatory reliability of the questionnaire scale before and after the dental prosthetic treatment. Test-retest statistical analysis of data for 27 subjects was conducted in accordance with recommendations from the reference [19, 24, 25].

In order to determine the influence of conducted dental prosthetic treatment on oral health-related quality of life in patients, we have calculated the average and standard deviation of the GOHAI scores and percentile representation of the value of responses to each question in the questionnaire before and after the treatment. In addition, Student's *t*-matched-test was used for determining the value of the difference in the responses to individual questions, as well as the GOHAI scores before and after the treatment.

Pearson's χ^2 test and the analysis of variance (ANOVA) were used to assess the relationship of sociodemographic (gender, age, education) and oral health indicators (oral hygiene, the need for dental prosthetic treatment, the number of extracted teeth) with the values of the GOHAI score.

Internal consistency was assessed by Cronbach's Alpha coefficient (Crα) and the discriminatory analysis of responses to individual questions before and after the treatment.

To test the validity of the discriminatory properties of the GOHAI score in relation to oral health (oral hygiene, the need for dentures and the number of extracted teeth) Spearman's Rank Correlation Coefficient (r) was used, and to determine the reliability of the questionnaire during the test-retest analyses Pearson's correlation coefficient and Kappa coefficient were used.

Results

Looking at the distribution of frequency and structure of the respondents' answers to individual questions in the GOHAI questionnaires before the treatment, the prevailing response was occasionally-often, then never-almost never and in the end very often-always. After the treatment, the proportion of respondents who answered to the questions with never-almost-never significantly increased, the number of those who responded with occasionally-often significantly reduced, while the number of respondents who gave answers very often-always was negligible (Table 1).

The correlation analysis of the GOHAI score before the treatment showed that there was a statistically highly significant correlation of values with the answers for 10 questions to the questionnaire ($p < 0.01$), for one question the relationship was statistically significant ($p < 0.05$) and for one question the significance of relationship was not stated, and for 6 questions Spearman's r was ≥ 0.7 (Table 2).

Table 1

Frequency distribution and structure of the responses to individual questions in the Geriatric Oral Health Assessment Index (GOHAI) questionnaire before and after the dental prosthetic intervention

Questions	Never (0)		Almost never (1)		Occasionaly (2)		Often (3)		Very often (4)		Always (5)	
Physical difficulties	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1. Is there any kind of food you cannot eat or cannot eat the desired amount?	5 (16.7)	23 (76.7)	3 (10.0)	5 (16.7)	9 (30.0)		9 (30.0)		1 (3.3)		3 (10.0)	2 (6.7)
2. Do you have difficulty eating ceratin food such as meat or a hard apple?	6 (20.0)	22 (73.3)	4 (13.3)	8 (26.7)	11 (36.7)		3 (10)		6 (20.0)			
3. Do you have difficulty swallowing certain food?	14 (46.7)	25 (83.3)	7 (23.3)	4 (13.3)	3 (10.0)		6 (20.0)	1 (3.3)				
4. Does the condition of your teeth and mouth prevent you from speaking as clearly as you wish?	9 (30.0)	25 (83.3)	8 (26.7)	5 (16.7)	9 (30.0)		1 (3.3)		3 (10.0)			
Pain/Discomfort												
5. Are you, due to the feeling of discomfort unable to eat all kinds of food?	9 (30.0)	27 (90.0)	5 (16.7)	3 (10.0)	9 (30.0)		7 (23.3)					
8. Do you use any medicatio9ns to reduce pain or discomfort, caused by the condition of your teeth and mouth?	12 (40.0)	24 (80.0)	6 (20.0)	5 (16.7)	8 (26.7)	1 (3.3)	2 (6.7)		2 (6.7)			
12. Are your teeth sensitive to cold, hot, or sweet food?	10 (33.3)	21 (70.0)	3 (10.0)	7 (23.3)	11 (36.7)	2 (6.7)		4 (13.3)				
Physical problems												
6. Do you avoid contact with other people because of the condition of your teeth and mouth?	17 (56.7)	26 (86.7)	6 (20.0)	4 (13.3)	5 (16.7)		1 (3.3)		1 (3.3)			
7. Are you unhappy with the condition of your mouth and teeth?	4 (13.3)	23 (76.7)	3 (10.0)	5 (16.7)	8 (26.7)	2 (6.7)	7 (23.3)		5 (16.7)		3 (10.0)	
9. Are you unhappy because of the problems with your mouth, teeth?	7 (23.3)	25 (83.3)	3 (10.0)	3 (10.0)	10 (33.3)	2 (6.7)	2 (6.7)		4 (13.3)		4 (13.3)	
10. Do you feel uncomfortable or stressed because of the condition of your mouth and teeth?	9 (30.0)	28 (93.3)	6 (20.0)	2 (6.7)	7 (23.3)		2 (6.7)		3 (10.9)		3 (10.0)	
11. Are you embarrased to eat in front of others because of the Condition of your mouth and teeth?	14 (46.7)	26 (86.7)	6 (20.0)	4 (13.3)	3 (10.0)		2 (6.7)		1 (3.3)		4 (13.3)	

Data in the table are presented as number (n) and percentage (%) of the patients.

Table 2

Correlation analysis in the Geriatric Oral Health Assessment Index (GOHAI) score before and after the treatment

Questions	Spearman's correlation coefficients (n = 30)	
	before the tretmant	after the tretmant
Is there any kind of food you cannot eat or cannot eat the desired amount?	0.300	0.642**
Do you have difficulty eating certain food, such as meat or a hard apple?	0.405*	0.627**
Do you have difficulty swallowing certain food?	0.588**	0.424*
Does the condition of your teeth and mouth prevent you from speaking as clearly, as you wish?	0.562**	0.627**
Are you, due to the feeling of discomfort, unable to eat all kinds of food?	0.700**	0.353
Do you avoid contact with other people because of the condition of your mouth and teeth?	0.751**	0.340*
Are you unhappy with the condition of your mouth and teeth?	0.633**	0.736**
Do you use any medications to reduce pain or discomfort caused by the condition of your teeth and mouth?	0.763**	0.389*
Are you worried about the problems with your mouth and teeth?	0.771**	0.470**
Do you feel uncomfortable or stressed because of the condition of your mouth and teeth?	0.800**	0.384*
Are you embarrassed to eat in front of others because of the condition of your mouth and teeth?	0.840**	0.517**
Are your teeth sensitive to hot, cold or sweet food?	0.487**	0.735**

***$p < 0.05$; **$p < 0.01$.**

When the same analysis was conducted after the treatment, it indicated the presence of highly significant relationship of the score with the answers to seven questions ($p < 0.01$), the significant relationship with the answers to 4 questions ($p < 0.05$), and for one question the significance of relationship was not stated, and for 5 questions Spearman's r was > 0.6 (Table 2). Consequently, the values of responses to individual questions in the GOHAI questionnairy decreased (Figure 1), and the values of Student's t-test of matched pairs differences was highly significant (Table 3).

Specialists in dental prosthetics, during the check-ups, found that in 12 (40.0%) patients oral hygiene was satisfactory, while in the remaining 18 (60.0%) that was not the case. It was also found that 12 (40.0%) of respondents needed fixed dentures, 9 (30%) needed mobile dentures, while 9 (30%) of the respondents needed both types of work. In the group of patients there were 3 (10.0%) edentulous, 16 (53.3%) respondents had 10 teeth extracted, while 11 (36.7%) patients had 11 to 25 teeth extracted (Table 4).

The values of Sperman`s correlation coefficient of the GOHAI score before the treatment and the parameters of oral health indicated that GOHAI score was associated ($p = 0.01$) with oral hygiene ($r = 0.468$) and with the number of extracted teeth ($r = 0.496$), and that it was also associated ($p = 0.05$)

Table 3

Student's matched questions and answers test in the Geriatric Oral Health Assessment Index (GOHAI) score before and after the prosthetic intervention

Questions	Difference between the GOHAI score before and after the treatment		
	$\bar{x} \pm SD$	t	p
Is there any kind of food you cannot eat or cannot eat the desired amount?	1.733 ± 1.999	4.750	0.000
Do you have difficulty eating certain food, such as meat or hard apple?	1.700 ± 1.343	6.934	0.000
Do you have difficulty swallowing certain food?	0.800 ± 1.375	3.188	0.003
Does the condition of your teeth and mouth prevent you from speaking as clearly, as you wish?	1.200 ± 1.324	4.966	0.000
Are you, due to the feeling of discomfort, unable to eat all kinds of food?	1.367 ± .217	6.150	0.000
Do you avoid contact with other people because of the condition of your mouth and teeth?	0.633 ± 1.129	3.072	0.005
Are you unhappy with the condition of your mouth and teeth?	2.200 ± 1.627	7.405	0.000
Do you use any medications to reduce pain or discomfort caused by the condition of your teeth and mouth?	0.967 ± 1.217	4.350	0.000
Are you worried about the problems with your mouth and teeth?	1.933 ± 1.701	6.227	0.000
Do you feel uncomfortable or stressed because of the condition of your mouth and teeth?	1.700 ± 1.664	5.596	0.000
Are you embarrassed to eat in front of others because of the condition of your mouth and teeth?	1.267 ± 1.837	3.777	0.001
Are your teeth sensitive to hot, cold or sweet food?	1.200 ± 1.584	4.148	0.000
GOHAI score	16.700 ± 12.490	7.318	0.000

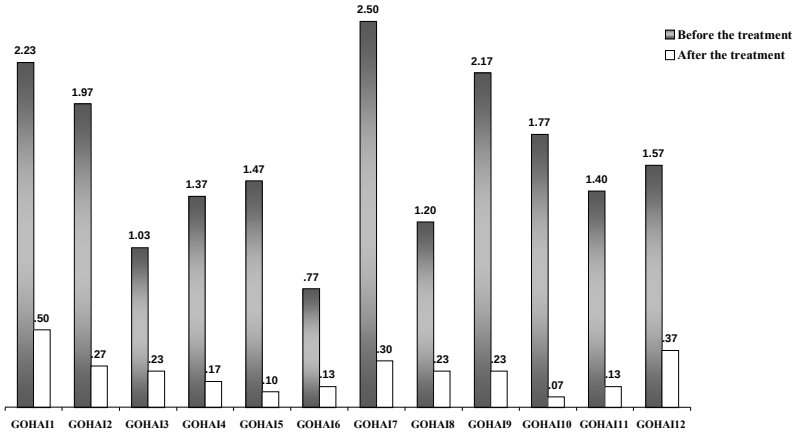

Fig. 1 – The values of the Geriatric Oral Health Assessement Index (GOHAI) score and responses to individual questions before and after the treatment (test-retest analysis).

Table 4

Correlation of the Geriatric Oral Health Assessment Index (GOHAI) score before the treatment with quality of oral hygiene required dentures, and extracted teeth

Variables	Patient n (%)	Spearman's rank correlation coefficient (r)
Oral hygiene		
satisfactory	12 (40)	0.468**
unsatisfactory	18 (60)	
Required dentures		
fixed	12 (40)	
mobile	9 (30)	0.392*
both types of dentures	9 (30)	
Extracted teeth		
edentulous	3 (10.0)	
up to 10	16 (53.3)	0.496**
11–25	11 (36.7)	

***p* < 0.05; ****p* < 0.01.**

with the type of required dentures ($r = 0.392$), confirming the validity of discriminatory properties of the GOHAI score in relation to oral health of the respondents (Table 4).

The sample included 30 respondents of various education, 13 (43.3%) men and 17 (56.7%) women, median age 67.87 ± 11.29. Without primary education was 1 (3.3%), with primary education 4 (13.3%), with secondary education or college 13 (43.3%), with university education 12 (40.0%) respondents. Observing the dependence of the GOHAI scores on sociodemographic characteristics of respondents, it appears that it was not statistically significant. The values of the applied tests were: in relation to gender, $\chi^2 = 25.249$, $p = 0.123$; in relation to age, $F = 1.354$, $p = 0.329$, and in relation to education level, $\chi^2 = 74.583$, $p = 0.9097$.

The value of the GOHAI score before the treatment was 19.44 ± 11.12, the range 4.00–44.00, while it decreased to 2.77 ± 3.83 in the interval 0.00–14.00 after the prosthodontic treatment.

When it comes to values of the GOHAI score before and after the treatment, Student's *t*-test of matched pairs showed that the average differences value was 16.700 ± 12.49 with a high significance at $t = 5.596$, $p = 0.000$ (Table 3).

Coefficient values before the treatment ($Cr\alpha = 0.878$) and after the treatment ($Cr\alpha = 0.788$) indicate that the applied GOHAI questionnaire is internally consistent and stable, according to quality criteria which were proposed for measuring the characteristics of health condition.

Discriminatory analysis, which grouped the values of respondents' answers to questions in GOHAI questionnaire, provided the result of classification before and after the treatment of 90%, thus confirming the reliability and value of usability of the applied scale.

Analysis of the reliability and validity of the GOHAI questionnaire (test-retest) was carried out by re-interviewing 27 subjects included in the sample two months after the control examination (test-retest reliability), and the correlation coefficients of individual samples were in the range of 0.64 to 0.97, while for the entire group was $r = 0.927$, and Kappa coefficient = 0.63.

Discussion

During the research of the basic version of the GOHAI questionnaire, the consistency and stability of the questionnaire was at $Cr\alpha = 0.79$ in a sample of 87 patients [20]. In the se-

ries of studies which were later pursued throughout the world, with translation and cultural adaptation of the GOHAI questionairre to different languages [14], the consistency and stability of the questionnaire was at similar levels in India (Crα = 0.88) in a sample of 500 respondents [26], France (Crα = 0.86) in a sample of 260 respondents [14], Germany (Crα = 0.92) [12], Sweden (Crα = 0.86) in a sample of 153 respondents [27], Lebanon (Crα = 0.887) [28], Malaysia (Crα = 0.79) [29] Latin America (Crα = 0.83) in a sample of 280 respondents [30] and in Arab countries (Crα = 0.83) [31] and Japan (Crα = 0.83) [21].

Our pilot research, regardless the fact that the sample size was small, established the value of Crα coefficient = 0.878 before the treatment and Crα = 0.788 after the treatment, indicating that the applied GOHAI questionnaire is internally consistent and stable and completely in accordance with the quality criteria that have been proposed for measuring the characteristics of psychometric questionnaires and surveys related to the health condition of respondents [15, 25].

When considering the question of reliability of the GOHAI questionnaire, and test-retest analysis, Pearson's correlation coefficient in the study of Hassel et al. [12] was in the range 0.36–0.98, for individual subjects, and for the whole group r = 0.89. In the test-retest analysis of Tubert-Jeannin et al. [32], which involved 32 subjects, individual correlation coefficients ranged from 0.51 to 0.87, while for the whole group r = 0.87. The same analysis of Mathur et. al. [8] was conducted on 29 subjects and individual correlation coefficients ranged from 0.748 to 0.946. Hägglin et al. [27] conducted a test-retest analysis in 47 patients and the correlation coefficient of GOHAI for the whole group was r = 0.64. The size of our group of patients in which the test-retest analysis was conducted, and the analysis of the results are in accordance with the mentioned researches.

Results of Tubert-Jeannin et al. [32], when it comes to the application of the GOHAI questionnaire, indicated that the internal correlation coefficients range was in the interval 0.40–0.78, while the values of the same parameters in the study Deshmukh and Radke [26] were in the range 0.50–0.83. In the research of Daradkeh and Khader [31] the internal correlation coefficients were in the range 0.53–0.77, for all questions except for the question 5, for which the correlation coefficient was 0.27, while in the researches of El Osta et. al. [29], the coefficients of internal correlation varied from 0.41 (question no. 12) – 0.79 (question no.10). Hassel et. al. [12] pointed out the internal correlation coefficients range 0.27–0.80. The coefficients of internal correlations of almost all other researches were in this framework, and it is necessary to note that all the researches were conducted only once [33–36].

Internal correlation coefficient values before the treatment in our study were in the range 0.40–0.84, with higher levels of statistical significance, except for the question number 1, for which the internal correlation was 0.30, and the significance of the relation was not stated, and the connection for 10 questions was highly significant. The emphasis must be placed on the fact that the correlation coefficients for the six questions were greater than 0.6. When the same analysis was conducted after the treatment, the internal correlation coefficients were in the range 0.35–0.74, pointing to the existence of a highly significant relationship recently with answers to seven questions, the existence of a significant relationship in terms of three questions, and for one question the significance of relationship was not stated (question 5). Therefore, values of internal correlation coefficients of our pilot study indicate the internal consistency of the questionnaire and show that they are completely in accordance with similar studies in this area.

Conclusion

Based on the obtained results we can conclude that the Serbian preliminary version of the Geriatric Oral Health Assessment Index questionnaire used in the pilot project confirmed the consistency, stability, and validity of the questionnaire. Inroduction to verification the pilot study confirmed the appropriateness of including the parameters and assessment of the quality of life of patients with dentures, before and after the intervention, as a measure of the success of the performed prosthodontic treatment.

There is a need for verification of the GOHAI instrument before recommendation for its mass use.

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Sweat rate and fluid intake in young elite basketball players on the FIBA Europe U20 Championship

Stepen znojenja i unos tečnosti kod mladih elitnih košarkaša na Prvenstvu Evrope FIBA U20

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Abstract

Background/Aim. Previous investigations in many sports indicated that continued exercise, especially in hot environments, can cause high sweat rate and huge water and electrolyte losses, thus impairing the performance of athletes. Most these studies were conducted during training sessions, but rarely during an official competition. Therefore, the aim of our study was to determine pre- and post-competition hydration, fluid intake and sweat loss of young elite basketball players during the FIBA Europe U20 Championship. **Methods.** The study included 96 basketball male players, (19 ± 0.79 years) of eight national teams. Ambient temperature was 30 ± 2ºC, humidity 55 ± 4% and the mean playing time in game 18.8 ± 10.5 min. The following parameters related to hydration status were measured: fluid intake, urine output, sweat rate, percent of dehydration, urine parameters (specific gravity, color and osmolarity), body mass and body surface area. **Results.** We found that the mean fluid intake was 1.79 ± 0.8 L/h, sweat rate 2.7 ± 0.9 L/h, urine output 55 ± 61 mL and the percentage of dehydration 0.99 ± 0.7%. According to urine osmolarity more than 75% of players were dehydrated before the game and the process continued during the game. The difference in body mass (0.9 ± 0.7 kg) before and after the game was statistically significant. There were statistically significant correlations between the sweat rate and fluid intake, urine osmolarity, body mass loss, body surface area and percentage of dehydration. Fluid intake correlated with the percentage of dehydration, body mass loss, urine specific gravity and urine color. The sweat rate, which varied between the teams, was the highest for centers when this parameter was calculated on the effective time in game. **Conclusion.** Most of the athletes start competition dehydrated, fail to compensate sweat loss during the game and continue to be dehydrated, regardless what kind of drink was used. These results suggest that hydration strategies must be carefully taken into account, not only by the players, but also by the coaches and the team doctors.

Key words:
basketball; water-electrolyte balance; sweating; dehydration; europe.

Apstrakt

Uvod/Cilj. Prethodna istraživanja u mnogim sportovima ukazuju da kontinuirano vežbanje, posebno pri visokim temperaturama, može da prouzrokuje obilan stepen znojenja i gubitak vode i elektrolita, smanjujući na taj način sportsku sposobnost. Mnoge od ovih studija sprovedene su u toku treninga, ali retko za vreme nekog zvaničnog takmičenja. Cilj ove studije bio je da se odredi stepen hidracije pre i posle utakmice, unos tečnosti i gubitak tečnosti putem znojenja kod mladih košarkaša tokom šampionata Evrope za mlađe od 20 godina. **Metode.** Studija je sprovedena na 96 košarkaša, starosti 19 ± 0,79 iz osam nacionalnih timova. Ambijetalna temeratura iznosila je 30 ± 2ºC, vlažnost 55 ± 4%, a prosečno aktivno vreme u utakmici 18,8 ± 10,5 minuta. Praćeni su sledeći parametri hidracije: količina popijene tečnosti, količina izlučenog urina, stepen znojenja, procenat dehidracije, parametri urina (specifična težina, boja i osmolarnost), telesna masa i površina tela. **Rezultati.** Našli smo da je prosečni unos tečnosti iznosio 1,79 ± 0,8 L/h, stepen znojenja 2,7 ± 0,9 L/h, količina izlučene tečnosti 55 ± 61 mL, a procenat dehidracije 0,99 ± 0,7%. Na osnovu osmolarnosti urina, više od 70% igrača bilo je dehidrirano pre utakmice i taj proces se nastavio u toku utakmice. Razlika u telesnoj masi (0,9 ± 0,7 kg) pre i posle utakmice bila je statistički značajna. Postojale su statistički značajne korelacije između stepena znojenja i količine unete tečnosti, osmolarnosti urina, gubitka telesne mase, površine tela i stepena dehidracije. Količina unete tečnosti korelisala je sa stepenom dehidracije, gubitkom telesne mase, specifičnom težinom urina i bojom urina. Stepen znojenja, koji se razlikovao između timova, bio je najviši kod centara kada je izračunovan u odnosu na efektivno vreme u toku igre. **Zaključak.** Većina košarkaša započinje takmičenje dehidrirana, ne uspeva da kompenzuje gubitak znoja u toku utakmice i nastavlja da bude dehidrirana bez obzira na vrstu unete tečnosti. Ovi rezultati ukazuju da strategija hidracije mora biti pažljivo razmatrana, ne samo od strane igrača već i od trenera i timskih lekara.

Ključne reči:
košarka; voda-elektroliti, balans; znojenje; dehidracija; evropa.

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Introduction

Sweat rate can be influenced by few factors such as environmental conditions, choice of clothing and exercise intensity and also can be significantly different between individuals. Exercise can stimulate high sweat rates and large water and electrolyte losses during continued exercise, particularly in warm-hot weather [1].

Individual characteristics, such as body weight, genetic predisposition, heat acclimatization state, level of aerobic fitness, hydration status and metabolic efficiency may influence sweat rates for a certain activity [2–5]. Heat acclimatization enhances an individual to reach higher and more sustained sweating rates. Also, aerobic exercise training has a moderate effect on enhancing sweating rate responses [2].

Continuous intensive or strenuous exercise in a hot environment presents a bigger challenge to the body's homoeostatic mechanisms than any other factor. The volume of consumed fluids should be greater than the volume of sweat lost in order to make provision for the ongoing obligatory urine losses [6]. In team sports, a number of factors such as awareness about the loss of fluid, fluid availability, the ability to intake the liquid, palatability liquid, gastrointestinal comfort, awareness of the hypohydration problems, fear of weight gain, fear of urge to urinate, affects the internal mechanisms that regulate fluid intake [7].

In its position stand, the American College of Sports Medicine (ACSM) recognized that consumption of beverages containing electrolytes and carbohydrates can help to sustain fluid electrolyte balance and exercise performance. Rehydration beverage should contain certain level of sodium (at least 10–20 mmol/L), potassium and, also, certain amount of carbohydrates (30–60 g/L) [8]. The water alone is sufficient for rehydration when food contains a sufficient amount of electrolyte which is lost through sweat [6].

Specific individual recommendations for fluid intakes should be calculated based on sweat rates, sport dynamics, and individual tolerance. Casa et al. [9] suggested a general rehydration protocol for basketball players: 200 mL at quarter breaks, 400 mL at half time and 100 mL at one timeout/half.

Tournament conditions in many team sports involve a daily schedule of matches played over a specified time culminating in grand finale. Failure to replace sweat losses during and between matches could lead to the situation of chronic or progressive hypohydration at the same time when optimal performance becoms more critical [7].

Up to now, most studies focused on the problems of dehydration in sport games have been performed during a training period, but rarely during an official competition. Therefore, our study was designed to investigate pre and post-competition hydration, fluid intake and sweat loss of young elite basketball players during the FIBA Europe U20 Championship. All these results should help to optimize and encourage fluid replacement for athletes during tournaments and to provide teams with feedback about their fluid intake habits.

Methods

Subjects

A total of 96 basketball players, the members of 8 national teams were assessed during the official FIBA Europe U20 Championship. The players were informed in writing about the aims of this study and the procedures performed during the project. Each of them signed consent for participation in the study.

Measurements

We measured weight and body composition of all the players pre-game and post-game by a Tanita, Body Composition Analyzer BC-418MA. Their anthropometric characteristics are given in Table 1. During the pre-game measurements the participants were dressed in minimal clothing. For the post-game measurements all of them had identical dry clothes. After the pre-game weight players were given labeled bottles containing fluid and instructed to drink until the post-game weighting, in order to calculate total water consumed. Players were advised to continue with the normal routine of fluid intake and to drink only from their bottles. Before the match, we measured the empty bottles and bottles full of liquid. In addition, during the game, the bottles before filling of liquid and after refueling were measured. Only after the measurement basketball players were returned bottles to continue with drinking. In half time, sterile urine vessels were provided in every toilet in the dressing rooms in order to measure the volume of urine. Three mornings urine samples were collected from each player as well as an urine sample after the game.

Full statistic data on each game was provided by official organizer. Temperature and humidity were measured with a UPM wireless weather station (model: ws290c), BIOS Weather^{TM}, (model: 312BC-RX).

Table 1

Anthropometric characteristics of the basketball players (n = 96) involved in the study

Parameters	$\bar{x} \pm SD$	Range
Age (years)	19 ± 0.79	16–20
Weight (kg)	90.6 ± 12.4	62–144
Height (cm)	196.3 ± 8.18	177–214
Body mass index (kg/m^2)	23.3 ± 2.56	10.9–34.4
Body surface area (m^2)	2.27 ± 0.19	1.91–2.83
Body fat (%)	9.4 ± 3.8	3–27
Muscle mass (kg)	81.8 ± 9.53	57–110

Hydration status was evaluated by the following parameters: urine specific gravity (USG) for which we used reagent strips (Combur 10 Test, Roche) and a refractometer (Atago Pal – 10s). Cut off value was 1.02 [10]; urine color (UC) by using a scale of eight colors (10) (cut off value was 4[10]); urine osmolality (UO) with a laboratory osmometer – UriSedilabUMAT, cut off value was 700 mOsm[8]). Sweat rate (SwR) was calculated using the change in mass adjusted for fluid intake and urine production: SwR = (pre-exercise body weight – post-exercise body weight + fluid intake – urine volume)/exercise time in hours percentage of dehydration (DEH) during the practice was estimated as the net body mass loss (kg) during the practice divided by the pre-practice body mass: we also measured % DEH = (body mass loss/pre exercise body mass) × 100, fluid intake (FI) and urine output (UO).

Statistical analysis

All data were presented as the mean ± SD. Associations between variables were investigated using Pearsons correlation analyses. In order to define statistically significant differences among the groups we used one-way ANOVA test. Statistical significance was accepted at $p < 0.05$.

Results

Hydration status

Hydration status was evaluated before and after the game by USG, UO and UC parameters. The results are presented in Figure 1A. Pre-game evaluation of all the players showed that the mean USG was 1024 ± 0.6, mean UO 883 ± 229 mOsm and mean UC 5.67 ± 1.12. According to cut-off values given in the methods, 80%, 75%, and 95% of studied subjects, respectively, were hypohydrated before the game. When these urine parameters were evaluated after the game, dehydration increased further based on increased USG (1026 ± 6) and UC (5.97 ± 1.37) but not UO (852 ± 228 mOsm) and accordingly the percentages of dehydrated players increased to 85% and 95%, respectively. However, statistical analysis did not confirm that these pre-post differences in any of the examined parameters were statistically significant.

When hydration status was analyzed depending on the team, it can be seen (Figure 1B) that all the hydration parameters of the team 6 were statistically significantly lower compared to other teams, indicated that the players of this team had lowest dehydration.

Other parameters associated with hydration including significantly body mass loss (0.9 ± 0.7 kg), percentage of dehydration (0.99 ± 0.7%) and urine output (55 ± 61 mL) are shown in Table 2.

Sweat rate

The mean sweat rate (Table 2) was 2.7 ± 0.9 L/h (range 0.23 L/h – 5.54 L/h). Figure 2 illustrates the distribution of sweat rate among players based on an arbitrary division. The highest percentages of players had sweat rate between 2 L/h and 3 L/h. There was no statistically significant difference in the sweat rate between various positions of players in the team during the complete period of game (Figure 3A). How-

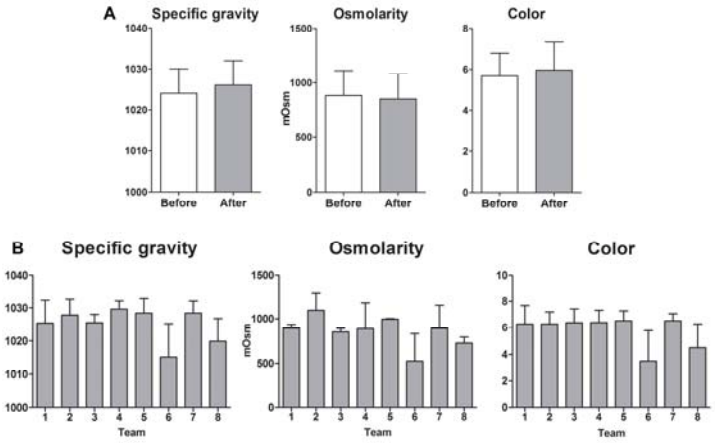

Fig. 1 – Urine parameters related to young elite basketball players before and after the game for all participants (A) and *per* teams (B).

Table 2

Parameters related to hydration assessment

Parameters	x̄ ± SD	Range
Body mass loss (kg)	0.9 ± 0.7	-1.0–2.9
Percentage of dehydration (%)	0.99 ± 0.7	-1.25–2.95
Total fluid intake (L)	1.87 ± 0.82	0.38–3.98
Fluid intake (L/h)	1.79 ± 0.8	0.4–19
Sweat rate (L/h)	2.7 ± 0.9	0.23–5.54
Urine output (mL)	55 ± 61	0–240
Temperature (°C)	30 ± 2°C	27.2–32.5
Relative humidity (%)	55 ± 4	48–58
Playing time in game (min)	18.8 ± 10.5	0.15–40

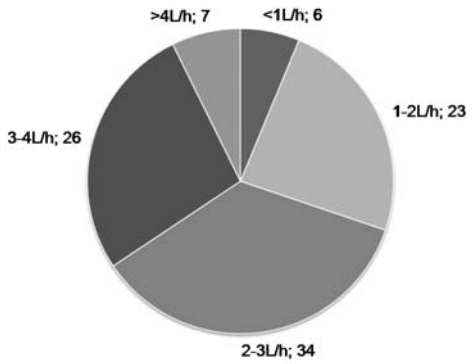

Fig. 2 – Distribution of sweat rate (L/h) among players (n = 96) based on arbitrary division.

ever, when sweat rate was analyzed by the team position in a playing time, it can be seen (Figure 3B) that the highest sweat rate was for center and the lowest for point guard and the difference was statistically significant.

Fluid intake

The mean total fluid intake was 1.87 ± 0.82 L and relative fluid intake 1.79 ± 0.8 L/h (Table 2). Total fluid intake and its composition in team is presented in Table 3. Figure 4 ilustrates the distribution of players depending on the quantitiy of drank fluid and shows that the largest percentages of players were in the group between 1 and 2 L/h of intaken fluid. No significant difference was seen in the quantitiy of drank fluid depending on the team position of the players (data not shown).

Correlations

The statistically significant correlations between sweat rate and: fluid intake ($p = 0.001$) are shown in (Figure 5), UO ($p = 0.001$), body mass loss ($p = 0.001$), (body surface areal-BSA) ($p = 0.001$) and percentage of dehydration ($p = 0.001$) were found.

The statistically significant correlations between fluid intake and: percentage of dehydration ($p = 0.001$), body mass loss ($p = 0.000$), USG ($p = 0.035$) and UC ($p = 0.004$) were

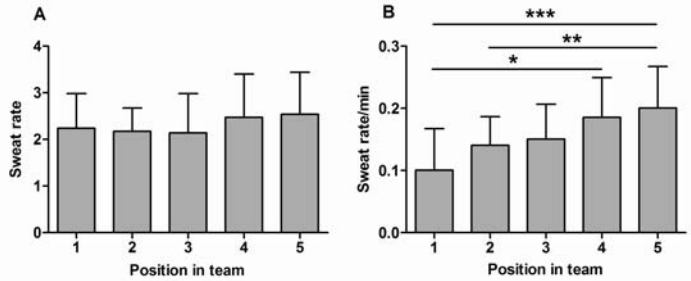

Fig. 3 – A) Sweat rate, and B) relative sweat rate/min depending on the position in team.
1. Point guard; 2. Shooting guard; 3. Small forward; 4. Power forward; 5. Center.

Table 3

Composition of beverages that players drink during the game

Team	Total fluid intake (L)	Water (L)	Isotonic drink (L)
1	1.55	1.55	0
2	1.35	1.35	0
3	1.52	1.15	0.37
4	1.19	0	1.19
5	2.44	1.66	0.77
6	2.11	1.18	0.93
7	2.07	2.06	0
8	1.96	0.85	1.11

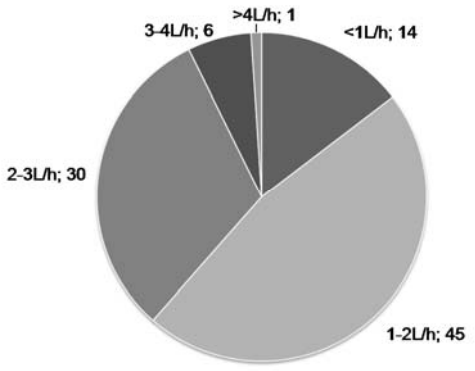

Fig. 4 – Distribution of fluid intake (L/h) among the players (n = 96) based on arbitrary division.

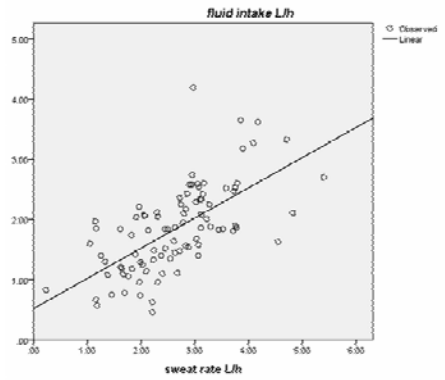

Fig. 5 – Correlation between fluid intake and sweat rate of young elite basketball players during the game.

obtained. No statistically signficant correlations between sweat rate and USG and between fluid intake and BSA in m^2 and UO were found.

Discussion

Basketball is a sport defined by bursts of high-intensity activity with intermittent rest periods and coupled with the large body sizes. This type of stop-and-go action is associated with heavy sweat losses and dehydration [11, 12]. Tournament could involve even the greater risk of dehydration since players may have difficulty to obtain fully rehydration between and after games. One study suggests that approximately 50% of individual and team-sport athletes are hypohydrated at the start of competition [13]. In our study 75–95% of all the basketball players were hypohydrated on the beginning of the competition and after the games, depending on the used urine parameters for evaluation of hydration status [8, 10]. Thus, one of the main feedbacks to the teams was that dehydration during competition is very high and because of that they should take care about their fluid intake habits.

Having in mind that basketball is playing in sport halls, under possible high, both, temperature and humidity, potential dehydration should be taken seriously [7]. During a regular basketball match maximal time of physical activity can be 80 min (warm up period 30 min, effective time in the game 40 min and warm up on the halftime 10 min). The mean playing time in our study was 18.8 minutes, but according to our results the players sweated not only during the time spent in the game but also while sitting on the bench, especially in such a warm environment.

In the sport of basketball, even though players have opportunities to drink during time-outs and when players are substituted, they may still be unable to maintain fluid balance [14]. Most athletes in the present study did not intake enough fluids to match sweat losses during the game and they were significantly dehydrated.

Sweat rates of athletes have been analysed in different studies and sports, and a wide range of results have been recorded. Average sweat rates from the scientific literature in athletes can vary from 0.5 L/h to more than 2.5 L/h[9]. The high sweat rate of 2.7 ± 0.9 L/h in our study could be caused by several reasons: high temperature of 30°C in sport hall, duration of competition (two weeks), day-to-day games and high exercise intensity.

The sweat rate in our study (2.7 ± 0.9 L/h) was higher than in a study of Broad et al. [15]. They studied the competition with a group of basketball players of similar ages to participants in our study. The sweat rate was 1.6 ± 0.37 L/h and fluid intake 1.08 ± 0.64 L/h. This study was conducted in summer conditions at the temperature of 23ºC and the humidity of 41%. A big difference between our and that study was temperature and humidity in sport hall where the games were played (in our study 30 ± 2°C, 55 ± 4%).

The sweat rate in our study was also close to the study of Bergeron et al. [5]. In that study sweat rates between 1.7 L/h and 2.4 L/h were recorded for single three set tennis matches in warm conditions (31.9 ± 0.5°C, RH 55 ± 0.2%).

In a report of Maughan et al. [16], during a training session, the sweat rate in 24 premiership soccer players, at 24–29°C was 1.35 ± 0.275 L/h. Shirreffs et al. [1] analysed 26 soccer players using the same methodology with the same duration of training session, but in much warmer conditions (32°C), and found out a mean sweat rate of 1.46 L/h, with a range from 1.12 to 2.09 L/h. Our results showed a higher sweat rate, compared to the previous studies. The main reason for that could be the fact that our study was conducted during competitions on tournament, not on trainings.

We found that centers had the biggest sweat rate when this parameter was calculated per min of the effective game. Similar results were obtained when we analysed sweat rate *per* $BSAm^2$. The highest sweat rate for a center could be explained with a high $BSAm^2$ and a bigger number of sweat glands, and therefore higher rates of sweating compared with smaller BSA athletes [17].

A total fluid intake of players in our study was 1.87 ± 0.82 L, which was not sufficient to compensate sweat losses. Despite of insufficient rehydration, mean percentage of dehydration was only 0.9%. During the competition fluid intake of our participants included sport drinks as well as water. There are few studies on fluid intake and sweat rate of basketball players on competition. Much more of them investigate those parameters during training sessions.

Our study found a statistically significant positive correlation between sweat rate and fluid intake. Such a correlation was previously identified by Palmer and Spriet [12]. and Silva et al. [18]. Different studies show that athletes are better hydrated when taking more sports drinks than water [19, 20]. In our study, out of 8 teams, only one used sport drink, 3 used water and 4 used a mix of water and sport drinks. There was a statistically significant difference for all the three urine parameters between the team 6 compared to other teams. The difference could be explained by the fact that the team 6 took a proper balance of water and sport drink during the game.

The fluid intake of young basketball players in our study was higher than in studies of Australian Institute of Sport during competition in summer season (23°C) in which fluid intakes were from 0.465 to 1.69 L [21] and in the studie of Broad et al. [15] who reported fluid intake 1.079 ± 0,615 L/h in male basketball players during practices and competitions. The fluid intake we found was also higher than in a study of Osterberg et al. [22] (1.0 ± 0.6 L) and Palmer and Spriet [12] (1.0 ± 0.1 L/h). One of the reasons for that could be the temperature in the venue and the level of competition.

Conclusion

Most of the athletes start competition dehydrated, fail to match sweat losses during the game and continue to be dehydrated. Quantity of water given to the teams is not adequate, having in mind the sweat rate and hydration status of the players. These fact must be considered seriously, not only by players, but also by the coaches and the team doctors. A hydration strategy, involving proper fluid and electrolyte replacement should be planned on an individual base and monitored carefully during practice or competition.

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ORIGINAL ARTICLE

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Efficacy of botulinum toxin type A in treatment of different forms of focal dystonias in the Serbian population: experience of the Botulinum Toxin Outpatients Department

Efikasnost terapije botulinskim toksinom tipa A u lečenju različitih formi fokalnih distonija u srpskoj populaciji: iskustvo Centra za botulinski toksin

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Abstract

Background/Aim. Botulinum toxin (BTX) irreversibly inhibits presynaptic acetylcholine release with subsequent relaxation of abnormally contracting muscles. It is an effective and well tolerated treatment with long-term benefit in a variety of movement disorders and other neurological and non-neurological disturbances. The aim of our study was to present our experience with BTX type A in treatment of different forms of focal dystonias. **Methods.** A hundred of patients with different focal dystonias (spastic torticollis, blepharospasm and graphospasm) from the Botulinum Toxin Outpatients Department, Clinic for Neurology, Clinical Center of Serbia, were included in the study. All the patients were examined and rated at baseline visit prior to BTX application and on the following visit, after 3–4 months, using self-assessment improvement questionnaire and standardized rating scales. **Results.** The improvement of ≥ 50% was presented in 68.2% of all (199) the analyzed applications. Independent predictors of good response to the therapy (improvement ≥ 50%) were male sex ($p = 0.011$), the presence of sensory trick ($p = 0.013$) and the total number of BTX applications ($p = 0.002$). The patients with spastic torticollis and blepharospasm showed a statistically significantly better BTX effect (improvement 57.3 ± 27.5% and 54.1 ± 28.3%), respectively than the graphospasm group (26.7 ± 25.6%). Most of the patients did not have therapy complications (81.4% and 72% in two applications). Side effects in the remaining patients (muscle weakness, dysphagia, ptosis, double vision, neck weakness and lacrimal dysfunction) lasted for 28.3 ± 18.6 days after the first treatment and 32.5 ± 36.2 days after the second one. **Conclusion.** BTX is safe and highly effective in long-term treatment of patients with different forms of focal dystonia, with only mild and well- tolerated side-effects.

Key words:
dystonia; botulinum toxins; serbia; questionnaires; quality of life.

Apstrakt

Uvod/Cilj. Botulinski toksin (BT) ireverzibilno inhibira presinaptičko oslobađanje acetilholina sa posledičnom relaksacijom prekomerno kontrahovanih mišića. To je efikasna i bezbedna terapija sa dugotrajnim povoljnim dejstvom kod brojnih bolesti nevoljnih pokreta, kao i kod drugih neuroloških i neneuroloških oboljenja. Cilj ovog istraživanja bio je predstavljanje našeg iskustva sa BT tipa A u lečenju različitih formi fokalnih distonija. **Metode.** U studiju je bilo uključeno 100 bolesnika sa različitim formama fokalnih distonija (spastični tortikolis, blefarospazam, grafospazam) lečenih u Centru za botulinski toksin Klinike za neurologiju, Kliničkog centra Srbije. Svi bolesnici su pregledani i ocenjeni pre primene BT, a zatim pri sledećoj poseti, nakon 3–4 meseca. Korišćeni su upitnik za samoprocenu poboljšanja i standardne skale za procenu težine bolesti. **Rezultati.** Poboljšanje ≥ 50% utvrđeno je kod 68,2% od ukupnog broja analiziranih primena BT (199). Nezavisni prediktori dobrog odgovora na terapiju (poboljšanje ≥ 50%) bili su muški pol ($p = 0,011$), prisustvo senzornog trika ($p = 0,013$) i ukupan broj aplikacija BT ($p = 0,002$). Efekat BT bio je statistički značajno bolji kod bolesnika sa tortikolisom i blefarospazmom (poboljšanje 57,3 ± 27,5%, odnosno 54,1 ± 28,3%), nego kod bolesnika sa grafospazmom (26,7 ± 25,6%). Većina bolesnika nije imala komplikacije terapije (81,4% i 72% u dve primene). Neželjeni efekti kod preostalih bolesnika (slabost mišića, disfagija, ptoza, diplopije, slabost vrata i poremećaj lakrimacije) trajali su 28,3 ± 18,6 dana posle prve primene i 32,5 ± 36,2 dana posle druge. **Zaključak.** Botulinski toksin je bezbedan i veoma efikasan u dugotrajnoj terapiji kod bolesnika sa različitim formama fokalnih distonija. Neželjeni efekti su blagi i dobro se podnose.

Ključne reči:
distonija; botulin toksini; srbija; upitnici; kvalitet života.

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Introduction

Botulinum toxin (BTX) irreversibly inhibits presynaptic acetylcholine release with subsequent relaxation of abnormally contracting muscles [1]. Although there are 7 different BTX serotypes named A, B, C, D, E, F and G, only BTX type A (BTX-A) has been widely studied for therapeutic purposes, and more recently, BTX type B [2]. BTX-A is an effective and well tolerated treatment with long-term benefit in a variety of movement disorders and other neurological and non-neurological disturbances which appear as a consequence of abnormal muscle contractions [1].

In a meta-analyses BTX-A therapy of spasmodic torticollis (TS) generated statistically significant clinical improvements on objective and subjective rating scales, as well as on subjective scales for pain relief in over 85% of cases [3, 4]. In blepharospasm (BS), BTX-A was found to be superior to placebo with the benefit reported by 90% of patients [5]. Good efficacy of BTX-A treatment in patients with focal task-specific dystonia (musician`s dystonia, occupational dystonia and writer's cramp) was proven in 67–93% of patients, but many of them discontinued therapy because it failed to meet their expectations or needs [6, 7], with mostly mild and transient weakness of the hand as an important side effect [8]. Follow-up studies showed a substantial 5-year benefit in most patients (90% in BS, 63% in TS, 56% in writer's cramp) with improvement maintained for up to 10 years [9].

The aim of our study was to present our experience with BTX-A in treatment of different forms of focal dystonias, treated at the Botulinum Toxin Outpatients Department of Clinic for Neurology, Clinical Center of Serbia.

Method

A hundred of patients with different focal dystonias from the Botulinum Toxin Outpatients Department, Clinic for Neurology, Clinical Center of Serbia, Belgrade were included in the study. The diagnosis of primary focal dystonia was made by the experienced movement disorders specialists, based on standardized criteria [10]. The study included patients with cervical dystonia, i.e. spasmodic torticollis (TS), blepharospasm (BS) and writer's cramp (i.e. graphospasm – WC).

The study was approved by the Ethic Commitee of Clinical Center of Serbia, Belgrade. Upon signing informed consent, the patients were interviewed in order to obtain demographic and clinical data on the disease course, therapy, previous BTX applications, complications of therapy, etc.

BTX type A (Dysport, 500 U) was used in this survey. All the patients were examined and rated at baseline visit prior to BTX application. They were asked to rate efficacy of previous application using self-assessment improvement questionnaire. Afterwards, the severity of symptoms was evaluated by specific rating scales: Toronto Western Spasmodic Rating Scale (TWSTRS) [11], Blepharospasm Disability Index (BSDI) [12], Jankovic Rating Scale (JRS) for Blepharospasm [13] and Writer's Cramp Rating Scale (WCRS) [14]. Muscle injection spots and BTX dosage for previous and actual application were noted. The next rating was performed on the following visit, 3–4 months after the baseline visit, when self-assessment improvement questionnaire was applied. Sustained benefit was defined as continued improvement of $\geq 50\%$ from baseline.

Our patients were injected by observation and palpation of specific muscles activity, without electromyography (EMG) guidance, as well as without ultrasound control. None of our patients were given "booster" injections because of the risk of antibody development. Data were analyzed using methods of descriptive statistic, χ^2 test, analysis of variance (ANOVA) and multivarant regression analysis.

Results

The study comprised 100 patients (48 with TS, 38 with BS and 14 with WC) whose demographic and clinical characteristics are summarized in Table 1. The patients with BS were statistically significantly older than the patients in other two groups ($p < 0.001$). The mean disease duration was similar in all the groups (8.3–11.0 years), sensory tricks were statistically significantly more frequent in the patients with TS (68.8%, $p = 0.001$), as well as it was dystonic tremor (45.8%, $p < 0.001$).

Table 1

Demographic and clinical characteristics of patients with focal dystonias

Variables	Spasmodic torticollis	Blepharospasm	Writer's cramp	*p*
Number	48	38	14	
Sex (m/f), n	17/31	12/26	3/11	0.613
Age*	51.1 ± 10.4 (24–72)	65.9 ± 8.2 (49–84)	50.2 ± 11.2 (31–67)	< 0.001
Age at onset*	40.5 ± 10.4 (15–63)	57.6 ± 9.8 (33–73)	40.2 ± 12.8 (17–62)	< 0.001
Disease duration*	11.0 ± 7.3 (2–32)	8.3 ± 6.2 (2–27)	10.0 ± 7.2 (2–30)	0.228
Number of applications of BTX‡	20.2 ± 15.2 (1–60)	21.0 ± 15.4 (2–80)	3.7 ± 4.2 (0–14)	0.001
Associated movement disorders†				
without	45 (95.8)	37 (97.4)	14 (100)	0.431
Dystonic tremor†	22 (45.8)	0 (0)	2 (14.3)	< 0.001
Sensory trick†	33(68.8)	19 (50)	2 (14.3)	0.001
Pain†	32 (66.7)	2 (5.3)	3 (8.1)	< 0.001
Positive family history†	14 (29.2)	1 (2.6)	1 (7.1)	0.002

***The values presenting the average duration and standard deviation in years with the range in brackets; †values presenting the number of patients with percentage in brackets; ‡values presenting the number of injections and standard deviation with the range in brackets; m – male; f – female; BTX – batulinum toxin.**

The patients with WC had statistically significantly longer latency to response (12.6 ± 9.4 days, $p < 0.001$) comparing to TS (7.2 ± 4.7) and BS (6.1 ± 4.9), as well as duration of therapeutic effect lasted significantly shorter in WC (1.9 ± 2.0 months, $p = 0.002$) than in TS (2.9 ± 1.1 months). Furthermore, the patients with TS and BS showed a statistically significantly better BTX effect (improvement 57.3 ± 27.5% and 54.1 ± 28.3%, respectively) than WC group (26.7 ± 25.6%) (Table 2).

Although most of the patients did not have therapy complications (81.4% and 72% in two applications), side effects in remaining patients lasted for 28.3 ± 18.6 days after the first treatment and 32.5 ± 36.2 days after the second treatment. Complications of the therapy appeared with the latency of 2–50 days after the first application and 1–30 days after the second one. Rare complications included: muscle weakness (7.2% and 14%, respectively), dysphagia (4.1% and 7%, respectively), ptosis (4.1% and 2%, respectively), double vision (3.1% and 2%, respectively), neck weakness (1%), lacrimal dysfunction (1%), and others (1%).

The improvement of ≥ 50% was presented in 68.2% of all (199) the analyzed applications (Figure 1). Impairment was reported in 1.5%, while 8% of applications had no effect, and improvement < 50% was noticed in 22% of all the applications.

Multivariate linear regression analysis showed that independent predictors of good response to the therapy (improvement ≥ 50%) were male sex ($p = 0.011$), the presence of sensory trick ($p = 0.013$) and the total number of BTX applications ($p = 0.002$) (Table 3).

Objective rating of clinical severity and subjective assessment of improvement showed a correlation in 2 TWSTRS domains: disability subscore (r = -0.294, $p = 0.05$) and total score (r = -0.286, $p = 0.05$).

Table 2

Botulinum toxin therapy characteristics in different forms of focal dystonia

Variable	Spasmodic torticollis	Blepharospasm	Writer's cramp	*p*
Number of applications	96	76	27	
Latency to response*	7.2 ± 4.7 (1–21)	6.1 ± 4.9(1–30)	12.6 ± 9.4 (5–30)	< 0.001
Duration of response†	2.9 ± 1.1 (0–6)	2.7 ± 1.1 (0–5)	1.9 ± 2.0 (0–7)	0.002
Self assess. improvement‡	57.3 ± 27.5 (50–100)	54.1 ± 28.3 (50–100)	26.7 ± 25.6 (0–75)	< 0.001

***The values presenting the average number and standard deviation in days with the range in bracket; † the values presenting the average number and standard deviation in months with the range in brackets; ‡ the values presenting the percentage of improvement and standard deviation with the range in brackets.**

Table 3

Multivariate linear regression analysis

Variable	Standardized β coefficient	95% Confidence Interval	*p*
Male gender	0.238	0.50–0.373	0.011
Sensory trick	0.231	0.41–0.344	0.013
Number of BTX application prior to testing	-0.290	-0.013– -0.003	0.002

Dependent variable: self-assessment questionnaire improvement ≥ 50% ; BTX – botulinum toxin.

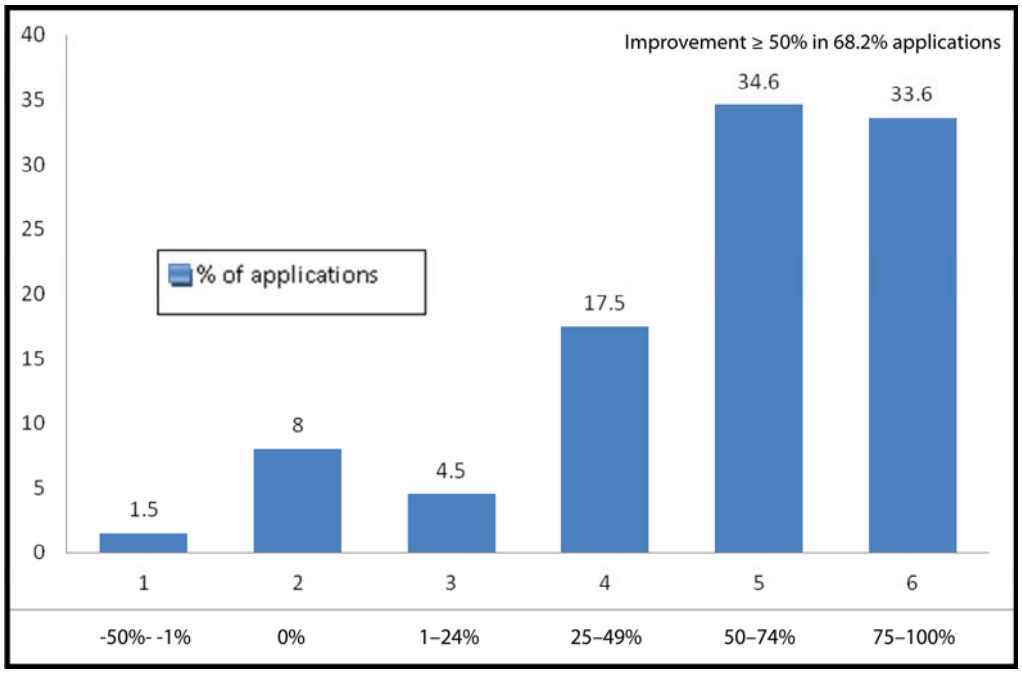

Fig. 1 – Self-assessment of improvement in percentage in 199 applications of botulinum toxin (BTX).

Discussion

We examined 100 patients with focal dystonia who were injected twice (overall 199 applications of BTX). The improvement of ≥ 50% in all analyzed treatment sections was present in about 70% of patients. Both applications achieved marked and similar functional and clinical improvement.

Our patients with TS estimated their mean improvement at about 57%. At the time of analyzed injection sections they had disease duration of about 11 years (2–32) with more than 20 previous injections of BTX A. Some of these TS patients were included in our previous study on BTX-A efficacy, which showed that 86% of patients improved [15]. Among 155 patients with TS treated with BTX-A injections over a period of 6 years, 78% continued the therapy, two thirds of which reported injections as "always helpful" [16]. Other studies on patients with TS treated for median of 5.5 years showed a high degree of patients' satisfaction evaluated by them and their neurologists (i.e. 67% of patients had good therapeutic effect) [17].

Our patients with BS found their mean improvement of about 54%. At the time of analyzed injection sections their disease duration was about 8 (2–27) years, with approximately 21 previous injections. According to the results of previous studies, BTX-A showed the best efficacy in patients with BS comparing to other forms of focal dystonia [9, 18, 19]. Bentivoglio et al. [19] demonstrated in a 15-year period follow-up study with 128 BS patients that in a six-point scale the mean efficacy of BTX-A was 3.9 ± 1.2. In another study with 73 BS patients, 96% of them, reported a significant relief of their symptoms.

A total of 20 patients with focal hand dystonia followed for up to 16 years had mild benefit in 50% of cases [20], while more than one third of musicians reported a long-term benefit and improvement in their performance ability [6]. In a study with 39 participants with WC, BTX-A injections had a significantly greater improvement compared to placebo, and about 50% of them were still treated after one year [8]. Moreover, reorganizational changes in the primary motor cortex have been shown in patients with WC receiving BTX-A injections [21]. In our WC group improvement was significantly less than in other 2 groups (26.7%), which is in accordance with other studies that showed worse BTX-A efficacy in patients with task specific dystonia comparing to other forms of focal dystonia [9].

Although our study was not longitudinal, it is important to stress that all our patients were regularly treated for a long period (1–80 injections *per* patients). There are only few studies with long-term follow-up [1]. Retrospective analysis of the long term efficacy of BTX-A over a 10-year period in 355 subjects with focal dystonia found that at 2 years the highest response rate of sustained benefit (defined as continued improvement of ≥ 50% from baseline) was in patients with BS (92%), TS (68%), and WC (57%), and that was similar at 5 years. Patients' satisfaction increased after 5 years of treatment with average reported benefit of 75.8% [9]. A slightly lower number of patients with sustained benefit in our study can be explained with the fact that the results are done for the whole group and that the patients with WC generally have worse treatment response.

We use a subjective, self-assessed improvement rating for detection of the level of improvement. Interestingly, these rating scales showed a correlation with the TWSTRS objective scale (total and severity subscores) [11]. The mean total duration of clinical improvement in our patients was 2.7 ± 1.3 months (range, 0–6). Latency to response was statistically significantly longer (12.5 days) and improvement and therapy duration inferior in the patients with WC than in the groups with TS and BS.

The frequency of complications in our group was low. Adverse events can be local (pain, edema, eryhema, ecchymosis, headache and short-term hyperaesthesia) and reactions following migration of the toxin into the adjacent muscles, as well as systemic reactions (nausea, fatigue, malaise, flu-like symptoms and rush). A total of 81% of our patients had no complications after the first treatment, and 72% after the second one, which is in accordance with the results of a meta-analysis on the safety of BTX-A in different indications, showing the overall rate of adverse events of approximately 25% in BTX-A treated subjects, compared with 15% in the control group [22]. The most frequent adverse events were muscle weakness, dysphagia, ptosis and double vision. Rare adverse events were neck weakness and lacrimal dysfunction. Complications in our patients were rare, mild and transient. Our results demonstrate that a total number of BTX-A applications, accompanied with male sex and the presence of sensory trick are the most significant predictors of improvement. Mejia et al. [23] followed 45 patients during 12 years and showed that both global efficacy score and the peak effect score, likewise duration of maximal response, improved comparing the first and last treatments, thus pointing out increasing long-term efficacy of BTX-A.

Conclusion

The results obtained in this study confirm that botulinum toxin is safe and highly effective in long-term treatment of patients with different forms of focal dystonia, with only mild and well-tolerated side-effects.

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Analysis of the factors influencing development of urinary tract infections in patients with spinal cord injuries

Analiza faktora razvoja infekcije urinarnog trakta kod bolesnika sa povredom kičmene moždine

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Abstract

Background/Aim. Urinary tract infections are still the most frequent complications in patients with spinal cord injury (SCI). The aim of this study was to analyze the factors influencing development of urinary tract infections during rehabilitation in patients with SCI. **Methods.** This retrospective case/control study including 540 patients with SCI which were rehabilitated in the Clinic for Rehabilitation "Dr Miroslav Zotović" between January 2000 and December 2009. We used patient files and other available medical documentation for obtaining information contained in this study, such as the manner of bladder emptying, the type of neurological disorder of the bladder, the neurological level and completeness of a lesion, the injury etiology, treatment method, secondary complications and associated injuries, kidney and bladder calculosis, age and sex. **Results.** Out of the total number of patients included in the study, 152 (28.1%) were without urinary tract infections, whereas 388 (71.9%) had urinary tract infections. There were 389 (72%) male and 151 (28%) female patients. The average age of patients without urinary tract infections was 51.0 ± 15.4 years, whereas the mean age of patients with urinary tract infections was 44.3 ± 16.9 years. The results of our study showed that the occurrence of urinary tract infections during rehabilitation in patients with SCIs was associated with the following factors: combined injuries (OR = 3.5), anemia (OR = 5.67), type of the bladder functional disorder (OR = 40–60) and crystals in urine (OR = 7.54). **Conclusion.** The physicians should take precautions and try to make the early diagnosis and rapid appropriate treatment of urinary tract infections in patients with SCI who also have functional bladder disorder, combined spinal injuries, anemia or urine crystals.

Key words:
spinal cord injuries; urinary tract infections; risk factors; urinary catheterization; multiple trauma; anemia; rehabilitation.

Apstrakt

Uvod/Cilj. Infekcije urinarnog trakta još uvek su najčešće komplikacije kod bolesnika sa povredom kičmene moždine. Cilj rada bio je da se ispitaju faktori koji mogu uticati na razvoj urinarnih infekcija u toku rehabilitacije bolesnika sa povredom kičmene moždine. **Metode.** Ova retrospektivna studija slučaja obuhvatila je 540 ispitanika sa povredom kičmene moždine koji su rehabilitovani u Klinici za rehabilitaciju „Dr Miroslav Zotović" u periodu od januara 2000. do decembra 2009. godine. Za dobijanje podataka koji su korišćeni u ovoj studiji kao što su način pražnjenja mokraćne bešike, vrsta neurološkog poremećaja mokraćne bešike, neurološki nivo i kompletnost lezije, etiologija povrede, način lečenja, sekundarne komplikacije, udružene povrede, kalkuloze bubrega i bešike, starost i pol, korišćene su istorije bolesti i ostala medicinska dokumentacija. **Rezultati.** Od ukupnog broja ispitanika koji su bili uključeni u ovu studiju 152 (28,1%) je bilo bez urinarnih infekcija, dok je 388 (71,9%) imalo urinarne infekcije. Bilo je 389 (72%) muškaraca i 151 (28%) žena. Prosečna starost ispitanika bez urinarnih infekcije bila je 51,0 ± 15,4, a bolesnika sa urinarnim infekcijama 44,3 ± 16,9 godina. Rezultati naše studije ukazuju na to da su faktori koji naj-

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više doprinose razvoju urinarnih infekcija tokom rehabilitacije bolesnika sa povredom kičmene moždine udružene povrede (OR = 3,5), anemija (OR = 5,67), način pražnjenja mokraćne bešike (OR = 40–60) i kristali u urinu (OR = 7,54). **Zaključak.** Kliničari bi trebalo da sprovedu ranu dijagnostiku i adekvatno terapijsko lečenje infekcija urinarnog trakta kod osoba sa povredom kičmene moždine i poremećajem pražnjenja mokraćne bešike, kombinovanim povredama, anemijom i kristalima u urinu.

Ključne reči:
kičmena moždina, povrede; urinarni trakt, infekcije; faktori rizika; kateterizacija urinarnog trakta; povrede, multiple; anemija; rehabilitacija.

Introduction

A spinal cord injury (SCI) is characterized by the loss of motor function and sensibility below the injury level and the loss of reflex activity [1].

The causes of SCIs include trauma, vascular disorders, tumors both of malignant and benign etiology, infections, development disorders, etc [2]. The patients with SCIs may suffer from many secondary complications, like urinary tract infections, decubital ulcers, cardiovascular and respiratory tract diseases, etc [3]. Urinary tract infections are the most frequent complications occurring after SCI and use to be the main cause of death in the past [4]. In addition, the risk of nosocomial infections is greater in patients with SCI than in patients without SCI. A patient is suffering from urinary tract infection if, apart from confirmed bacteriuria, has one of the following clinical symptoms: increased body temperature (> 38°C), stomach pain, urinary incontinence, increased spasticity, suprapubic pain, frequent urination, dysuria, unpleasant urine odor, etc [5]. If these symptoms are missing, a patient has asymptomatic bacteriuria. The asymptomatic bacteriuria should not be treated with antibiotics [6].

The loss of reflex activity after SCI is characterized by partial or complete loss of sphincter control (urination and defecation). Incontinence, vesicoureteral reflux, permanent catheter use, performance of intermittent catheterization or self–catheterization are only some of the factors which may influence the development of urinary tract infections [7]. Additionally, these factors may also influence the reoccurrence of urinary tract infections both during rehabilitation and in the post-rehabilitation period [8]. Some studies say that urinary tract infections are the most frequent cause for rehospitalization after SCIs [9]. Knowing the factors which may influence the development of urinary tract infections, we can create a prediction model and thus decrease the frequency and adverse influence of urinary tract infections on the duration of rehabilitation and quality of life [10].

The aim of this study was to determine factors influencing the development of urinary tract infections in patients with SCIs during rehabilitation.

Method

This retrospective case/control study enrolled 592 patients with SCIs hospitalized at the Clinic for Rehabilitation "Dr Miroslav Zotović" in Belgrade, Serbia between January 2000 and December 2009. The cases were the patients with developed urinary tract infections, and the controls were the patients in which urinary tract infections did not develop. The cases and controls were matched according to the age and sex.

The patients meeting the following criteria were included in the study: diagnosed with SCIs and hospitalized at the Clinic for Rehabilitation "Dr Miroslav Zotović" in Belgrade, suffering from bladder emptying disorder caused by SCIs, and hospitalized longer than 30 days.

The criteria for exclusion from the study were: any kind of deterioration of the basic disease causing termination of the rehabilitation process, the patients younger than 18 years.

Based on the exclusion criteria, 52 patients were excluded from the study, and the remaining 540 were taken into account for statistics and drawing conclusions.

The presence of urinary tract infection was confirmed by urine culture with antibiogram. The following laboratory analyses were done in patients diagnosed with urinary tract infection: complete blood count with erythrocyte sedimentation rate, leukocyte formula, blood biochemistry, urine biochemistry and urine sediment analysis. Blood biochemistry included: the level of urea, creatinine, uric acid, serum albumins and proteins, alkaline phosphatase, and serum iron level.

The following potential risk factors for urinary tract infections in patients with SCIs were noted if present: method of bladder emptying, type of neurological disorder of the bladder, neurological level and completeness of the lesion, injury etiology, treatment method, secondary complications and combined injuries, bladder and/or kidney calculi, age and sex. The information was obtained from the patient's files and other available medical documentation of the patients.

The subjects were duly informed about the methodology of this study and voluntary signed a consent form. The study was approved by local Ethics Committee (Clinic for Rehabilitation "Dr Miroslav Zotović") under the registration number 03-1882/2013.

During hospitalization the patients underwent the series of tests for evaluating the type of neurogenic injury of the bladder after SCI and the presence of neurological consequences: full urodynamic testing [11] for evaluating the bladder functional condition, to diagnose of bladder and kidney calculi were diagnosed with radiographic and ultrasound examination, American Association Impairment Scale (AAIS) test for evaluating the neurological level and completeness of lesion [12, 13], Modified Ashworth Score (MAS) test for evaluating the degree of spasticity [14], Manual Muscle Test (MMT) for evaluating the muscle strength [15].

For the primary data analysis, we used descriptive statistical methods, statistical hypothesis testing and the methods for analyzing the relationship between the outcome and potential predictors. We used the following descriptive statistical met-

hods: the measures of central tendency (arithmetic mean), variability measures (standard deviation) and relative numbers. For testing the hypotheses on the difference between frequencies, we used the following: $\chi 2$-square test, Fisher's test of exact probability and McNemar's test; *t*-test and Mann-Whitney test were used for testing the hypotheses on the arithmetic mean differences. Logistic regression was used as the method for analyzing binary outcome and potential predictors.

Statistical hypotheses were analyzed at the level of significance of 0.05.

Results

Out of the total number of the patients included in the study, 152 (28.1%) were without urinary tract infections, whereas 388 (71.9%) had urinary tract infections.

There were 389 (72%) male and 151 (28%) female patients. Exactly 73.2% of the male patients and 26.8% of the female patients had urinary tract infection. The difference in the prevalence of sexes between the studied groups was not statistically significant ($p = 0.338$).

The mean age was found to be 46.2 ± 16.7 years. The average age of patients without urinary tract infections was 51.0 ± 15.4 years, whereas the mean age of patients with urinary tract infections was 44.3 ± 16.9 years. There was a significant age difference between the studied groups ($p < 0.001$): urinary tract infections occurred more frequently in younger patients. These and other characteristics of the patients with SCI are shown in Table 1.

Out of the total number of patients, 195 (36.1%) had a complete and 345 (63.9%) had an incomplete SCIs. Urinary tract infections were present in 61.4% of the patients with incomplete SCIs and in 90.3% of the patients with complete SCIs.

Urinary tract infections occurred more frequently in the patients with complete SCIs ($p < 0.001$).

In all the patients with SCI, the most common was complete lesion American Spinal Injury Association (ASIA) A (195 patients, or 36.1%), followed by the ASIA C (201 patients, or 37.2%), ASIA B (94 patients, or 17.4%) and ASIA D type (50 patients, 9.3%). The patients without urinary tract infections most frequently had ASIA C level on admission (92 patients, 60.5%), whereas the patients with urinary tract infections most frequently had ASIA A level on admission (176 patients, 45.4%). There was a significant difference in the frequency of ASIA levels on admission between the studied groups ($p < 0.001$).

In the patients with SCI, the most common were thoracic injuries (42.8%), followed by cervical (35.0%) and lumbar ones (22.2%). Urinary tract infections were present in 68.3% of the patients with cervical injuries, in 79.7% of the patients with thoracic injuries, and in 62.5% of the patients with lumbar injuries. There was significant difference in the frequency of certain neurological level of injury on admittance between the studied groups ($p < 0.001$). Urinary tract infections were more frequent in patients with thoracic spine injuries.

Out of the total number of patients, 132 (24.4%) had

Table 1

Characteristis of the patients with spinal cord injuries

Parameter	Patients		*p*
	with UTI (n = 388)	without UTI (n = 152)	
Age (years), $\bar{x}$ ± SD	44.3 ± 156.9	51.0 ± 15.4	< 0.001
Sex, n (%)			
male	284 (73.2)	105 (69.1)	0.338
female	104 (26.8)	47 (30.9)	
Etiology of injury, n (%)			
traumatic	264 (68)	57 (37.5)	< 0.001
non-traumatic	124 (32)	95 (62.5)	
Completeness of injury, n (%)			
complete	176 (45.4)	19 (12.5)	< 0.001
incomplete	212 (56.4)	133 (87.5)	
Level of injury, n (%)			
cervical	129 (68.3)	60 (31.7)	
thoracic	184 (79.7)	47 (20.3)	< 0.001
lumbar	75 (62.5)	45 (37.5)	
Length of stay (days), $\bar{x}$ ± SD	166.7 ± 89.5	96.4 ± 66.6	< 0.001

UTI – urinary tract infections.

Out of the total number of patients 321 (59.4%) had traumatic SCI, and 219 (40.6%) non-traumatic injuries. Urinary tract infections were present in 68.0% of the patients with traumatic injuries and in 32.0% of the patients with non-traumatic injuries. The difference in the frequency of urinary tract infections between the studied groups was significant ($p < 0.001$). Urinary tract infections occurred more frequently in patients with traumatic SCI.

bladder injury with urgent urination, 203 (37.6%) had hyperreflexive, and 205 (38.0%) hypotonic bladder. The patients without urinary tract infections most frequently had bladder functional disorder with urgent urination (73.0%), whereas the patients with urinary tract infections most frequently had hyper-reflexive bladder (49.5%). There was a significant dif-

ference in the frequency of bladder functional disorders between the studied groups ($p < 0.001$).

Various methods of bladder emptying were used: intermittent self-catheterization in 278 (51.5%) of the patients, intermittent catheterization in 69 (12.8%) of the patients, tapping in 28 (5.2%) patients, permanent catheter in 31 (5.7%) of the patients and spontaneous emptying (urgent urination) in 134 (24.8%) of the patients. In patients without urinary tract infections, the most common method of bladder emptying was spontaneous one (urgent urination) (72.4%), whereas in those with urinary tract infections, the most frequent method was intermittent self-catheterization (62.9%). There was a significant difference in the methods of bladder emptying between the studied groups ($p < 0.001$).

There were 127 (23.5%) patients with combined SCIs, and 413 (76.5%) without combined injuries. Exactly 13.8% of the patients without urinary tract infections had combined injuries, whereas the number of patients with urinary tract infections and combined injuries was 27.3%. There was a significant difference in frequency of combined injuries between the studied groups ($p = 0.001$).

Out of the total number of patients, 291 (53.9%) were not anemic, whereas 249 (46.1%) of them were. Exactly 10.5% of the patients without urinary tract infections had anemia, whereas the number of patients with urinary tract infections and anemia was 60.2%. There was a significant difference in the rate of anemia between the studied groups ($p < 0.001$).

In 209 (38.7%) patients urine crystals were not found, whereas in 331 (61.3%) of them were. In 16.4% of the patients without urinary tract infections urine crystals were found, whereas the number of patients with urinary tract infections and urine crystals was 78.9%. There was a significant difference in the frequency of urine crystals between the studied groups ($p < 0.001$).

The average duration of rehabilitation for all the patients was 147.0 ± 89.4 days. The minimal duration of rehabilitation was 28 and the maximal was 533 days. The average duration of rehabilitation in the patients with urinary tract infections was 166.7 ± 89.5 days, and in patients without urinary tract infections the average duration was 96.4 ± 66.6 days, which made significant difference ($p < 0.001$). The rehabilitation lasted much longer in the patients with urinary tract infections.

The model of multiple logistic regression included those predictors which had statistical significance at the level of 0.05 within the model of simple logistic regression, as well as those predictors which were considered, based on the previous research, as potentially significant for the development of urinary tract infections during rehabilitation. The model contained 13 predictors which were compared in 540 patients, and 387 of them had the result which was of interest to the study. The entire model (with all the predictors) was statistically significant ($p < 0.001$) (Table 2).

Logistic regression was used for analyzing the relationship between binary results and potential predictors.

In the model of multiple logistic regression, the statistically significant predictors for the development of urinary tract infections during rehabilitation were the following: combined injuries (B = 1.259; $p = 0.009$), type of bladder functional disorder [hyper -reflexic bladder (B =

Table 2

Multiple logistic regression model

Independent variable	B	*p*	OR	95% confidence interval	
				lower limit	upper limit
Age	-0.005	0.638	1.00	0.97	1.02
Gender	0.566	0.128	1.76	0.85	3.65
Etiology of injury	-0.132	0.739	0.88	0.40	1.90
Combined spinal cord injury	1.259	0.009	3.52	1.37	9.08
Urinary infections before rehabilitation	1.275	0.221	3.58	0.46	27.62
Type of lesion	0.120	0.790	1.13	0.47	2.74
Pressure ulcers during rehabilitation	1.014	0.171	2.76	0.65	11.78
Spasticity during rehabilitation	0.682	0.134	1.98	0.81	4.83
Neurological level of injury					
cervical			reference category		
thoracic	0.730	0.141	2.08	0.79	5.49
lumbar	0.033	0.955	1.03	0.33	3.21
Type of bladder emptying					
spontaneous			reference category		
intermittent self-catheterization	-1.057	0.440	0.35	0.02	5.07
intermittent catheterization	-0.137	0.925	0.87	0.05	15.05
tapping/Crede maneuver	-1.619	0.283	0.20	0.01	3.81
permanent catheter	0.167	0.917	1.18	0.05	27.67
Type of functional bladder disorder					
none			reference category		
hyper-reflexive bladder	4.078	0.002	59.01	4.47	779.85
hypotonic bladder	3.603	0.011	39.69	2.29	586.92
Anemia	1.739	< 0.001	5.69	2.64	12.26
Urine crystals	2.020	< 0.001	7.54	3.84	14.80

4.078; $p = 0.002$) and hypotonic bladder (B = 3.603; $p = 0.011$) in relation to bladder injury with urgent urination as a reference category], anemia (B = 1.739; $p < 0.001$) and urine crystals (B = 2.020; $p < 0.001$)

Discussion

Urinary tract infections frequently follow SCI, but chances for their development were 3.5 times higher in our patients with combined SCIs. This is not surprising, since severity of SCI was already linked to increased complication rate, including the urinary tract infection [16]. More severe SCIs, more difficult to treat and the patients with such injuries are longer hospitalized, which increases risk of all kinds of nosocomial infections including urinary tract ones [17].

Our results show that patients with SCI and hyper-reflexic bladder have much more chances to develop urinary tract infection. Hyperreactivity of the bladder involves strong detrusor contractions uncoordinated with relaxation of the internal urethral sphincter, which create turbulent urethral urine flow with elements of retrograde urethrovesical passage of urine. It was noted in a study on young girls with hyperreactivity of the bladder that urinary tract infection are much more frequent than in girls without the hyperreactivity [18]. On the other hand, as shown in our study, hypotonic bladder is also a risk factor for urinary tract infection, since delayed bladder emptying gives time to microorganisms to replicate and reach bladder mucosa. Urinary tract infections are more frequent in all patients with hypotonic bladder, regardless the cause [19].

Anemia is a well-known risk factor for infections at multiplicity of sites in human organism. Decreased oxygenation of tissues disrupts natural barriers for infection, and the immune system is less efficient in such circumstances. Surgical patients with anemia more frequently develop postoperative infections than those with normal hemoglobin level [20]. As shown in our study, the patients with SCI and anemia are much more likely to develop urinary tract infection than patients without anemia; this underlines necessity for early discovery and rapid treatment of iron-deficiency and other types of anemia in patients with SCI, in order to prevent emergence of urinary tract infections. Our results are similar to the results of previous studies on the relationship of anemia and SCIs. Grossman et al. [21] in their study show that anemia is one of most frequent complications in acute SCI patients followed by pneumonia, pleural effusion, cardiac dysrhythmia, and severe bradycardia. In a study by Cuttitta et al. [22] the risk factors of asymptomatic bacteriuria, and the association between different clinical and laboratory parameters and asymptomatic bacteriuria were examined. Their study showed that obesity and iron deficiency anemia were independent risk factors for asymptomatic bacteriuria.

The association between urine crystals and urinary tract infections observed in our patients with SCI could have been expected, since urine crystals are characteristics of concentrated and stagnant urine. Insufficient intake of water by patients with SCI and slowed bladder emptying create concentrated and stagnant urine, which is suitable medium for growth of microorganisms. The association of urine crystals and urinary tract infections has already been shown in many different patient groups [23, 24]. Torzewska et al. [25] in their in *vitro* study hypothesized that one of the reasons for recurrence of the disease may be the ability of bacteria to invade urothelial cells, persist in the host cells and serve as potential reservoirs for infection. An *in vitro* model was used in this study to analyze intracellular growth and crystallization in the presence of *Proteus mirabilis*, *Klebsiella pneumoniae* and *Escherichia coli*. Their results show that *Proteus mirabilis* has an ability to form crystals inside the host cells. Under these conditions bacteria are protected from antibiotic killing, which leads to persistent and recurrent infections. They also suspect that this phenomenon may play an important role in kidney stones formation.

There are certain limitations of our study, which may indirectly affect the obtained results. The study was conducted as a single-centre study, so local patterns of SCI patients care could mask some of the factors which could influence urinary tract infection rate. Besides, the study included both patients with traumatic and non-traumatic SCIs, which differ in many ways one from another. Future studies should concentrate on each of these two populations separately, in order to reveal some specific risk factors, but only after a sufficient number of patients is provided.

Conclusion

The patients with spinal cord injury and functional bladder disorder, combined spinal injuries, anemia or urine crystals are under increased risk for the development of urinary tract infections. When one or more of these factors are present in those with spinal cord injury, the physicians should take precautions and try to make the early diagnosis of urinary tract infections. This is the way to reduce the incidence of urinary tract infections and provide continuity in implementation of kinesitherapy which is often stopped due to these complication. It also will reduce the length of rehabilitation and increase quality of life of those with spinal cord injury.

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Diuretic ^{99m}Tc DTPA renography in assessment of renal function and drainage in infants with antenatally detected hydronephrosis

Značaj diurezne ^{99m}Tc DTPA scintigrafije u proceni renalne funkcije i drenaže kod dece sa prenatalno otkrivenom hidronefrozom

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Abstract

Background/Aim. The controversy over the postnatal management of infants with antenataly detected hydronephrosis (ANH) still exists. We presented the results of diuretic ^{99m}Tc diethylenetriamine pentaacetic acid (DTPA) renography in 30 infants with the antenatal diagnosis of unilateral renal pelvic dilatation. The aim of this study was to assess the renal function determined by the pattern of drainage and split renal function (SRF) on diuretic renography and to correlate these findings with anteroposterior pelvic diameter (APD) estimated by ultrasonography. **Methods.** A total of 30 infants with 60 renal units (RU) (25 boys and 5 girls, median age 6.0 months, range 2–24) presented with unilateral hydronephrosis on ultrasound in the newborn period, underwent DTPA diuretic renal scintigraphy (F+15 protocol). The median APD evaluated on perinatal ultrasound was 15 mm (range 5–30). The postnatal associated clinical diagnosis were pelviureteric junction obstruction (PUJ), simple hydronephrosis, megaureter, vesicoureteral reflux (VUR) and posterior urethral valves in 11, 10, 6, 2 and 1 infant, respectively. Images and Tmax/2 after diuretic stimulation on the background subtracted renographic curves were used as the criteria for classifying the drainage as good, partial, and poor or no drainage. The SRF was calculated with the integral method. **Results.** Good drainage was shown in 36/60, partial drainage in 13/60 and poor or no drainage in 11/60 RU. The SRF > 40% was observed in 55/60 RU, with no RU showing SRF lower than 23.5%. In infants with severe ANH the obstruction was not excluded in 94.1%. **Conclusion.** Diuretic renography in antenatally detected hydronephrosis should be a useful tool in postnatal follow up, especially in differentiating nonobstructive hydronephrosis from obstructive. It is also importanat to assess and monitor the SRF. Our results suggest that even in the presence of partial or no drainage, SRF may not be significantly impaired.

Key words:
infant; hydronephrosis; prenatal diagnosis; radioistope renography.

Apstrakt

Uvod/Cilj. Još uvek postoje kontroverze o načinu postnatalnog praćenja dece sa prenatalno dijagnostikovanom hidronefrozom. U ovom radu prikazali smo rezultate diurezne ^{99m}Tc *diethylenetriamine pentaacetic acid* (DTPA) scintigrafije bubrega kod 30-oro dece sa antenatalnom dijagnozom dilatacije bubrežne karlice. Cilj ove studije bio je procena renalne funkcije na osnovu stepena pražnjenja bubrega nakon diuretske stimulacije i separatnog klirensa, kao i korelacija ovih nalaza sa anterioroposteriornim prečnikom (*anterioposterior pelvic diameter* – APD) bubrežne karlice dobijenog ultrazvukom. **Metode.** Diurezna DTPA scintigrafija bubrega (F+15 protokol) urađena je kod 30-oro dece (25 dečaka i 5 devojčica, uzrasta 2–24 meseca, medijana 6 meseci) sa 60 renalnih jedinica (RU) kod kojih je perinatalno ultrazvučno utvrđena hidronefroza lakog do teškog stepena. Medijana APD iznosila je 15 mm (5–30 mm). Postnatalno udružene kliničke dijagnoze bile su opstrukcija pelviureteričnog spoja (PUJ) kod 11, hidronefroza kod 10, megaureter kod 6, vezikoureteralni refluks (VUR) kod 2 i zadnja valvula uretre kod jednog deteta. Na osnovu scintigrama i vrednosti Tmax/2 nakon diuretske stimulacije na renografskim krivuljama renalna drenaža je klasifikovana kao dobra, parcijalna i loša ili odsutna. Separatni klirens je računat metodom integrala. **Rezultati.** Dobra renalna drenaža dobijena je kod 36/60 RU, parcijalna kod 13/60 RU i loša ili odsutna kod 11/60 RU. Separatni klirens > 40% dobijen je kod 55/60 RU, dok

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ni kod jedne RU separatni klirens nije bio manji od 23,5%. Opstrukcija se nije mogla isključiti kod 94,1% dece sa teškom hidronefrozom. **Zaključak.** Diurezna scintigrafija bubrega preporučuje se kao korisna metoda u postnatalnom praćenju i terapijskom odlučivanju kod dece sa prenatalno dijagnostikovanom hidronefrozom, posebno u izdvajanju neopstruktivne hidronefroze od opstruktivne. Takođe, važna je procena i praćenje vrednosti separatnog klirensa. Naši rezultati ukazuju da čak i kod parcijalne ili odsutne renalne drenaže, separatni klirens ne mora biti značajno smanjen.

Ključne reči:
novorođenče; hidronefroza dijagnostika, prenatalna; renografija, radioizotopska.

Introduction

The widespread ultrasound screening during pregnancy has resulted in increasing recognition of antenatal hydronephrosis (ANH). Depending on the diagnostic criteria and gestation, the prevalence of antenatally detected ANH ranges from 0.6% to 5.4% [1–3]. The causes of ANH vary from transient benign conditions – transit hydronephrosis, which resolves by birth or during infancy to conditions that can significantly affect renal function. The outcome of ANH depends on the underlying etiology, so it is very important to determine these causes [4]. The definition and grading of ANH is based on anteroposterior pelvic diameter (APD) of the fetal renal pelvis [5]. It is an objective parameter, although it varies with gestation, maternal hydration and bladder distension. ANH is present if the APD is ≥ 4 mm in the second trimester and ≥ 7 mm in the third trimester [4]. ANH is further graded as mild, moderate and severe depending on the size of the measured APD. While fetuses with minimal pelvic dilatation (5–9 mm) have low risk of postnatal pathology, the APD ≥ 15 mm at any gestation represents severe hydronephrosis and requires close follow-up [6–9]. Antenatal management includes antenatal ultrasound monitoring, which is usually repeated every 4–6 weeks, but its frequency depends on the gestation at which ANH was detected, as well as its severity and the presence of oligohydramnios [5].

Almost 80% of the fetuses diagnosed in the second trimester show resolution or improvement of findings with the low likelihood of postnatal pathology [10]. Patients with persistence or worsening hydronephrosis in the third trimester show higher rates of postnatal pathology and require more frequent monitoring. Also, more frequent monitoring is required for fetuses with findings that suggest lower urinary tract obstruction. It is recommended that additional prenatal ultrasound evaluation is done at 16–20 weeks pregnancy in fetuses with the ANH detected [5]. It includes evaluation of lower urinary tract obstruction, renal dysplasia and extrarenal structural malformations. The controversy about the postnatal management of infants with the ANH still exists. It is emphasized that an ultrasound in the first few days of life underestimates the degree of pelvic dilatation due to dehydration and a relatively low urine output. Despite this limitation, an early ultrasound, 24–48 hour after birth, is necessary in the neonates with suspected lower urinary tract obstruction, oligohydramnios and bilateral severe hydronephrosis or severe hydronephrosis in a solitary kidney [4]. In others, the first ultrasound examination should ideally be delayed until the end of the first week. An ultrasound at 6 weeks is more sensitive and specific for obstruction. The presence of the two normal postnatal renal ultrasounds excludes the presence of the significant renal disease including dilating vesicoureteral reflux (VUR) [11]. It is recommended that the assessment of the severity of postnatal hydronephrosis is based on the anteroposterior diameter of the renal pelvis. The diuretic renal scintigraphy is important in postnatal evaluation of these infants, particularly in distinguishing kidney with the poor drainage from the nonobstructive hydronephrosis with the good drainage.

The aim of this study was to assess the renal function determined by the pattern of drainage and split renal function (SRF) on diuretic renography and to correlate these findings with the APD estimated by ultrasonography.

Methods

A total of 30 infants with 60 renal units (RU) (25 boys and 5 girls, median age 6.0 months, range 2–24) presented with unilateral mild to severe hydronephrosis on ultrasound in newborn period underwent diethylentriaminepentaacetic acid (DTPA) diuretic renal scintigraphy (F+15 protocol). The postnatal associated clinical diagnosis were pelviureteric junction (PUJ) obstruction, simple hydronephrosis, megaureter, VUR and posterior urethraL valves in 11, 10, 6, 2 and 1 infant, respectively. The median anteroposterior pelvic diameter evaluated on perinatal ultrasound was 15 mm (range 5–30). In 32/60 RU APD was ≥ 5 mm, while in 28/60 RU APD was < 5 mm. The diuretic renal scintigraphy was performed during 30 minutes (60 frames, 30 seconds each, matrix size 128 × 128) after *iv* injection of ^{99m}technetium labeled DTPA using the dose of 1.8 Mbq/kg in posterior projection. The single-head "Orbiter-Siemens" gamma camera filtered with low energy all-purpose collimator and with the Pegasys computer was used. To asses renal drainage 15 minutes after starting the study, 0.50 mg/kg furosemide *iv* was injected. Images and Tmax/2 after diuretic stimulation on the background subtracted renographic curves were used as the criteria for classifying the drainage as good (Tmax/2 < 10 min), partial (Tmax/2 from 10 min–20 min) and poor or no drainage (Tmax/2 > 20 min). The SRF was calculated with the integral method, and the range of 45–55% was considered as normal finding. We used statistical program SPSS version 20 for analyzing the descriptive statistic of our findings.

Results

We classified hydronephrosis into three groups according to ultrasound measurement of the renal pelvis diameter: mild (APD 5–9.9 mm) in 5/60 RU, moderate (APD 10–14.9 mm) in 10/60 RU and severe (APD ≥ 15 mm) in 17/60 RU.

Good or almost good drainage was shown in 36/60, partial drainage in 13/60 and poor or no drainage in 11/60 RU. SRF > 40% was observed in 55/60 RU, with no RU showing SRF lower than 23.5% (Figure 1). Significant obstruction was excluded in 39/60 RU (Figure 2). In infants with severe ANH obstruction was not excluded in 94.1%. We considered the correlation between the pattern of the drainage and the magnitude of SRF (Figures 3 and 4), as well as the correlation between the APD and the grading of SRF (Table 1).

Discussion

In our study the most frequent cause for ANH detected by prenatally ultrasound was PUJ obstruction in 33% of in-

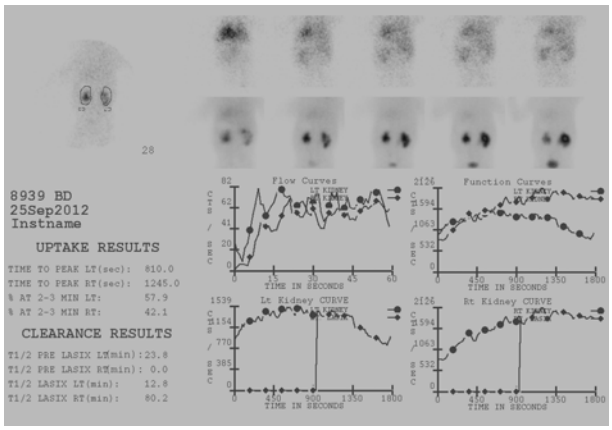

Fig. 1 – Diuretic renal scintigraphy 20MBq ^{99m}Tc diethylenetriamine pentaacetic acid (DTPA) in a 6 month-old infant: Unilateral right hydroneprhosis was diagnosted prenataly by ultrasound; slowing in drainage on the left side and almost no drainage on the right side, without diuretic response; relative function of the right kidney is not significantly impaired [split renal function (SRF) 42.1%].

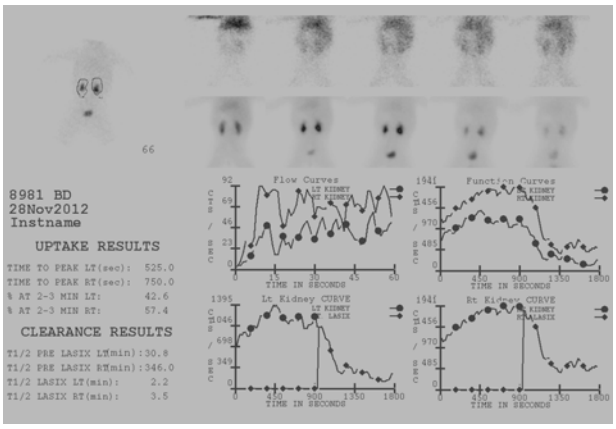

Fig. 2 – Diuretic renal scintigraphy 20 MBq ^{99m}Tc diethylenetriamine pentaacetic acid (DTPA) in a 4-month-old infant: rapid drainage on both sides after diuretic stimulation – significant obstruction is excluded; no signs of vesicoureteral reflux (VUR) were detected by micturating cystoureterogram (MCUG).

Fig. 3 – Magnitude of split renal function (SRF) in different patterns of drainage on the right side.

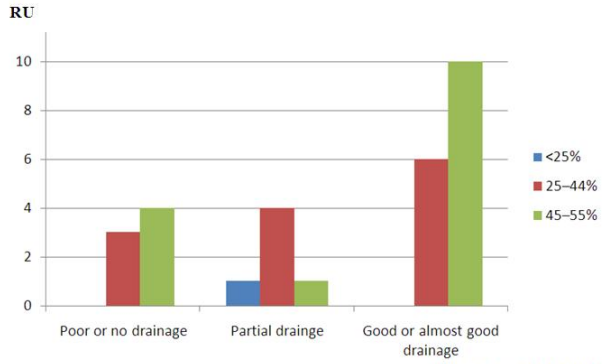

Fig. 4 – Magnitude of split renal function (SRF) in different patterns of drainage on the left side.

Table 1

Split renal function (SRF) grading according to anteroposterior (AP) pelvic diameter

AP pelvic diameter	SRF grading (%)			Total
	≤ 30	30–44	≥ 45	n (%)
Mild, n (%)	0 (0.0)	0 (0.0)	5 (100.0)	5 (100.0)
Moderate, n (%)	0 (0.0)	2 (20.0)	8 (80.0)	10 (100.0)
Severe, n (%)	3 (17.6)	4 (23.5)	10 (58.8)	17 (100.0)
Total, n (%)	3 (9.4)	6 (18.8)	23 (71.9)	32 (100.0)

n – number of renal units.

fants. According to our findings the risk of postnatal pathology strongly correlated with the magnitude of the fetal APD. We considered the pathological finding of the obstruction which could not be excluded if we found the curve that rises continuously over 20 minutes or appears as a plateau, despite the furosemide and post micturition. Our findigs show that the risk of detecting obstruction for severe hydronephrosis was 94.1%. In line with our study in one meta-analysis on 1,308 neonates from 17 studies, Lee et al. [6] found that the risk of postnatal pathology increased with the degree of antenatal pelvic dilatation, from 11.9% for mild, 45.1% for moderate and 88.3% for severe hydronephrosis.

There is no worldwide consensus on the exact timing of renography in the postnatal period. The European Society of Pediatric Radiology recommends two ultrasounds scans three months apart before obtaining renal scintigraphy, whereas in the United States a single ultrasound scan is deemed sufficient [12]. Our patients, at the time of diuretic renography had at least one postnatal ultrasound scan. The latest recommendations suggest indications and the exact timing for the procedure of diuretic renal scintigraphy. Sinha et al. [5] recommend that infants with moderate to severe hydronephrosis (APD > 10 mm), or mild hydronephrosis with ureteric dilatation and no evidence of VUR, undergo diuretic renography. Since immaturity of the renal function results in reduced radiotracer uptake, these authors suggest that renography should be done at 6–8 weeks of life, but may be performed earlier in patients with severe hydronephrosis and cortical thinning, and the procedure may be repeated after 3–6 months in infants where ultrasound shows worsening of pelvicalyceal dilatation. We suggest that the timing of the repeated procedure is not definite and that it varies with the patient's age, initial renal function and the persistence or worsening of ultrasonographic findings.

Diuretic renography allows detection non obstructive hydronephrosis, and estimating SRF. Initial SRF below 35–40% in the kidney with poor drainage signifies impaired function [13].

Our results suggest that even in the presence of partial, poor or no drainage, SRF may not be significantly impaired. Possible explanation for this finding is the delayed elimination of radiotracer. Our results confirm the conclusion of Moon et al. [14] study that other features, including ipsilateral supranormal SRF (≥ 55%) and prolonged time to clear 50% of radionuclide (Tmax/2 >20 minutes) [15,] can indicate obstruction. On the other hand, our study confirms a strong correlation between the APD and grading SRF.

In the absence of prospective controlled studies, there is a variability in practice regarding the use of antibiotics in children with moderate to severe obstructive hydronephrosis. The rates of urinary tract infections (UTI) in patients with severe obstructive hydronephrosis due to PUJ obstruction or megaureter varied from 0–4.3% to 19–36.2% in different studies [16, 17]. Coelho et al. [18] report that infants with the postnatal renal pelvic APD of 10 mm or more have significantly greater risk of UTI (relative risk 2.6, 95% confidence interval 1.2–5.8) comparing to those with mild hydronephrosis. We suggest that infants with the postnatally confirmed moderate or severe hydronephrosis (APD >10 mm) or dilated ureter should receive antibiotic prophylaxis, as well as all the patients detected to have VUR while awaiting evaluation.

Infants with lower urinary tract obstruction are immediately referred to the surgeon, while surgery is considered also in those with bilateral hydronephrosis or with solitary kidney showing worsening dilatation and deterioration of function [5]. While most experts suggest that pyeloplasty should be considered in patients showing poor drainage and differential function below 40% [19], others propose surgery at differential function below 35% [20], or obstructed renogram with prolonged Tmax/2 > 20 minutes [21]. Other indications for surgery include the presence of pain, palpable renal lump or recurrent febrile UTI [22]. The presence of large APD exceeding 20–30 mm predicts the need for surgery in 50–55% patients [19]. We emphasize the importance of monitoring the magnitude of SRF, because the decrease of SRF indicates deterioration and possible need for surgical correction in order to prevent significant damage of renal function.

Conclusion

Although ANH is mostly benign condition and has favorable outcome, it can also cause a significant morbidity. For this reason we want to stress the importance of prenatal ultrasound screening in pregnancy. The purpose of our study was to describe the clinical outcomes of infants with ANH and to contribute to the definition of postnatal evaluation of these patients. Based on our results and practice, we also want to emphasize the importance of diuretic renography, especially in distinguishing nonobstructive hydronephrosis from obstructive, and in the assessment and monitoring of SRF. Our results suggest that even in the presence of partial or no drainage, the SRF may not be significantly impaired.

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The effect of antipsychotic drugs on nonspecific inflammation markers in the first episode of schizophrenia

Efekat antipsihotika na nespecifične markere inflamacije u prvoj epizodi shizofrenije

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Abstract

Background/Aim. Immune system disorder, including inflammation, takes a significant place when considering still unclear etiology of schizophrenia. The aim of this study was to determine the blood levels of nonspecific inflammation markers in the first episode of schizophrenia and their relation to the therapy response. **Methods.** In this study we determined the blood levels of nonspecific inflammation markers: white blood cells count (WBC), C-reactive protein (CRP), erythrocytes sedimentation rate (ESR) and the elements of differential white blood cell counts (or the leukocyte formula): granulocytes (Gra), lymphocytes (Lym) and monocytes (Mon), in the first episode of schizofrenia, in 78 patients hospitalized at the Clinic for Psychiatric Disorders "Dr Laza Lazarević" in Belgrade. The levels were measured at admission to the clinic, as well as after 4 weeks of antipsychotic treatment. The Positive and negative syndrome scale for schizophrenia (PANSS) was applied to measure the severity of psychopathology and response to the treatment. **Results.** During the first episode of schizophrenia, before initiation of antipsychotic treatment, the frequency of abnormal values was high (≥ 25% of the patients) for the following non-specific inflammation markers: WBC, CRP, ESR and Gra, in the leukocyte formula, but dropped after 4 weeks of antipsychotic treatment at the level of high statistical significance for WBC and Gra ($p < 0.001$). The ESR remained unchanged in as many as 50% of the patients even after 4-week antipsychotic treatment, at the level of statistical significance in the non-responders compared to the responders ($p = 0.045$). **Conclusion.** The obtained results indicate that in the first episode of schizophrenia the blood levels of non-specific inflammation markers (WBS, CRP, ESR and Gra from the leukocyte formula) were high in the subpopulation of patients with the tendency towards normalization of inflammation parameters after a 4-week antipsychotic treatment.

Key words:
schizophrenia; antipsychotic agents; inflammation mediators; sensitivity and specificity; predictive value of tests.

Apstrakt

Uvod/Cilj. U razmatranju još uvek nepoznate etiologije shizofrenije, disfunkcija imunskog sistema koja uključuje i inflamaciju zauzima značajno mesto. Cilj našeg rada bio je da se odrede koncentracije nespecifičnih markera zapaljenja u krvi, u prvoj epizodi shizofrenije i njihova povezanost sa terapijskim odgovorom na antipsihotike. **Metode.** U radu smo određivali koncentracije nespecifičnih markera zapaljenja u krvi: leukocita (WBC), C-reaktivnog proteina (CRP), sedimentacije eritrocita (SE) i elemenata leukocitarne formule: granulocita (Gra), limfocita (Lym) i monocita (Mon), i to u prvoj epizodi šizofrenije, kod 78 hospitalizovanih bolesnika u Klinici za psihijatrijske bolesti „Dr Laza Lazarević" u Beogradu. Njihove koncentracije određivali smo pri prijemu i četiri sedmice nakon antipsihotičke terapije. Težinu psihopatologije i farmakoterapijski odgovor pratili smo primenom Skale pozitivnih i negativnih sindroma shizofrenije (Positive and negative syndrome scale for schizophrenia – PANSS). **Rezultati.** U prvoj epizodi shizofrenije, pre uvođenja antipsihotika, postojala je visoka učestalost abnormalnih laboratorijskih vrednosti (≥ 25% bolesnika) sledećih ne-

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specifičnih markera inflamacije: WBC, CRP i SE, kao i Gra u leukocitarnoj formuli, ali i smanjenje svih njih nakon četiri sedmice antipsihotičke terapije, na nivou visoke statističke značajnosti za WBC i Gra ($p < 0.001$). Sedimentacija eritrocita ostala je povećana kod čak 50% bolesnika i nakon 4-sedmičnog antipsihotičkog lečenja, na nivou statističke značajnosti kod onih koji nisu reagovali na terapiju u odnosu na one koji jesu ($p = 0.045$). **Zaključak.** Dobijeni rezultati pokazuju da u prvoj epizodi shizofrenije kod subpopulacije bolesnika postoje povećane vrednosti nespecifičnih markera inflamacije u krvi (WBC, CRP , SE i Gra iz leukocitarne formule), sa tendencijom njihove normalizacije nakon četiri sedmice antipsihotičkog tretmana.

Ključne reči:
shizofrenija; antipsihotici; zapaljenje, medijatori; osetljivost i specifičnost; testovi, prognostička vrednost.

Introduction

Schizophrenia is a heterogeneous disorder with still unclear etiology that affects about 1% of the world population. Numerous theories have been considering the possible causes of this devastating disease [1].

Recent researches related to neuroinflammation in schizophrenia give an increasing importance to prolonged microglial activation [2–4], when pro-inflammatory cytokines and free radicals lead to apoptosis of cortical neurons and oligodendrocytes as well as changes of the synaptic organization in the brain [5, 6].

Increased serum concentrations of various cytokines and their soluble receptors, as well as interleukin-6 (IL-6), soluble interleukin-6 receptor (sIL-6R), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α) were observed in schizophrenic patients [7].

The effect of antipsychotics in terms of reduction and normalization of various proinflammatory immune parameters is an important factor which contributes to the clinical efficacy in the treatment of psychotic symptoms [8–10].

Inflammation in schizophrenia is also associated with the increased production of prostaglandin E2, and the increased expression of cyclooxygenase-2 (COX-2), of which the inhibitors can have a significant role in the treatment of schizophrenia, particularly in the early stage of the disease [11].

There is growing evidence of significant effects of pro- and anti-inflammatory cytokines in the tryptophan/kynurenine metabolism when the increased production of kynurenine acid leads to glutamatergic hypofunction and consequent dopaminergic dysfunction in schizophrenia [12]. In the subpopulation of psychotic patients there is a high degree of comorbidity with chronic inflammatory and autoimmune disorders, which suggests a common immune disorder background [13].

The proteins and immunoglobulins of the acute phase are nonspecific markers of the immune system changes. Their levels may be affected by a variety of conditions, infection, inflammation and stress. As isolated parameters they cannot be directly linked to the development of schizophrenic psychoses but can be used as an additional parameter in explaining the role of specific immune subsystems.

The aim of our study was to establish the blood levels of nonspecific inflammation markers [white blood cells (WBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)] and the elements of the leukocyte formula) in patients with the first episode of schizophrenia, who up to then did not take antipsychotics (druge naive), as well as the effect of antipsychotic treatment after four weeks of treatment in correlation with clinical treatment response by implementing the Positive and negative syndrome scale for shizophrenia (PANSS).

Methods

The study included 78 patients hospitalized at the Clinic of Psychiatric Disorders “Dr. Laza Lazarevic” in Belgrade, during a 6-month period. At admission to the Clinic all subjects met the criteria of the International Classification of Diseases, 10th revision, for the first episode of schizophrenia (F 20). The patients signed the consent to participate in the study abiding by the principles of Good Clinical Practice and prior approval of the Ethics Committee of the Clinic. The study protocol was in compliance with the Declaration of Helsinki.

The inclusion criteria were the age between 18 and 45 years, both genders, and that the patients had not previously received antipsychotic drugs (drug naive).

The exclusion criteria were comorbidity with inflammatory, neurodegenerative, malignant diseases, congestive heart disease and infectious diseases, as well as patients who were identified as alcohol or psychoactive substance abusers.

The patients were divided into 3 groups depending on the applied antipsychotic therapy, the group I of patients treated with first-generation antipsychotics (FGAs), a total of 38 patients; the group II of 22 patients treated with second-generation antipsychotics (SGAs), and the group III of 18 patients treated with combined antipsychotic therapy (antipsychotic combination of the first and second generation antipsychotics).

The protocol procedures implied three planned visits. The following activities were conducted at admission: clinical psychiatric exploration that included a structured clinical interview in order to evaluate the diagnosis of schizophrenia according to the criteria of the International statistical classification of diseases and related health problems, 10th revision (ICD-10); application of the PANSS for the assessment and clinical monitoring of the disease course and pharmacotherapeutic response; as well as the physical examination including measuring of vital parameters (heart rate, arterial blood pressure, respiratory rate per minute, body temperature); venous blood sampling after a 12-hour overnight fast, between 8 and 8.30 a.m., prior to antipsychotic therapy (leukocytes, lymphocytes, monocytes, granulocytes, ESP, CRP) .

The attending psychiatrist prescribed antipsychotic treatment for the patients, pursuant to the Good Clinical Practice Guidelines.

The applied drug dosages were as follows: haloperidol – from 2 to 15 mg/pd (approximately 12.7 mg/pd at admission, average 8.8 g/day at dismissal), risperidone – from 2 to 6 mg/day (approximately 3.6 mg/day); olanzapine – from 2 to 20 mg/day (approximately 8.3 mg/day), and clozapine – from 25 to 125 mg/day (approximately 67.3 mg/day).

The patients were hospitalized during the entire treatment. All study procedures from the first visit (day 0), except for the already completed questionnaires, were also conducted after 30 days of hospital treatment, as well as at the final third visit. The second visit, two weeks after admission included clinical exploration.

The laboratory hematologic tests were carried out using a hematology analyzer ABX MICROS 60-OT (UK).

The primary obtained data were analyzed by the descriptive statistical methods and the application of the regression model. As for the descriptive statistical methods, the central tendency measures (arithmetic mean and median), the variability measures (standard deviation and variation interval) and the data structures expressed in percentages were applied. The methods for testing the difference of numerical data (scores on the PANSS scales, hematological and biochemical variables) included *t*-test and one-way analysis of variance. When conditions for the application of parametric statistical methods were not met we applied the Mann-Whitney test and the Kruskal-Wallis test. For testing the differences of categorical data (gender, education, marital status, treatment, and categorically transformed numeric data) Pearson's χ^2-test and Fisher's exact probability test were applied. The repeated measurements of continuous numeric data were analyzed using the repeated measures analysis of variance, and when appropriate conditions were not met, we applied the Wilcoxon test. Statistical hypotheses were tested at the level of significance of 0.05.

Results

This study included 45 (57.7%) female, and 33 (42.3%) male patients out of a total of 78.

The socio-demographic characteristics indicate that in relation to gender, there was a statistically significant difference between males and females with regard to education (p = 0.002) and employment (p = 0.028) (Table 1).

Table 1

Socio-demographic characteristics of the study participants

Characteristics	Male (n = 33)	Female (n = 45)	*p*
Age (years), n (%)	16 (49)	13 (29)	0.192
18–30	9 (27)	19 (42)	
31–40	8 (24)	13 (29)	
≥ 41			
Married, n (%)	4 (12)	10 (22)	0.251
Education, n (%)			0.002**
Elementary school	3 (9)	7 (16)	
High school	26 (79)	18 (40)	
University	4 (12)	20 (44)	
Employment, n (%)	5 (15)	17 (38)	0.028*
Heredity, n (%)	11 (33)	14 (31)	0.835
Method of hospitalisation, n (%)			0.085
voluntary	18 (55)	33 (73)	
forced	15 (45)	12 (27)	
Cigarete smoking, n (%)	15 (45)	19 (42)	0.776
DUP, n (%)			
up to 30 days	7 (21)	13 (29)	0.443
2–6 months	8 (24)	14 (31)	
> 6 months	18 (55)	18 (40)	

***p* < 0.05 ; ****p* < 0.01; DUP – duration of untreated psychosis.**

Figure 1 shows the percentage of abnormal laboratory values of nonspecific inflammation markers (WBC, Gra, Lym, Mon, ESR, CRP), at admission and after a 4-week antipsychotic treatment. In our study, abnormal values were defined as those higher than the reference range.

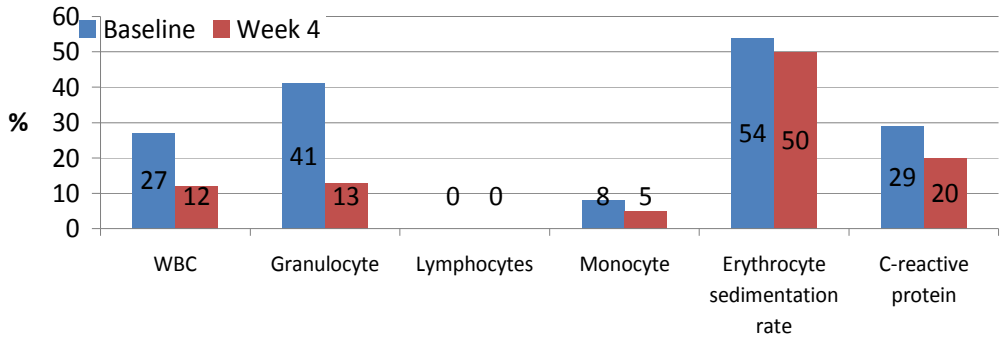

Fig. 1 – The percentage of patients with abnormal laboratory values of nonspecific inflammation markers at admission and after 4 weeks of antipsychotic treatment.

Note: abnormal is defined as levels of values higher than the reference range: white blood cells (WBC) – 3.5–10 × 10^9/L; granulocytes – 43.0–76.0%; lymphocytes – 17.0–78.0%; monocytes – 4.3–10.0%; erythrocyte sedimentation rate – 2–12 mm/h; C-reactive protein – 0–5 ng/L.

With regard to the observed values of PANSS scores (total and subscales: positive, negative and general psychopathology) and nonspecific markers of inflammation at the admission and after four weeks antipsychotic treatment, there was no statistical significance only when ESR and C-reactive protein were concerned (p = 0.970 and p = 0.359, respectively) (Table 2).

The responders had statistically less values at the PANSS total score and subscales compared to the non-responders after 4 weeks of antipsychotic treatment ($p < 0.001$), while there were no statistically significant differences in the therapy response among the antipsychotic therapy groups (p = 0.215) (Table 3).

The differences between the therapy responders and non-responders in relation to the nonspecific inflammation markers at admission and after 4 weeks of antipsychotic therapy showed a statistical significance with regard to ESR (p = 0.045) (Table 4).

Table 2

The values of positive and negative syndrome scale for schizophrenia (PANSS) scores and nonspecific markers of inflammation at admission and after 4 weeks of antipsychotic treatment (control visit)

Parameters	Baseline ($\bar{x}$ ± SD)	Control visit ($\bar{x}$ ± SD)	p
PANSS positive subscore	25.4 ± 5.7	12.2 ± 5.7	< 0.001**
PANSS negative subscore	21.8 ± 5.8	12.9 ± 6.3	< 0.001**
PANSS general psychopathology	52.9 ± 6.5	29.9 ± 11.5	< 0.001**
PANSS total score	100.1 ± 13.0	55.0 ± 21.8	< 0.001**
WBC ($\times 10^9$/L)	9.1 ± 3.1	7.8 ± 5.4	< 0.001**
Granulocytes (%)	72.3 ± 11.0	65.9 ± 9.1	< 0.001**
Lymphocytes (%)	22.5 ± 8.9	27.9 ± 7.7	< 0.001**
Monocytes (%)	5.2 ± 2.9	6.7 ± 4.4	0.001*
ESR (mm/h)	18.1 ± 16.2	17.9 ± 17.1	0.970
C-reactive protein (ng/L)	7.8 ± 20.9	3.7 ± 2.1	0.359

***p* < 0.01; ****p* < 0.001; WBC – white blood cells; ESR– erythrocyte sedimentation rate.**

Table 3

The differences between the therapy responders and non-responders in relation to the antipsychotic groups and positive and negative syndrome scale for schizophrenia (PANSS) scores at admission and after 4 weeks of antipsychotic treatment

Characteristics	Responders (n = 36)	Non-responders (n = 42)	p
Antipsychotic therapy, n (%)			0.215
first generation antipsychotics	14 (38)	24 (57)	
second generation antipsychotics	11 (31)	11 (26)	
antipsychotic combination	11 (31)	7 (17)	
Scores at baseline, $\bar{x}$ ± SD			
PANSS positive subscore	26.1± 5.3	24.8 ± 6.0	0.460
PANSS negative subscore	22.8± 4.9	20.9 ± 6.4	0.119
PANSS general psychopathology	53.4 ± 4.8	52.5 ± 7.7	0.845
PANSS total score	102.3 ± 10.8	98.2 ± 15.5	0.405
Scores at week 4, $\bar{x}$ ± SD			
PANSS positive subscore	8.6 ± 2.1	15.3 ± 6.1	< 0.001*
PANSS negative subscore	8.7 ± 3.0	16.4 ± 6.3	< 0.001*
PANSS general psychopathology	21.0 ± 4.9	37.6 ±10.0	< 0.001*
PANSS total score	38.4 ± 7.8	69.2 ± 19.7	< 0.001*

***p* < 0.001; Antipsychotic combination – combination of the first and second generation antipsychotics.**

Table 4

The differences between the therapy responders and non-responders in relation to nonspecific inflammation markers at admission and after 4 weeks of antipsychotic treatment

Characteristics	Responders (n = 36)	Non-responders (n = 42)	p
Values at baseline, $\bar{x}$ ± SD			
WBC ($\times 10^9$/L)	9.5 ± 3.6	8.7 ± 2.5	0.584
Granulocytes (%)	71.5 ± 11.4	72.8 ± 10.6	0.690
Lymphocytes (%)	23.1 ± 9.5	22.0 ± 8.3	0.902
Monocytes (%)	5.4 ± 2.9	5.1 ± 2.9	0.627
ESR (mm/h)	15.8 ± 15.4	20.0 ± 16.4	0.193
C-reactive protein (ng/L)	9.6 ± 27.9	5.8 ± 10.6	0.518
Values at week 4, $\bar{x}$ ± SD			
WBC ($\times 10^9$/L)	8.8 ± 7.6	6.9 ± 1.7	0.186
Granulocytes (%)	67.0 ± 10.2	64.8 ± 7.9	0.355
Lymphocytes (%)	27.0 ± 8.8	28.6 ± 6.6	0.387
Monocytes (%)	6.0 ± 2.2	7.4 ± 5.6	0.216
ESR (mm/h)	15.1 ± 15.6	20.8 ± 18.1	0.045*
C-reactive protein (ng/L)	3.5 ± 2.2	7.3 ± 20.3	0.161

***p* < 0.05 ; WBC – white blood cells; ESR – erythrocyte sedimentation rate.**

The combination of the first and second generation antipsychotics had weaker influence on nonspecific inflammation markers comparing to the first generation antipsychotics and second generation antipsychotics after a 4-week treatment, showing a statistical significance with regard to the value of WBC and lymphocytes, but no statistical significant changes in the blood concentrations of granulocytes and monocytes (Table 5).

Table 5

Effect of different groups of antipsychotics on nonspecific inflammation markers after four weeks of treatment

Antipsychotics	Baseline ($\bar{x} \pm SD$)	Control ($\bar{x} \pm SD$)	*p*
First generation antipsychotics			
WBC ($\times 10^9$/L)	9.1 ± 2.9	7.3 ± 2.1	0.001***
Granulocytes (%)	71.4 ± 11.6	66.8 ± 8.9	0.007**
Lymphocytes (%)	23.8 ± 9.9	27.1 ± 7.4	0.029*
Monocytes (%)	4.9 ± 2.3	6.0 ± 2.4	0.008**
ESR (mm/h)	19.3 ± 17.6	20.0 ± 18.2	0.579
C-reactive protein (ng/L)	6.7 ± 12.6	4.0 ± 2.5	0.872
Second generation antipsychotics			
WBC (x 10^9/L)	9.2 ± 3.3	8.7 ± 9.5	0.005**
Granulocytes (%)	72.9 ± 10.0	63.2 ± 8.4	0.001***
Lymphocytes (%)	21.± 8.0	29.6 ± 7.4	0.001**
Monocytes (%)	5.2 ± 2.8	8.8 ± 7.0	0.002**
ESR (mm/h)	20.2 ± 18.5	15.5 ± 16.9	0.133
C-Reactive protein (ng/L)	13.8 ± 35.1	3.5 ± 2.1	0.089
Antipsychotic combination			
WBC ($\times 10^9$/L)	9.0 ± 3.2	7.5 ± 2.1	0.050*
Granulocytes (%)	73.6 ± 11.7	67.2 ± 10.0	0.065
Lymphocytes (%)	20.5 ± 8.0	27.2 ± 8.7	0.025*
Monocytes (%)	5.,8 ± 4.1	5.5 ± 2.1	0.943
ESR (mm/h)	12.9 ± 6.8	16.1 ± 14.8	0.437
C-reactive protein (ng/L)	3.1 ± 2.4	3.4 ± 1.5	0.636

***p* < 0.05; ***p* < 0.01; ****p* < 0.001; WBC – white blood cells; ESR – erythrocyte sedimentation rate; Antipsychotic combination – combination of the first and second generation antipsychotics.**

due to antipsychotic therapy [16]. Prompted by many years of our clinical experience in work with psychotic patients and the numerous studies supporting the hypothesis that inflammation is involved in the etiopathogenesis of psychotic disorders [9, 15, 17], we came to the idea to do our study.

Having defined the blood levels of nonspecific inflammation markers (WBC with leukocyte formula, CRP, ESR) in patients with the first episode of schizophrenia before initiation of antipsychotic therapy, we discovered increased values of nonspecific inflammation markers in this subpopulation of patients (WBC in 27%, Gra in 41%, CRP in 29%, ESR in 54%, of patients). None of the patients had any subjective or objective indicators of acute infection syndrome, which was confirmed by internal examination. During a 4-week of hospital treatment no patient received antibiotic or anti-inflammatory therapy.

Discussion

In treatment of the first psychotic episode, the clinician's attention should be drawn to both the psychological and somatic symptoms as well as the laboratory parameters. Careful evaluation is especially important in the purpose of excluding many potential somatic and neurological causes of psychosis.

Research data indicate that the disorders of various body systems in schizophrenia (inflammation and immune processes, metabolic disorders, fatty acids metabolism, plasma antioxidants) do not have to be of secondary character, but may be an inherent part of schizophrenic disease itself [14]. Studies on antipsychotic-naive patients with first-episode psychosis find that inflammation is present already at this stage. Some of these abnormalities resolve after the initiation of treatment, suggesting that they are state markers of acute psychosis, but other abnormalities persist [15]. For this reason continuous monitoring of laboratory parameters is imposed as necessary already at the very beginning of the treatment, in order to clearly distinguish those abnormalities that are direct consequences of the disease itself from the disorders

Literature data related to research of nonspecific inflammation markers in schizophrenia are mainly focused on single inflammation markers.

WBC count is a well-established and widely used inflammatory marker [18]. Some studies support the fact that leukocytosis is found in patients with acute psychosis, and that under the antipsychotic therapy effect the number of leukocytes decrease [19, 20]. According to some authors higher white blood cell counts are associated with the increased risk for metabolic syndrome and more severe psychopathology (especially negative symptoms and symptoms of anxiety and depression, independent of age, gender, race, age of illness onset, smoking status and antipsychotic agent used [18, 21].

The results of one study indicate that a relative lymphopenia in the context of a relative granulocytosis appe-

ars to mark familial vulnerability for schizophrenia [20]. Recent researches indicate that the absolute levels of total lymphocytes were significantly increased in drug-naive first-episode psychosis [22]. Increased blood monocytes have been reported in schizophrenia [13, 23], and another study on intraindividual changes in blood monocyte levels found an association between monocytosis and worsening of psychotic symptoms [24]. The results of our study have shown a statistically significant decrease in WBC and Gra values after four weeks of antipsychotic treatment. In our study, however, monocytosis was present in only 8% of the patients at admission in the acute phase of the disease, while all patients had the lymphocyte counts within the reference laboratory range, which is not in compliance with the literature data.

C-reactive protein as acute phase protein is well-known as a nonspecific inflammation marker. Some recent studies have proved CRP increase in patients with schizophrenia [25, 26]. While according to some authors the elevated serum levels of C-reactive protein in schizophrenia are associated with the severity of cognitive impairment but not of psychiatric symptoms measured by the PANSS scale [27], the results of other authors indicate that elevated serum levels of CRP are associated with higher total scores on the PANSS, as well as higher scores on the Negative symptom subscale and the General psychopathology subscale of the PANSS [28]. A large number of studies have also confirmed the significant connection of increased CRP and some metabolic syndrome components (body mass index, HDL cholesterol) in schizophrenic patients [26]. Our study proved that 29% of the patients had increased CRP at admission, while CRP remained increased in 20% of the patients after four weeks of antipsychotic treatment.

Erythrocyte sedimentation rate is used as an indirect measure of the concentration of acute phase proteins [29]. One study revealed ESR increase in 17% of the patients with acute psychosis which decreased to normal values after eight weeks of antipsychotic treatment in 2/3 of those patients, with the reduction of psychopathological manifestations [30]. According to the claims of those authors, possibly those who had a high ESR, without a known physical illness, represent a subgroup of schizophrenia, thus ESR might possibly serve as a biological indication of the remission and relapse of the disease [30]. Waist circumference as a clinical measure of abdominal obesity, is reported to be significantly associated with an increased ESR. According to these authors there is also a strong correlation between systolic and diastolic and increased erythrocyte sedimentation which might potentially have everyday clinic significance by highlighting the correlation between blood pressure and intensity of inflammatory processes, which can be found in the background of metabolic syndrome development in patients suffering from schizophrenia [29].

Our study proved a high erythrocyte sedimentation rate in even 50% patients after a 4-week of antipsychotic treatment, that decreased only after applying the second generation antipsychotics. Whether ESR is a possible biological marker of early response to treatment, or is it a potential predictive marker of relapse in chronic patients, remains an issue for further prospective research in this direction.

The underlying mechanisms relating inflammation as reflected by elevated levels of WBC and CRP to schizophrenia are not well understood [21]. Also, there is no clear understanding as to how inflammatory-related pathways can precipitate the onset of psychiatric symptoms [31].

According to literature data, schizophrenic patients with high levels of inflammatory markers should be carefully monitored for metabolic syndrome. It dominantly prevails in schizophrenic patients and is correlated with low level chronic inflammation [26]. Monitoring for metabolic changes may be important within the first eight weeks of treatment, as changes can be determined very early in antipsychotic treatment [32]. Moreover, strategies to reduce inflammation may prevent metabolic syndrome in patients with schizophrenia who take atypical antipsychotic medication [18]. Early detection and consequent prevention of the metabolic syndrome is aimed to decrease diabetes and cardiovascular diseases risk that are the leading cause of mortality in schizophrenic patients [16].

In addition, it has been suggested that antidepressants, mood stabilizers and antipsychotic drugs act on inflammation-related pathways and therefore measuring levels of inflammation-related proteins in blood may be useful in monitoring treatment responsiveness [31]. Hence, we came to the idea that measuring levels of blood non-specific inflammation markers before and after antipsychotic treatment might be useful for measuring responsiveness to drug treatment.

Clinically significant response to therapy implies at least 50% reduction of the PANSS score [33]. According to this criterion, the results of our study show that there were 36 responders, or 46% patients, and 42 non-responders or 54% patients. They are in compliance with data from the literature which indicate that 40–50% patients have no optimum therapy response to antipsychotics [34]. Observing the nonspecific inflammation markers in correlation to the therapy response by implementing the PANSS scale, our results show that only the erythrocyte sedimentation values were statistically significantly higher in the non-responders compared to the responders.

Several studies have investigated the effects of antipsychotics on inflammation. Given the association between inflammation and schizophrenia, antipsychotics would be expected to have an anti-inflammatory effect. However, the anti-inflammatory effects of antipsychotics vary based on whether the antipsychotic is typical or atypical [35]. To date, there have been conflicting reports regarding the effects of antipsychotics on cytokine levels, and no antipsychotic has been shown to have consistent anti-inflammatory action [36].

The literature has largely ignored possible direct (not explained by metabolic syndrome) effects of antipsychotics on CRP and other inflammatory markers [37]. According to the results of our study, after 4 weeks of antipsychotic treatment there was a decrease of blood levels of non-specific inflammation markers (WBC, Gra, ESR, CRP), but the different antipsychotic therapy groups had different effects on

certain non-specific inflammation markers. Variable blood levels of nonspecific inflammation markers after antipsychotic treatment could possibly explain their still undefined mechanism of action in schizophrenia.

Lately, several trials have been conducted investigating the potential of anti-inflammatory agents to improve symptoms of schizophrenia [17, 15]. With regard to their usage and efficacy in adjuvant antipsychotic therapy in schizophrenia, the literature in this field is fraught with significant heterogeneity, including contradictory findings. Some of them claim that the results of aspirin addition to antipsychotic treatment seem promising, provided information on the efficacy on symptom severity [17], while the results of the other study indicate that adjunctive nonsteroidal anti-inflammatory drugs (NSAIDs) for schizophrenia may not benefit patients treated with first-line antipsychotics judged by the PANSS total score change. However, due to a limited database, further controlled studies are needed, especially in first-episode patients [38].

The limitations of our study relate to the relatively short follow-up period and assessment of a limited number of nonspecific inflammation markers, as well as the lack of personal experience related to the anti-inflammatory therapy application in the purpose of antipsychotic therapy augmentation.

Conclusion

The results of our study show that there is a subpopulation of patients in first-episode schizophrenia with increased values of nonspecific inflammation markers (WBC, CRP, ESR), tending to their normalization after 4-weeks of antipsychotic treatment.

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Importance of angle correction in transcranial color-coded duplex insonation of arteries at the base of the brain

Značaj korekcije ugla insonacije u transkranijalnom kolor dupleks ispitivanju krvnih sudova na bazi mozgu

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Abstract

Background/Aim. Transcranial color-coded duplex (TCCD) sonography allows visualization of the vessels being examined and measurement of the angle of insonation. The published literature suggests that blood vessels are insonated at the angle lower than 30 degrees, hence no correction for the angle is necessary. The aim of this study was to determine the availability of intracranial blood vessels for insonation, and the percentage of arteries and their segments which can be insonated at the angles lower than 30 degrees. **Methods.** The study included 120 patients (mean age 51). For each of the segments the angle of insonation was registered based on TCCD vizualization, and hemodynamic parameters were measured. The angle of insonation was measured using combined B-mode and color Doppler vizualization, as the angle between the direction of the ultrasound beam and the axis of the shown arterial segment. **Results.** The total success rate of insonation was 86.33% (1,554 out of 1,800). The mean angle of insonation value in all the examined arterial segments was 42 degrees. The insonation angle was higher than 30 degrees in about three quarters of the examined segments, especially in the A2 segment of the anterior cerebral artery (98%), the P1 segmet of the posterior cerebral artery (87%) and in the terminal internal carotid artery (83%). The A1 segment of the anterior cerebral artery showed the best insonation conditions with the angle of insonation lower than 30 degrees in 53% of the cases. **Conclusion.** The presented results of angles of insonation measurements for the anterior, middle and posterior cerebral arteries and their segments, as well as the terminal portion of the internal carotid artery clearly indicate that their average values in tested segments were very often higher than 30 degrees, which can cause an error in blood flow velocity measurement that cannot be ignored. The results confirm the necessity of correcting flow velocity values on the basis of the angle of insonation in TCCD sonography.

Key words:
ultrasonography, doppler, transcranial; ultrasonography, doppler, duplex; blood flow velocity; carotid artery, internal; anterior cerebral artery; middle cerebral artery

Apstrakt

Uvod/Cilj. Transkranijalna kolor kodirana dupleks (TCCD) sonografija omogućuje prikaz ispitivanih krvnih sudova i merenje ugla insonacije. Literatura govori u prilog tome da su uglovi insonacije uglavnom ispod 30 stepeni i da zbog toga nije potrebno raditi korekciju brzine. Cilj rada bio je da se utvrdi dostupnost intrakranijalnih krvnih sudova za insonaciju i procenat arterija i njihovih segmenata koji se mogu insonirati pod uglom manjim od 30 stepeni. **Metode.** Ispitivanjem je obuhvaćeno 120 bolesnika (srednje starosti 51 godine). Za svaki od segmenata registrovan je ugao insonacije na osnovu TCCD prikaza i merene su hemodinamske karakteristike. Ugao insonacije meren je kombinovanim B-mod i kolor doppler prikazom, kao ugao između pravca ultrazvučnog talasa i osovine prikazanog arterijskog segmenta. **Rezultati.** Insonacija je bila uspešna kod 86,33% insoniranih segmenata (1 554 od 1 800). Srednja vrednost ugla insonacije svih arterijskih segmenata bila je 42 stepena. Ugao insonacije bio je veći od 30 stepeni kod oko tri četvrtine ispitivanih segmenata, a naročito u A2 segmentu prednje moždane arterije (98%), P1 segmentu zadnje (87%) i u terminalnoj unutrašnjoj karotidnoj

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arteriji (83%). Segment A1 prednje moždane arterije pokazao je najbolje uslove za insonaciju, sa uglom manjim od 30 stepeni kod 53% bolesnika. **Zaključak.** Prikazani rezultati merenja uglova insonacije za prednju, srednju i zadnju moždanu arteriju i njihove segmente, kao i terminalnu unutrašnju karotidnu arteriju, jasno pokazuju da su njihove srednje vrednosti često veće od 30 stepeni, što može uzrokovati grešku u merenju brzine protoka koja se ne može zanemariti. Rezultati potvrđuju potrebu za korekcijom vrednosti brzine protoka u odnosu na ugao insonacije u TCCD sonografiji.

Ključne reči:
ultrasonografija, dopler, transkranijumska; ultrasonografija, dopler, dupleks; krv, brzina protoka; a. carotis interna; a. cerebri anterior; a. cerebri media.

Introduction

Modern neurosonological examination consists of Doppler sonography of cervical blood vessels and transcranial Doppler sonography of intracranial blood vessels. Although transcranial examination was one of the first attempts of medical use of ultrasound, it became a routine neurological diagnostic tool only since 1982 [1]. Transcranial Doppler sonography is performed by two types of devices, standard (blind) transcranial Doppler (TCD) and transcranial color-coded duplex (TCCD) sonography [2, 3]. Standard TCD does not show a live image of the structures being examined, but displays only the Doppler signal, therefore intracranial arteries are identified by indirect parameters such as: depth of the sample volume, position of the probe, flow direction and the reaction to the compression tests [4]. TCCD displays the image of blood vessels and measurement of the blood flow velocity. There is also the possibility to correct for the angle of insonation. The approaches to insonation for TCCD are not different from the standard TCD. The most important approach to displaying the anterior circulation, posterior cerebral arthery and veins is the transtemporal acoustic window [5]. Insonation approach to vertebrobasal structures is the transforaminal window [6].

Blood flow velocity and its changes during the cardiac cycle represent the basic data which are analyzed by Doppler sonography. Blood flow velocity is determined using a Doppler sonography computer, by processing the of differences frequency in emitted and received ultrasound waves which are reflected from erythrocytes in blood vessels (Doppler shift) using the Doppler formula. It is important to have in mind that the calculated velocity of blood flow can be accurate only if the Doppler probe is placed in such a way that the ultrasound beam is in the direction of blood flow. If the ultrasound beam is directed at the angle wider than 0 degree to a blood vessel, real blood flow velocity is equal to the measured velocity divided by the cosine function of the angle between the ultrasound beam and the long axis of a blood vessel. The cosine function ranges from 1 (when the angle of insonation is zero) to 0 (when the angle of insonation is 90 degrees).

Based on radiological and anatomical considerations, when TCD was introduced, it was assumed that the angle of insonation of the arteries at the base of the brain is similar and relatively small, so the normal values of flow rate were defined accordingly [7]. Given the lack of visual control, identification of certain vessels, and in particular their specific segments depends mostly on the experience of the examiner. The possibility of doing angle correction was available only for extracranial blood vessels with duplex ultrasound examination. With the introduction of TCCD, which involves the simultaneous use of the B-mode and color Doppler image and pulse wave Doppler signal, the possibility of measuring the insonation angle became available also in transcranial examination. Since then several works showing the importance of correcting the velocity by the angle of insonation have been published [8–10], including a smaller number of articles outlining the angle of insonation values for particular artery [usually only for the middle cerebral artery (MCA) and on a relatively small number of respondents)] [7].

Having in mind importance of transcranial ultrasound examination, and the significance of correct blood flow velocity measurements for evaluation of many pathological states, the possibility of using angles of insonation greater than 30 degrees, which largely alter the real blood velocity measured, cannot be ignored.

The aim of this study was to determine the availability of intracranial blood vessels for insonation, and the percentage of areteries and their segments which are insonated at angles lower than 30 degrees.

Methods

This study was conducted from March 2004 to March 2005 at the Clinic of Neurology the Military Medical Academy in Belgrade, Serbia. Inclusion criterion for the patients was that the neurological finding was negative for any pathological process of any origin in the brain, or significant heart disease. The subjects in which examination revealed hemodynamically significant stenotic or occlusive changes either in precranial, or intracranial arterial segments, as well as patients who did not have a sufficient temporal acoustic window were excluded from the study.

All the patients accepted precranial and transcranial Doppler sonography examination to be done, and signed the informed consent form.

Examination of patients was performed using a “Toshiba SSA 370A, Power Vision 6000” ultrasound, in the supine position after taking basic personal and medical history, blood pressure and pulse measurements.

Doppler sonography of blood vessels was done using a 7.5 to 11 MHz probe in B-mode and color Doppler mode to establish morphological abnormalities (if present), degree of lumen reduction, and the flow characteristics in the carotid and vertebral arteries: systolic [peak systolic velocity (PSV)] and

diastolic flow velocity [end diastolic velocity (EDV)] and the diameter of the vessel from which the machine calculates the mean flow velocity (MEANV), resistance index (RI), cross sectional area (AREA) and cardiac output flow (FLOW) [11].

Transcranial examination of the arteries at the base of the brain was carried out with a 2 MHz probe through the temporal and occipital acoustic window [6]. Figure 1 shows the Willis arterial polygon and in particular its components and branches: the distal portion of the internal carotid artery (ICA), anterior cerebral artery (ACA), MCA and posterior cerebral artery (PCA), which is a typical image in TCCD examination of intracranial arteries through the temporal window. The examination through temporal a window was carried out in the axial plane, usually through the middle and posterior part of this window.

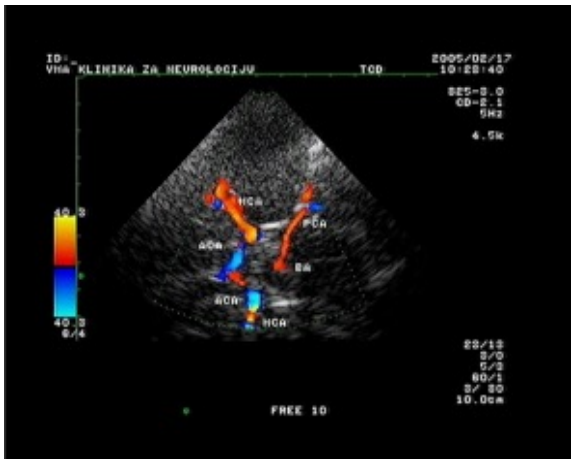

Fig. 1 – The Willis arterial polygon as can be seen by transcranial color-coded duplex sonographic examination of intracranial arteries through the temporal window.

It began with B-mode presentation of brain structures in mesencephalic plane and continued with color Doppler in order to obtain a better view of the terminal internal carotid artery (TICA), proximal precommunicating (A1) and postcommunicating (A2) segments of the ACA, the horizontal sphenoid (M1) and insular (M2) segments of MCA and the proximal precommunicating (P1) and distal mesencephalic (P2) segments of PCA [10]. For each of the registered segments angle of insonation based on TCCD, visualization and hemodynamic parameters (PSV, EDV, MEANV and RI) were measured. The angle of insonation was measured using combined B-mode and color Doppler visualization, as the angle between the direction of the ultrasound beam and axis of the shown arterial segment.

Statistical analysis was performed using the free PSPP Software version 0.8.4.

In order to determine the significance in the difference of the distribution of age between male and female patients, as well as the distribution of insonation angles between the left and right intracranial blood vessels *t*-test was used. Continous variables were summarized as mean ± standard deviation. The angle measurement results of the examined arteries and their segments were grouped by the angle of insonation into those less than and equal to 30 degrees, and those above 30 degrees.

Results

A total of 120 patients were examined. Sixty five (54%) were men, and fifty five (46%) women. The mean age of the patients was 51 ± 16 years. There was no statistically significant difference between the age of men and women (data not shown).

A total of 1,800 insonations were attempted. The rate of success of insonation of different arteries and their segments during the transcranial ultrasound examination is shown in Table 1. The total success rate of insonation was 86.33%. In almost all the patients (97.08–99.16%) arteries were insonated successfully. Their segments had lower insonation rates, ranging from 70% for the P1 segment of the PCA, to 82.50% for the A2 segment of the ACA.

Table 1

The success rates of transcranial insonation of different arteries and their segments

Artery/Segment	Right (n)	Left (n)	Total n (%)
Terminal ICA	118	119	237 (98.75)
ACA	116	117	233 (97.08)
ACA A1	97	100	197 (82.08)
ACA A2	99	99	198 (82.50)
MCA	119	119	238 (99.16)
PCA	118	116	234 (97.50)
PCA P1	89	79	168 (70.00)
PCA P2	86	84	170 (70.83)
BA	117		117 (97.50)
Intracranial VA	116	117	233 (97.08)
Total	721	833	1554 (86.33)

ICA – internal carotid artery; ACA – anterior cerebral artery; A1 – precommunicating, and A2 – postcommunicating segments; MCA – middle cerebral artery; PCA – posterior cerebral artery; P1 – proximal precommunicating, and P2 – distal mesencephalic segments; BA – basilar artery; VA – vertebral artery.

The mean angle of insonation value in all the examined arterial segments was 42 degrees. The proportion of insonation angles lower than 30 degrees and higher than 30 degrees for all the examined arteries and their segments is shown in Table 2. TICA was insonated at the angles less than and equal to the 30 degrees in only 17% of cases, while in 83% of cases the angle of insonation was higher than 30 degrees.

Table 2

Proportions of arteries and their segments insonated at angles below or above 30 degrees

Artery/Segment	Angle of insonation (%)	
	≤ 30°	> 30°
TICA	17	83
ACA A1	53	47
ACA A2	2	98
MCA	21	79
PCA P1	13	87
PCA P2	49	51
Total	25	75

TICA – terminal internal carotid artery; ACA – anterior cerebral artery; A1 – maximal precommunicationg, and A2 – postcommunicating segments; MCA – middle cerebral antery; PCA – posterior cerebral artery; P1 – proximal precommunicating, and P2 – distal mesencephalic segments.

The A1 segment of the ACA was insonated at the angle lower than 30 degrees in 53% of the cases, while the A2 segment of the same artery was insonated at the angle lower than 30 degrees in only 2% of the cases. In approximately 75% of the respondents, the angle of insonation of the proximal A1 segment of the ACA was less than 45 degrees, while for the A2 segment about 80% of respondents were examined at an angle greater than 45 degrees, and in 23% the angle was greater than 60 degrees.

In 21% of cases the MCA was insonated at the angle lower than 30 degrees.

The PCA was examined under various conditions depending on the segment. P1 and P2 segments of the PCA were insonated at angles lower than 30 degrees in 13 and 49% of the cases, respectively. In approximately 58% of respondents the angle of insonation in the P1 segment was greater than 45 degrees. The postcommunicating segment P2 was insonated at a more favorable angle at about 30 degrees where the noted flow velocity was higher and the flow direction was away from the transducer. In about 78% of the patients the angle of insonation was less than 45 degrees.

In around three quarters of examined segments, the angle of insonation was higher than 30°.

Discussion

This study examined 120 patients without any cerebral pathologies to measure the angles of insonation while evaluating blood flow through cerebral blood vessels. Out of 1,800 potential insonations, 1,554 were successful, and the angles, ranging from 1 to 80 degrees, were obtained.

The average value was about 42 degrees. The literature generally considers the values of the angle of insonation to be less than 30˚, which was presented by Eicke et al. [8]. The range in their results, which was 0–70 degrees, is similar to the results of this study. The basic assumption of the authors that spoke in favour of these results was that the angle of insonation was usually less than 30˚ thereby the error in measuring the velocity does not exceed the value of around 15%. According to the presented results: cos 42˚ = 0.74, which makes the error of about 25% in measuring the velocity without corrections. The results of measurements of the angles of insonation of intracranial arteries show that they were greater than 30˚ in 75% of the examined segments.

Only in 17% of the cases, the angle of insonation of the terminal segment of the ICA was smaller than 30˚ and higher than 60˚ in the same percentage. It is a short arcuate segment of the ICA syphon in which the flow direction was toward the probe in one part, and away from the probe in another one. No data on the angles of insonation in this segment was found in the literature.

The ACA was insonated under different circumstances depending on the segment. The proximal A1 segment with excellent insonation conditions was insonated at the angle lower than 30 degrees in 53% of the patients. According to our results, this segment had the most favourable insonation conditions. Our results are similar to those presented by Bartels et al. [10] and Martin et al. [12], and differ significantly from the results of Baumgartner et al. [9]. The segment A2 was examined under a very unsatisfying angle in most cases. In 98% of the cases the angle of insonation was higher than 30˚. In the literature, no data on the insonation conditions of this segment through temporal window was found. More favorable conditions could be reached through the frontal acoustic window that has been described previously [13].

The MCA is the artery that is the most studied, using both conventional TCD, as well as more contemporary TCCD sonography. The results obtained by measuring the angles of insonation using this method can vary depending on the author. The most favorable results were measured by Baumgartner et al. [9], while Krejza et al. [14] and Tsuchiya et al. [7] obtained significantly higher values. About 20% of the examined segments of the MCA were lower than 30 degrees, and other 80% were greater, although in only 5% the angle was higher than 60 degrees. The PCA insonation conditions differ significantly in proximal and distal segments, which is consistant with its anatomical course. Segment P1, P2a and P2P and P3 segment in part are accessible to Doppler sonographic examination. In Doppler sonographic terminology two segments of PCA are present: P1 in which blood flow is directed towards the probe and P2 where flow direction is away from the probe. The segment P1 corresponds to precommunicating and anterior mesencephalic segments. It is usually insonated in a more distal part, close to the point where flow of blood changes direction in relation to the probe. The result obtained by Bartels et al. [10] was close to this while the angles received by Baumgartner et al. [9] and Martin et al. [12] were significantly lower. The results of the two authors are close to those obtained in the distal P2 segment, which has much more favorable insonation conditions, in about 49% of the segments the angle was lower than 30 degrees. The authors whose results can be found in the available literature did not specifically analyze the insonation conditions of the segments P1 and P2.

Conclusion

The presented results of the angles of insonation measurements for the anterior, middle and posterior cerebral arteries and their segments, as well as the terminal portion of the internal carotid artery clearly show the values which are very often higher than 30 degrees. This situation can cause an error in blood flow velocity measurement that should not be ignored. The results confirm the necessity of correcting flow velocity values on the basis of the angle of insonation in transcranial color-coded duplex sonography.

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Epidemics of the central nervous system infections caused by West Nile virus in the territory of the South Bačka District, Vojvodina, Serbia

Epidemija infekcija centralnog nervnog sistema virusom Zapadnog Nila na teritoriji Južnobačkog okruga, Vojvodina, Srbija

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Abstract

Background/Aim. West Nile virus (WNV) is a neurotropic RNA virus particle which belongs to the *Flaviviridae* family, genus *Flavivirus*. It is sustained in arthropods within the transmission cycle between the mosquitoes and birds. Most commonly (80% of cases) WNV infections are asymptomatic among people. Less than 1% of patients develop neuroinvasive forms of the disease – meningitis, encephalitis, or acute flaccid paralysis. The aim of the research was to determine most common clinical and laboratory manifestations, to emphazise the presence of comorbidities and outcomes of treatment among patients with WNV infection. **Methods.** This retrospective study, which was conducted in the period from January 1, 2012 to December 31, 2013, evaluated 32 patients who were diagnosed with WNV infection based on clinical findings, laboratory, and serological tests. To assess statistical significance we used χ^2, and *t*-test. **Results.** The study involved 22 (69%) males and 10 (31%) females aged from 31 to 65 years. On admission, there were 16 (50%) febrile individuals, 27 (84.4%) with positive meningeal signs, 17 (53.2%) with pathological neurological signs, and 10 (31.3%) with consciousness disorders. WNV infection was confirmed by the method enzyme linked immuno sorbent assay (ELISA) in all the patients, while Reverse Transcription Polymerase Chain Reaction (RT-PCR) test was positive in 3 (30%) of the tested patients. Cardiovascular comorbidities dominated in 7 (21.9%) of the cases. Full recovery was accomplished in 87.5 % of the cases. **Conclusion.** The results of our study show that the absence of meningeal signs and fever on the day 7 of hospital treatment are indicators of good course and prognosis of neuroinvasive forms of WNV infection. Comorbidities do not increase the risk of disease. ELISA test is a sovereign diagnostic method. In most cases, after the administered symptomatic therapy, the complete recovery of patients was achieved.

Key words:
west nile virus; central nervous system viral diseases; diagnosis; treatment outcome; prognosis; serbia.

Apstrakt

Uvod/Cilj. Virus *West Nile* (WNV) je neurotropna RNA virusna partikula koja pripada porodici *Flaviviridae*, rodu *Flavivirus***.** Održava se u artropodama u okviru transmisivnog ciklusa između komaraca i ptica. WNV infekcije su asimptomatske kod 80% ljudi. Manje od 1% inficiranih osoba razvije neuroinvazivni oblik bolesti**,** meningitis, encefalitis ili akutnu flakcidnu paralizu. Cilj rada bio je da se utvrde najčešće kliničko-laboratorijske manifestacije, prisustvo komorbiditeta i ishod lečenja kod obolelih od WNV infekcije. **Metode.** U ovu retrospektivnu studiju, sprovedenu u periodu od 1. 1. 2012. do 31. 12. 2013. godine, bila su uključena 32 bolesnika, kojima je na osnovu kliničkog nalaza, laboratorijskih i seroloških analiza dijagnostikovana WNV infekcija. U svrhu ocene statističke značajnosti korišćeni su χ^2 i *t*-test. **Rezultati.** U studiju je bilo uključeno 22 (69%) bolesnika muškog pola i 10 (31%) žena, starosti 31 do 65 godina. Povišenu temperaturu na prijemu imalo je 16 (50%) bolesnika, pozitivne meningealne znake 27 (84.4%), patološke neurološke znake 17 (53,2%), a poremećaj stanja svesti 10 (31,3%). WNV infekcija je kod svih obolelih potvrđena metodom e*nzyme linked immuno sorbent assay* (ELISA). Istovremeno *reverse transcription polymerase chain reaction* (RT-PCR) test bio je pozitivan kod 3 (30%) testirana bolesnika. Od komorbiditeta, najčešće su registrovane kar-

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diovaskularne bolesti, kod 7 (21,9%) bolesnika. Potpuni oporavak postignut je kod 87,5% bolesnika. **Zaključak.** Rezultati naše studije pokazali su da su odsustvo febrilnosti i pozitivnih meningealnih znakova sedmog dana hospitalizacije pokazatelji dobrog ishoda neuroinvazivnih oblika WNV infekcije, a da prisustvo komorbiditeta ne povećava rizik od obolevanja od ove bolesti. ELISA predstavlja vrhunsku metodu za postavljanje dijagnoze. Kod najvećeg broja obolelih simptomatska terapija dovodi do potpunog oporavka.

Ključne reči:
virus zapadnog nila; nervni sistem, centralni, virusne bolesti; dijagnoza; lečenje, ishod; prognoza; srbija.

Introduction

West Nile Virus (WNV) is a small neurotropic RNA virus particle that belongs to the *Flaviviridae* family, genus *Flavivirus*. It is sustained in arthropods within the transmission cycle between the mosquitoes and birds. WNV is the most widely spread arbovirus type in the world [1].

Birds are natural reservoirs and hosts of WNV infection, while human population and other mammals could be occasionally infected after being bitten by infected mosquitoes [2]. WNV was originally identified in 1937 in the endemic region of Uganda in a female patient with moderate febrile condition. It is the cause of sporadic infection cases in Europe and epidemics in endemic regions of Africa, southern Asia, and northern Australia. Starting from 1996, the WNV infection has been getting in significance in western countries causing massive epidemics or smaller cluster epidemics of WNV encephalitis in Europe [2–4].

In the United States of America (USA), the WNV was recognised for the first time in 1999 when it became the key public health problem spreading across several countries. Today, this flavivirus represents the leading cause of neuroinvasive arboviral disease in the USA and it is responsible for the occurrence of focal seasonal epidemics. Until 2010 almost 1.8 million people in the USA were infected with WNV, and 12,851 cases of meningoencephalitis and 1,308 lethal outcomes of infection were reported [2, 4, 5].

In 80% of cases, the WNV infection is asymptomatic. The symptoms occur in insignificant number of patients (around 20%), most frequently in the form of acute system febrile condition – West Nile fever. It is believed that in clinical manifested cases the incubation period lasts from 3 to 14 days. Monitoring of the described epidemics indicates the presence of febrile condition (body temperature above 39°C accompanied by fatigue, anorexia, nausea, vomiting, myalgias, artralgia, headache, macculopapulose rash and swollen lymph glands). The disease lasted for 3 to 6 days. In the epidemics occurring before 1999, there were also reported myocarditis, pancreatitis, and fulminant hepatitis cases. Less than 1% of patients with WNV infection develop a neuroinvasive form of disease that is manifested as meningitis, encephalitis, or acute flaccid paralysis and accompanied by higher morbidity and mortality [4–6].

Transitional viremia occurs in patients infected with WNV directly prior to the occurrence of initial symptoms, *ie* in the early disease stage (5–14 days after inoculation) and it lasts for 4 to 9 days. The production of neutralising antibodies leads to termination of viremia and transiting of the disease in the symptomatic phase. Prolonged viremia is found in immunocompromised patients who cannot develop an adequate immune response in the presence of virus particles. Specific immunoglobulins of IgM class are detected in the patient's serum 2 to 8 days after the occurrence of initial disease symptoms and this is accompanied with the increase of WNV-specific IgG antibodies [5].

WNV genome is a single-stranded positive-polarity ribonucleic acid (RNA) molecule with approximately 11,000 nucleotides built up by 3 structural and 7 non-structural proteins. Out of five WNV lineages, lineages 1 and 2 are most widely spread in the world. Until 2004, only the WNV of lineage 1 circulated across Europe. That same year the WNV particle of lineage 2 was isolated for the first time in Hungary in the sample taken from the infected goshawk [7].

The establishing of etiological WNV infection diagnosis is based on a routine use of serological tests: enzyme-linked immunosorbent assay (ELISA), immunofluorscence assay (IFA), neutralization test (NT), and hemagglutination-inhibition test. The neutralization tests is considered most specific, it is difficult to perform, time consuming and it can be performed only in Biosecurity Level 3 (BSL-3) laboratories. Serological ELISA test is currently most widely used since it is fast, easy to perform, cheap and it enables detecting of immunoglobulin, *ie* IgM and IgG WNV antibodies [8]. The reverse transcription polymerase chain reaction (RT-PCR) is used to detect viral RNA during the acute disease phase. Sequencing of RT-PCR products can confirm the presence of WNV particle [9].

WNV is transferred onto people primarily *via* infected mosquito's bites (genus *Culex*) [5, 9, 10].

The WNV infection epidemics in human population in the territory of the Republic of Serbia were reported for the first time in the period from August to October 2012 in northern parts of the country, including the AP Vojvodina [3, 7, 11].

The detection and understanding of a complex group of factors that condition the creating of the focus of WNV infection and consequential spreading of this pathogen in the environmental are of the key significance for forecasting and mitigating of future epidemics [1].

The aims of this retrospective study were to determine the most frequent subjective health problems and clinical manifestations in patients with neuroinvasive forms of WNV infection, the presence of comorbidities in patients, the significance of laboratory and serological methods compared to other procedures in diagnostics, as well as the length of treatment, treatment outcomes and sequelae.

Methods

The study of WNV infection was conducted as retrospective study by analysing medical documentation of patients

who were treated at the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina in the period from January 1, 2012 to December 31, 2013. The study included 32 patients diagnosed with neuroinvasive form of WNV infection based on clinical findings, laboratory analyses, and serological tests.

In all the patients we analysed clinical (febrile condition), laboratory indicators of inflammation process [sedimentation value, C-reactive protein (CRP) and leukocytes, liquor findings] depending on age and sex, on the day of admission and on the day 7 of hospital treatment.

We analysed the presence of febrile condition among patients at admission and on the day 7 of hospitalisation classifying them either as afebrile (body temperature up to 37°C), sub-febrile (body temperature from 37 to 38°C), or febrile (body temperature above 38°C)**.** Lumbar puncture was performed at admission and on the day 7 of hospital treatment in all the patients within the analysed sample.

We monitored clinical and demographic indicators (patients' age, the presence of health problems on admission of patients to hospital treatment, duration of hospitalisation, place of residence).

Etiological diagnostics was carried out for all patients in the studied sample by means of isolation and identification of the virus and virus antigens from the patients' material using the method ELISA and/or by means of RT-PCR methods that were carried out at the Institute of Public Health of Vojvodina, Virology Department.

The results of the study were processed using standard statistical methods. The χ^2 and *t*-test were used for the purpose of statistical significance evaluation.

Results

Retrospective analysis showed that 32 patients with the diagnosed neuroinvasive form of WNV infection were hospitalised at the Clinic for Infectious Disease of the Clinical Center of Vojvodina in Novi Sad within a two-year period from the beginning of January 2012 to the end of December 2013. The distribution of patients by age showed the predominance of patients belonging to the working population [aged from 31 to 65 – 19 (59%)]. There were 3 (10%) patients younger than 30 and 10 (31%) geriatric patients (older than 65). Analysing the representation by gender among the observed patients we found 22 (69%) males and 10 (31%) females. The existence of statistically significant difference in distribution of patients by gender was not found, neither among the studied age groups ($p > 0.001$).

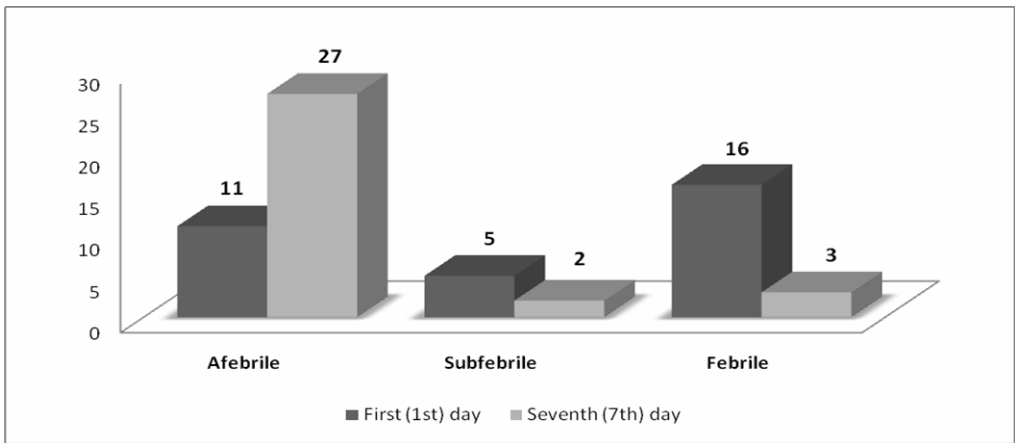
Febrile condition was present at admission in 21 (65.6%) patients, 5 (15.6%) were subfebrile and 16 (50%) febrile. On the day 7 of hospitalisation, we registered 27 (84.4%) afebrile, 2 (6.2%) subfebrile and 3 (9.4%) febrile patients. We presented the obtained results in Figure 1. Statistically significant difference was found between the number of febrile persons at admission and on the day 7 of hospital treatment ($p < 0.001$).

When it comes to indicators of inflammatory response, we analysed the CRP, total leukocytes count as well as lymphocytes values. In our sample there was no registered leukocytosis either at admission or on the day 7 of hospital treatment. Lymphocytosis was present in 2 (6.25%) cases at first as well as the day 7 of hospitalisation. The elevation of CRP at admission showed in 14 (43.75%) patients and in 12 (37.5%) patients on the day 7 of hospital treatment. There was no statistically significant difference between the analysed parameters ($p > 0.001$).

Etiological diagnostics was carried out at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina by applying serological testing of patients. ELISA test was carried out for all hospitalised patients and all the findings were positive (100%). Out of a total of 32 patients, PCR was carried out in 10 (31.25%) of them. Positive PCR findings for the presence of WNV genome were found in 3 (30%) of the patients out of the total number of tests that were carried out.

At the moment of patients' admission, positive meningeal signs were present with the total share of 27 (84.4%). The frequency of individual symptoms within the meningeal syndrome at admission was registered as – headache (78.1%), vomiting (34.4%), neck rigidness (43.8%), and photophobia (6.25%).

Neurological signs were determined in 17 (53.2%) of the cases and consciousness disturbances in 10 (31.3%) cases. On the day 7 of hospital treatment, we registered the



***p* < 0.001**

Fig. 1 – The presence of febrile condition at admission and on the day 7 of hospital treatment.

absence of consciousness disturbances and each individual symptom within the meningeal syndrome. The presence of neurological signs on the day 7 of hospitalisation was confirmed in 4 (12.5%) of the cases. We found a statistically significant difference between the share of the analysed clinical parameters (meningeal signs, neurological signs, consciousness disturbances) among individuals at admission and on the day 7 after the admission to hospital treatment ($p < 0.001$).

A percentage share of cerebrospinal fluid parameters representation is presented graphically in Figure 2.

We determined the presence of comorbidity and predisposing factors of the disease occurrence in the patients. Out of the total number of patients, the presence of accompanying diseases was registered in 12 (37.5%) of the cases. The highest frequency in the patients with comorbidity was exhibited by cardiovascular diseases (21.9%). We presented the distribution of the analysed groups of diseases in a graphic form (Figure 3) and found no statistically significant difference in the share of the analysed groups of diseases. The largest number of patients were released from the hospital treatment fully recovered, 28 (87.5%). Of the remaining 4 (12.5%) patients, 2 (6.25%) developed sequelae. Lethal outcome was registered in 1 (3.13%) of the cases.

Ciota et al. [12] confirmed that transmissive potential of *Culex pipiens* mosquito population decreased after WNV of the MP02 lineage infection despite high sustainability of those chains in the host's organism.

The development of WNV infection in endemic regions is linked with multiplication of population of *Culex* genus mosquitoes (*Culex pipiens, Culex restuans,* and *Culex tarsalis*) that are primary vectors. The above-mentioned mosquito genus pullulates and reproduces itself in stale, dirty, and putrid collections of ground water areas and flood prone regions, landfills and septic pits that are located within the residential infrastructure [13].

Within the project titled "*Detekcija virusa Zapadnog Nila kod populacije komaraca na teritoriji Republike Srbije*" ("Detection of the West Nile virus among the mosquito population in the territory of the Republic of Serbia"), the Institute for Biocides and Medical Ecology from Belgrade performed the tests on mosquitoes in the territories of municipalities of Novi Sad and Temerin in September, 2013. The traps were set at potential sites and live mosquitoes of *Culex* genus were isolated under the professional supervision of doctors of veterinary medicine. They were transported in adequate conditions and subsequently tested by PCR method. Out of 10 sites in the

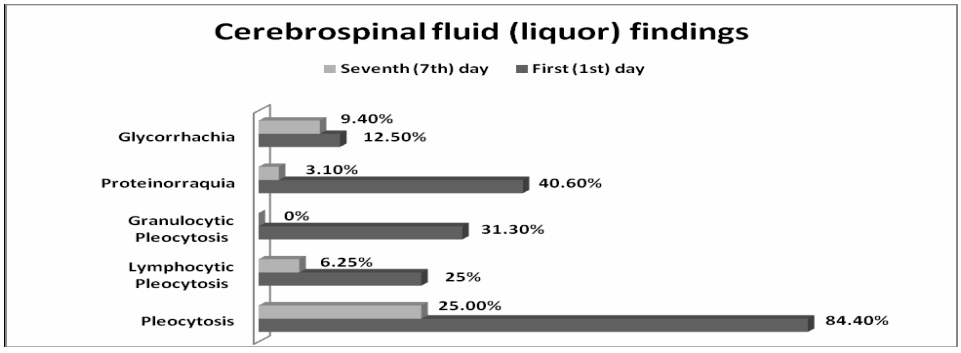


Fig. 2 – Registered values of the findings for cerebrospinal fluid (liquor).

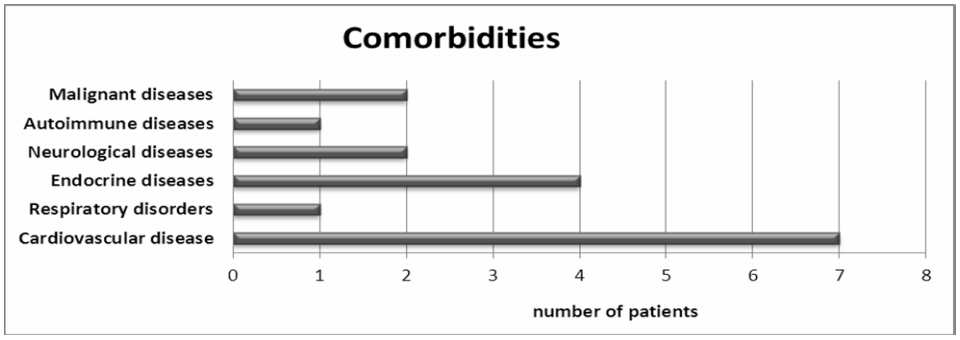


Fig. 3 – The share of registered groups of comorbidity among patients.

Discussion

Arboviruses, RNA viruses with huge inherent evolution potential, have been evolving very slowly until now. The life cycle and survival of the viruses of this group relies on vertical transmission that reduces their pathogen potential for the vertebrate host [12].

territory of Novi Sad, the presence of the viral genome was proved at 5 sites (50%). In the municipality of Temerin, the result of the PCR method was positive to the presence of the viral genome at one site out of 5 (20%) tested [14].

In the sample of 32 patients who were treated at the Clinic for Infectious Diseases of the Clinical Center of Vojvodina during 2012 and 2013 and included in our study,

there were 15 (59.4%) of the patients with the residence in the territory of the municipality of Novi Sad and 9.4 (3/32) in the territory of municipality of Temerin. Our results are in compliance with the data obtained in the analysis of mosquitoes from the same territory.

Thanks to the data of the Institute for Public Health from Novi Sad and Institute for Biocides and Medical Ecology from Belgrade, we produced the map of the analysed sites in the territory of the municipality of Novi Sad and residence of citizens with positive laboratory findings.

The European and American researchers agreed that more than 90% of infections caused by arboviruses show seasonal character and occur in the period from July to September [15, 16]. Both epidemic waves analysed in our study were recorded in the period from the beginning of August to the end of September 2012 and 2013.

In a long-term period from 1999 to 2012, more than 36,000 cases of WNV infection in human population were reported to the Centre for Disease Control and Prevention (CDC). Epidemiological department of this institution assess that starting from 1999 there were 2 to 4 million infected people in the territory of the USA, while some 400,000 to 1 million of infected people had some sort of symptomatology [14]. In the territory of the Republic of Serbia, there are no records on the number of infected people and the number of people with the West Nile virus.

In the Climatological Analysis of the Republic Hydrometeorological Service of Serbia that was conducted by Smailagić et al. [17], it was confirmed that the summer of 2012 was one of the hottest within the last few years. The obtained climatological data correlate with multiplication of the *Culex* genus mosquito population, and the occurrence of the first registered cases of infection with the West Nile virus in the territory of the Republic of Serbia.

The US CDC registered 5,245 cases of WNV infection until the end of November, 2012, including 256 lethal outcomes for the current year. Nasci [18] states in his report that 51% of the total number of infected individuals have neuroinvasive form of the disease (meningitis/meningoencephalitis). This has been the highest annual incidence of WNV infection diseases in the USA since 2003.

Immediately after the WNV RNA lineages were isolated from the serum of goshawk in the territory of Hungary and after it was proven they circulated in the blood of the infection reservoir (birds, horses) in the territory of Hungary, Austria, and Italy, a sharp wave of epidemics among humans occurred in the territory of Greece in 2010. During two seasons, there were 273 registered cases of neuroinvasive forms of West Nile virus infection in Greece [19].

In our region, all registered cases of WNV infection had neuroinvasive forms of the disease. The data from our study indicate the presence of neuroinvasive form of WNV infection (meningitis/meningoencephalitis) in all the patients within the analysed sample. It is assumed that the number of persons infected with WNV was much higher in both epidemic waves, during 2012 and 2013. Only those with the most severe forms of clinical picture were hospitalised and serologically tested.

Thanks to cooperation between the Clinic for Tropical and Infectious Diseases of the Clinical Center of Serbia and Institute for Virology Torlak, the report was complied and the epidemic of WNV infection in the territory of Belgrade and its surroundings was evaluated in 2012. Within this study, Popović et al. [3] emphasise the predominance of neuroinvasive forms of the disease. The results of our study are in compliance with the data from the study published by this group of authors from Belgrade.

The group of authors from Belgrade believe that the number of infected persons within the WNV epidemics in 2012 was higher than the registered one and that a significant number of patients with febrile condition resembling to influenza remained undiagnosed. It is assumed that these patients with nonspecific inflammatory symptomatology did not ask for medical assistance or they reported to the institutions of primary health care where they were treated symptomatically [3].

The results of national studies are in compliance with the conclusions of wider professional public. Kwan et al. [20] determined in the study that encompassed a six-year period (2004–2010) that the number of reported cases of West Nile fever and neuroinvasive forms of WNV infection in the territory of the state of California was not a representational sample compared to the entire population. The above-mentioned authors indicate the reduced number of performed serological tests in persons with febrile condition, which reduces a total number of reported disease cases.

According to the estimates of the US CDC, 30 to 70 cases of moderate neuroinvasive forms of disease could be added to each reported case of neuroinvasive form of WNV infection [15].

Epidemiological analysis of WNV infections in Texas was carried out in January 2013 for a multi-annual period from 2002 to 2011 and it was determined male Caucasian individuals aged 54 on the average dominate among the reported cases (59%) [21]. The data of the US CDC show insignificant differences in age and gender distribution in the territory of the USA – the share of male individuals among the ill makes 56% with the average age of 56 [15] . Our results in the analysed sample indicate the predominance of male individuals (68.75%) with a high frequency of diseases among the working population aged 30 to 65 (59.38%), which is in compliance with the conclusions of the American authors.

Nolan et al. [21] point to the mortality rate of 6.3% among patients with neuroinvasive WNV infection in the territory of Texas. The report from June 2013 describes that mortality or people with similar diseases in the entire territory of the USA reaches 5%, with the average age of 77 years [15]. Contrary to American studies, our study indicates a lower mortality rate among the patients – lethal outcome was found in 3.13% (1/32) of cases in the analysed sample.

PCR can be useful diagnostic procedure during an early stage of WNV infection. After seroconversion, it is much more likely that etiological diagnosis will be established *via* serological tests [22]. PCR testing was performed in 31.25% of patients in our study. A positive result was obtained in 30% of the analysed samples.

South African researchers Zaayman et al. [22] from the University in Pretoria described a lower incidence of positive PCR results stating 2 factors that conditioned a small number of positive PCR WNV tests: 1) arbovirus viremia lasts for a short time and cerebrospinal fluid retains a small number of viruses, and 2) certain percentage of falsely negative results will occur if the samples are not stored properly. Turkish experts pointed out that they did not obtain a single positive result of RT-PCR to the presence of WNV RNA in the analysed sample [23].

Serological testing using the method ELISA was performed in all the patients who were included in our study, which enabled etiological diagnosing of the disease. The obtained results are in compliance with the data of the Clinic for Tropical and Infectious Diseases of the Clinical Centre of Serbia described by Popović et al. [3] in their study. A larger number of European authors emphasise the significance and advantages of using the method ELISA for final diagnosing and determining of the causes of infection [19, 22, 23].

Garcial-Bocanegra et al. [24] believe that supervision over the domestic horses can be useful for monitoring of WNV genome circulation in endemic region, which is important from the aspect of public health. These attitudes are based on the established seroprevalence in horses who have not got ill and determined elevated frequency of neurological symptoms among horses compared to birds and humans [25].

Birds are the main natural WNV reservoirs. Local mobility of domestic birds and huge distances that migratory birds travel could contribute to spreading of the WNV infection [26–29]. Veterinary monitoring related to WNV infections was performed in the territory of the Republic of Serbia within the recent years. Petrović et al. [7] from the Scientific Institute for Veterinary Medicine in Novi Sad performed epidemiological monitoring and testing over domestic birds and migratory birds from the territory of the AP Vojvodina in the period from January to September 2012. The WNV presence and circulation among birds in the territory of the Republic of Serbia was confirmed serologically for the first time in 7 (8%) of the analysed samples.

The preventive procedures aimed at the prevention of WNV infection occurrence imply public-health measures that will preclude mosquito bites in human population. The fact is that vaccines against domestic forms of arbovirus are not available. Forms of prevention of arbovirus infections rely on the activities of social community and personal efforts to reduce the vector population. Education of population on significance of prevention and methods of implementation of personal protection measures (*via* posters, leaflets, media campaigns) and implementation of system measures directed to the vector-mosquito population (destruction of habitats, larvae, and adult mosquitoes, use of insecticides) would enable combating new epidemic waves of WNV infection. The relevant bibliography states that the use of repellents correlates with the reduction of the risk of development of WNV infection [3, 5, 17]. During the analysed epidemic wave in 2013, only the insignificant number of patients (3.13%) carried out personal protection measures and used repellents.

Conclusion

The absence of meningeal signs and fever on the day 7 of hospital treatment are indicators of good course and prognosis of neuroinvasive forms of WNV infection. The presence of comorbidities does not increase the risk of development of neuroinvasive forms of WNV infection. ELISA test is a sovereign diagnostic method compared to RT-PCR test that can serve as an indicator of acute infection. In most cases, after administering symptomatic therapy, the complete recovery of patients is achieved.

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Palliative embolization of renal tumors

Palijativna embolizacija tumora bubrega

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Abstract

Background/Aim. Palliative embolization of renal tumors is the method of choice in the treatment of advanced inoperable renal cell carcinoma in patients with hematuria and pain. Patients with small tumors in the remaining solitary kidney who refuse surgery are suitable for this type of therapy as well as patients with centrally located inoperable tumors. The prerequisite for successful capillary embolization is the closure of the main arterial trunk with metal spirals. **Methods.** In the period from 2000 to 2010 we conducted 42 palliative embolizations. The average age of the patients was 75 years, including 26 men and 16 women. In 8 of the patients the intervention was repeated and in one with severe AV shunts embolization was performed 4 times. Embolization was performed with alcohol, Ivalon 150–250 μm and with metal coils. **Results.** No serious complications were observed during and after the intervention. Fourteen patient were still alive then and among the deceased patients the average survival time was 13.5 ± 10.8 months with the range of 1 to 56 months. The minimal survival time was 1 month with a maximum survival time of 56 months. **Conclusion.** Our results are consistent with data in the literature. Survival in patients without metastases was longer than in those with metastases, as confirmed by the 14 of the patients from the study. An additional therapeutic safety in the treatment of small cancers is provided with a combination therapy of embolization and radiofrequency thermoablation.

Key words:
kidney neoplasms; carcinoma, renal cell; hematuria; palliative care; embolization, therapeutic; radiography, interventional.

Apstrakt

Uvod/Cilj. Palijativna embolizacija tumora bubrega je metoda izbora u lečenju uznapredovalih inoperabilnih karcinoma bubrega kod bolesnika sa hematurijom i bolovima. Bolesnici sa malim tumorom na preostalom bubregu su pogodni za ovaj vid terapije, ukoliko ti bolesnici odbijaju operativni zahvat ili ukoliko tumor leži centralno, tako da operativna enukleacija nije moguća. Preduslov za uspešnu embolizaciju je kapilarna embolizacija, a glavno arterijsko stablo se zatvori metalnim spiralama. **Metode.** U periodu 2000–2010 godine kod 42 bolesnika urađena je palijativna embolizacija. Prosečna starost bolesnika iznosila je 75 godina. U studiju je bilo uključeno 16 žena i 26 muškaraca. Kod 8 bolesnika intervencija je ponovljena, a kod jednog, sa izraženim AV-šantovima, embolizacija je urađena četiri puta. Embolizacija je rađena alkoholom, Ivalonom 150–250 μm i metalnim spiralama. **Rezultati.** U toku intervencije i nakon intervencije nije bilo teških komplikacija. Ukupno 14 bolesnika još su živi, a kod umrlih prosečno preživljavanje iznosilo je 13,5 ± 10,8 meseci. Minimalno preživljavanje iznosilo je jedan mesec, a maksimalno 56 meseci. **Zaključak.** Naši rezultati podudaraju se sa podacima iz literature. Preživljavanje kod bolesnika bez metastaza duže je nego kod bolesnika sa metastazama, što potvrđuje i 14 bolesnika iz naše studije. Dodatnu sigurnost u terapiji malih karcinoma pruža kombinovana terapija: embolizacija i radiofrekventna termoablacija.

Ključne reči:
bubreg, neoplazme; hipernefrom; hematurija; lečenje, palijativno; embolizacija, terapijska; radiografija, interventna.

Introduction

Renal cell carcinoma is a rare tumor which accounts for 1.2–3% of all newly diagnosed cancers. Men are afflicted twice as often as women. It usually occurs between the 5th and the 7th decade of life. Renal cell carcinoma is the third most common cancer in the field of urology following bladder cancer and prostate cancer. The therapy of choice is nephrectomy or enucleation surgery of smaller tumors if feasible, depending on tumor location.

Percutaneous transarterial embolization of the kidney is a minimally invasive therapeutic procedure by which complete or partial radiological nephrectomy can be performed.

Percutaneous renal embolization was first performed experimentally on animals in 1969 by Lalli et al. [1]. In 1973

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Almgard et al. [2] reported embolization of a patient with renal cell carcinoma.

Since then, not only the embolization technique has changed, but also the diagnosis of renal cell carcinoma was revolutionized by the introduction of computed tomography (CT) and magnetic resonance imaging (MRI). Palliative embolization is the treatment of choice for patients with advanced kidney cancer in whom surgery is technically unfeasible or if they have a high surgical risk. The main indication for the implementation of embolization in these patients is hematuria, which can be treated successfully with this method regarding aspects of technique and clinical outcome. Furthermore these patients often have severe pain because of the infiltration of the organ as well as the surrounding anatomical structures, in which case embolization is also an adequate paintherapy. Metastases are not a contraindication to implementing this procedure regarding the two above mentioned indications.

In asymptomatic patients without distant metastases the complete necrosis of the tumor is achieved by capillary embolization so that a portion of these patients are converted from the inoperable to the operabable status.

Another indication for palliative embolization is a small tumor in the remaining solitary kidney in which partial resection of the kidney is not technically possible or in patients who refuse surgery on the remaining kidney.

Patients with advanced metastases and small tumors of the kidney are also indications for embolization. Embolization is performed on the capillary level. For capillary embolization alcohol (96%), Ethibloc and polyvinyl alcohol foam – Ivalon (150–250 μm) are used. Alcohol is the cheapest and most efficient embolization agent. The disadvantage of using alcohol as the only agent to embolize is that it must be applied to a blocked blood vessel in order to prevent reflux or otherwise must be made visible so as to be able to make sure, that reflux can be seen.

After capillary embolization is completed, the occlusion of the central main stem of the renal artery is performed by using spirals.

Methods

This study included 42 patients in whom palliative embolizations of renal tumors were performed in the period from 2000 to 2010. The average age of patients was 75 years, including 26 men and 16 women. Indications for embolization were as follows: inoperability demonstrated by CT or MRI, surgery contraindicated due to poor general health status, surgery refusal, tumors in both kidneys, and tumor in the remaining kidney after unilateral nephrectomy.

All the patients were diagnosed by CT scan or MRI. CT examinations were biphasic with a portal venous phase after 65 sec and an urographic phase after 10 min. As contrast agent we used Xenetix 350. The contrast medium was applied by using an injector with a flow rate of 3.5 mL /sec.

MRI was performed with T2 and T1 sequences without contrast agent and after the contrast agent (Dotarem®) had been injected manually in a quantity according to the patients weight (0.1 mg/kg). After administration of the contrast medium the T1 sequence with fat suppression (T1 VIBE) was done to make it easier to recognize the contrast discoloration of pathological structures.

After selective angiography has been performed, the embolization procedure follows. Embolization was done at the first capillary level. The embolic materials used in this study at the capillary level were alcohol or Ivalon® (150–250 μm particles).

For the intervention 96% alcohol was used. In the beginning of the study the alcohol embolization was done by balloon occlusion using latex balloons. In the second half of the study 1/3 of lipiodol was added (to alcohol so as to make it visible), as occluding catheters were not availably anymore. Some patients underwent combined embolization with alcohol and Ivalon® – the so-called "sandwich" technique. The advantage of using Ivalon® is that the supplying blood vessel need not to be blocked. Upon completion of capillary embolization, control angiography was performed. If renal tumor is sufficiently embolized, central embolization of the major supplying vessel (inlet vessel) should be done using metal coils. In the study, Tornado® spirals provided by Cook Medical were used.

After having embolized the tumor on the capillary and more central level, aortography was performed to confirm the complete embolization and to show the state of the surrounding nontargeted blood vessels.

Results

The major symptoms that lead the patients to their physicians are shown in Table 1.

Table 1
The major symptoms and radiological findings in the studied patients

Parameter	Patients, n = 42 (100%) n (%)
Symptoms	
pain	13 (31,0)
hematuria	16 (38,1)
weight loss > 5 kg	18 (42,9)
Radiological findings	
vein infiltration	5 (11.9)
necrosis	30 (71.4)
infiltration of the surrounding organs	6 (14.3)

One of the patients was diagnosed with bilateral tumors, so unilateral nephrectomy was done and superselective embolization was performed on the remaining kidney.

In 7 of the patients after nephrectomy tumor was discovered in the remaining kidney. Some of these patients refused surgery or the tumor had the central location, making partial nephrectomy impossible.

Angiographic findings of small residual tumor on the right kidney before and after embolization as well as CT scan after a year are shown in Figure 1.

The mean tumor size was 7.35 cm. The smallest tumor measured 2 cm and the largest measured 15 cm. There were 11 tumors found in the left kidney and 31 tumors in the right kidney. Radiologic findings are shown in Table 1.

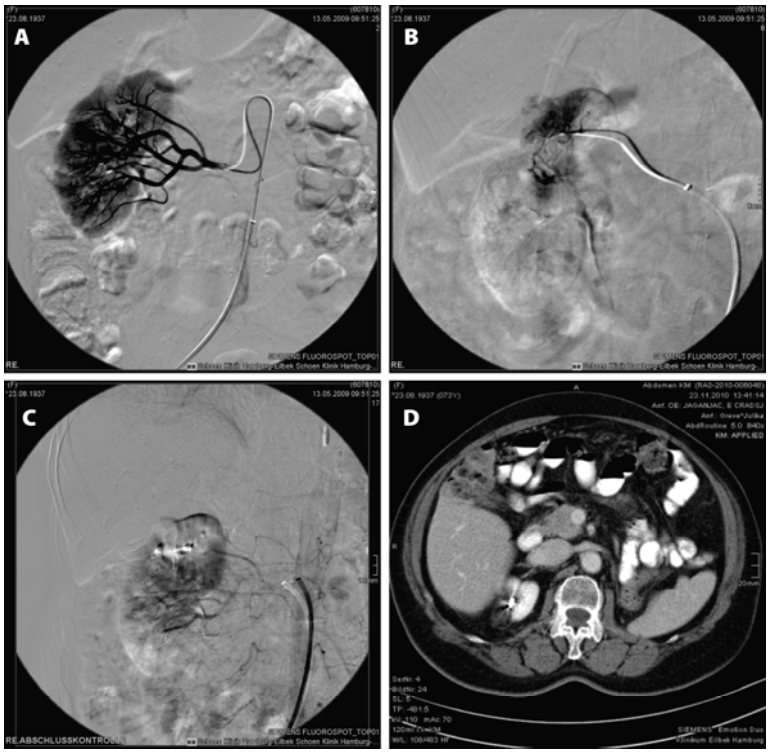

Fig. 1 – Small residual tumor on the right kidney: a) and b) – angiographic findings; c) angiographic finding after embolization; d) computed tomography scan after a year showing scar tissue in the kidney.

Lymph node metastases were present in 10 patients and 11 patients had distant metastases. Two of the patients had infiltration of the hollow system.

In 8 of the patients embolization was repeated, and in one it was repeated 3 times, due to severe AV shunts.

Metastatic tumor of the left kidney and CT of lung showing metastases, and angiographic findings before and after embolization are shown in Figure 2.

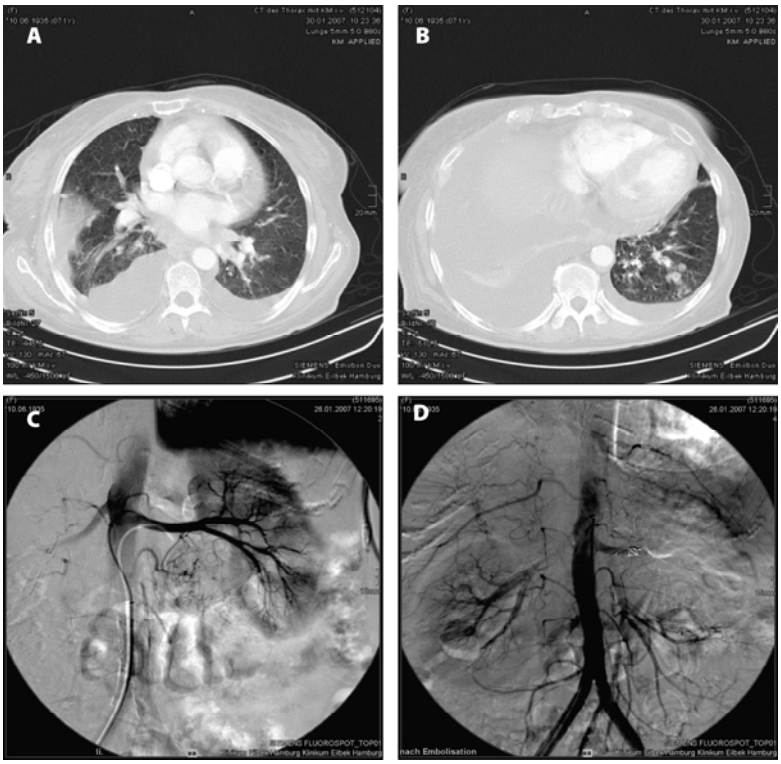

Fig. 2 – Metastatic tumor of the left kidney: a) and b) – computed tomography scan of lung showing metastases; c) angiographic finding before, and d) angiographic finding after embolization.

After intervention approximately 2/3 of the patients showed the symptoms of a postembolization syndrome. Elevated body temperature could be documented in 31 patients (73.8%). A total of 27 (64.3%) patients had postinterventional pain. In 15 (35.7%) patients nausea or vomiting was reported.

Figure 3 shows survival in months in patients receiving palliative embolization. The median survival time was 13.5 ± 10.8 months, with the shortest survival being 1 month and the maximal one 56 months. Fourteen patients were still alive. The patients in this group were in the stage M0. In 2 of the patients not included in the study with small tumors (2 cm), angiographic findings were negative, so that embolization could not be performed. These patients underwent thermoablation.

Figure 4 shows CT scan in a patient with a small tumor on the left kidney and negative angiographic findings.

Figure 5 shows renal tumor immediately after embolization and a few days after embolization with air development.

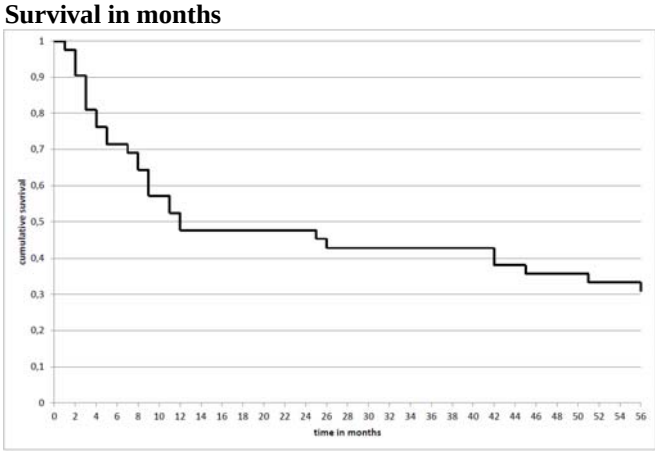

Fig. 3 – The survival time in patients receiving palliative embolization of renal tumors.

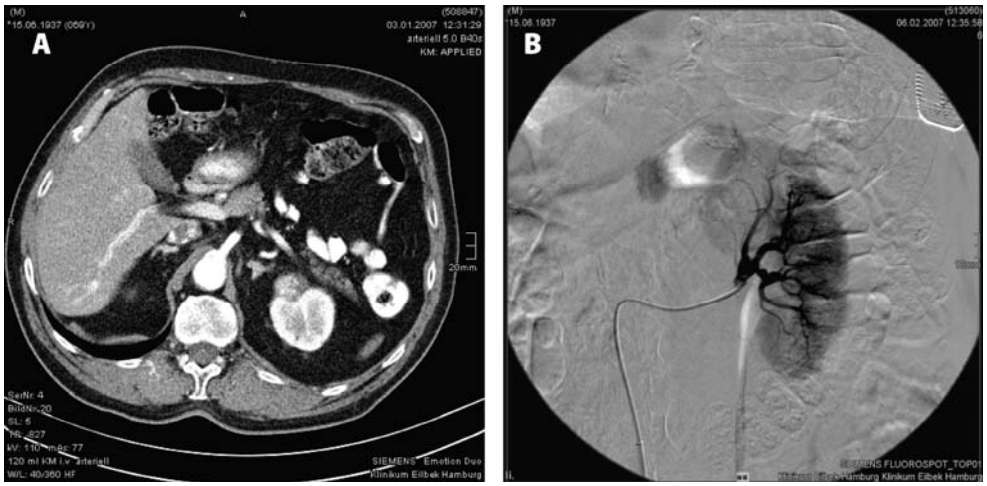

Fig. 4 – a) Computed tomography scan in a patient with small tumor on the left kidney; b) negative angiographic finding in the same patient.

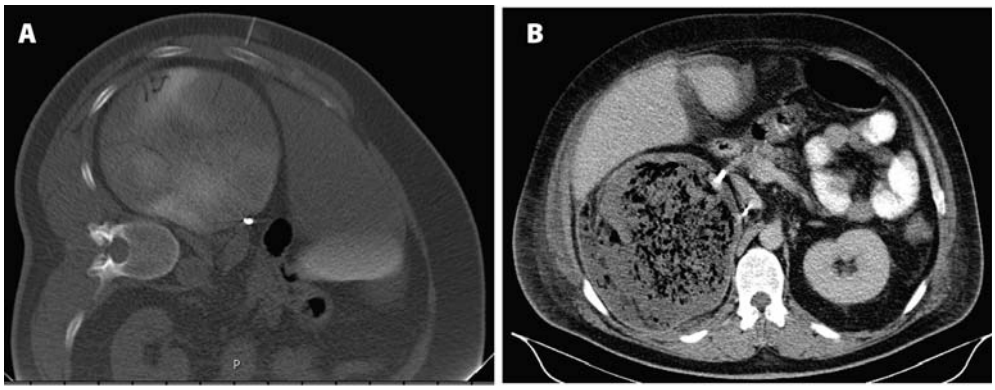

Fig. 5 – Computed tomography scan of renal tumor: a) immediately after embolization; b) a few days after embolization, with the development of air.

Discussion

Palliative embolization is a feasible treatment for patients with inoperable kidney cancer, especially in patients with hematuria or severe pain. These palliative, therapeutic goals are achieved in our patients, which is consistent with the literature [3, 4].

Capillary embolization in the case of inoperability in asymptomatic patients without distant metastases can induce complete tumor necrosis, showing that embolization is equivalent to nephrectomy. In patients who undertook nephrectomy and were diagnosed with the second tumor on the remaining kidney embolization is one of the therapeutic options. According to our experience patients having a solitary kidney (only one remaining kidney) very often refuse total nephrectomy of. It should be noted that in our hospital surgery is the method of choice if enucleation of the tumor is possible. In case of unfavorable location of the tumor, when operational enucleation is impossible, superselective embolization is offered as therapeutic option in which healthy tissue of the remaining kidney is preserved, and patients do not have to undergo hemodialysis.

Advanced inoperable tumors often develop AV shunts which may be disastrous for the patient, as blood flow through these shunts is very large and often leads to right heart failure. Intervention in these patients is not simple and often needs to be repeated at least once. The literature on the survival rate of patients undergoing palliative embolization is controversial. According to Hansmann et al. [5] in 9 patients that underwent palliative embolization the survival was 3 years in 3 patients with metastases and 6 years and 4 months in 6 patients without metastases.

In the Onishi et al. [4] study the average survival time of patients in the group that underwent palliative embolization with alcohol was extended to 229 days, compared to the mean survival time of 116 days in the control group without embolization.

Kauffmann et al. [6] report on the extension of lifespan in 6 patients with metastases by the mean value of 3 years. The embolizations were performed with capillary Ethibloc.

In another study Demirci et al. [7] report that a group of patients with kidney tumors and metastases that was treated with an adjuvant therapy using interferon-alpha and 5-fluorouracil in combination with nephrectomy, had approximately the same survival rate as a group of patients treated only with embolization.

Hallscheidt et al. [8] states that in a group of 7 patients with metastases, there was no significant prolongation of life when treated with palliative tumor embolization. In 6 patients without metastases median survival was 2.3 years. The same study reports that other studies have shown the average survival time of 4 to 11 months after palliative embolization.

In their publication from 2007 Maxwell et al. [9] report the median survival time of 6 months in their group of 19 patients. These authors did not distinguish between patients with or without metastases. This group of patients was very heterogeneous, also including patients with local recurrence after nephrectomy.

Vaicekavicus and Pranculis [10] state that one patient with lung metastases out of the group of 8 patients undergoing palliative embolization lived for 4 years.

Munro et al. [11] report in a retrospective study including 25 patients, that restaging was assessed in a period from 27 to 39 months in 14 patients. The results were as follows: in a patients the tumor size increased, in 7 patients the tumor remained unchanged, and in 5 patients there was a decrease of the tumor size.

In patients without metastases there were no cases of newly appearing metastases during the follow-up time. In two patients with metastases a regression of the metastases occurred. All embolizations in this group were performed with absolute alcohol and spirals.

Superselective embolization is performed in cases of small tumors in the remaining kidney (< 4 cm) when surgery is not possible. Additional security for those patients is offered by ablative therapeutic methods such as radiofrequency ablation. We prefer superselective embolization if abnormal blood vessels can be found at angiography. Following embolization, thermoablation is to be performed after 24 hours under CT supervision. In our opinion this approach is a lot more convenient for the patient and for the interventional radiologists. Because renal cell carcinoma is extremely well vascularized tumor, there is a certain risk of bleedings occurring when puncturing the tumor, which must be treated surgically. We exclude this complication by previous embolization. It is interesting to note that in two patients out of our group of patients with small renal tumors the size of 2 cm we did not have positive angiographic findings. In both patients only thermoablation was performed, which passed without complications. One of the above-mentioned two patients had multiple metastases to the spine that were surgically treated, and histopathology confirmed that the metastases originated from a clear-cell renal tumor.

At the beginning of the era of embolization a complication rate of about 4% was described in the literature [12]. During the beginnings of embolization a group of authors in Germany applied alcohol without using occlusion catheter, leading to more serious complications. Löhr and Ross [13] describe death during embolization of tumors of the right kidney with alcohol, probably due to massive pulmonary embolism. In this patient an AV malformation was identified, so the authors assume a direct crossing of alcohol into the venous circulation, which led to pulmonary embolism. Several authors [14–16] report bowel infarction as a complication during embolization. Besides, Laarmann et al. [17] and other authors [18] also report gonadal damage during kidney embolization. Individual descriptions of paraplegia as a result of renal embolization exist in literature [19, 20].

In recent studies, also, it has been reported that after embolization approximately 75% of the patients develop postembolization syndrome that lasts shortly [21]. The same authors report further complications such as hematoma or dislocation of spirals. The administration of corticosteroids before the intervention can reduce the occurrence of postembolization syndrome [22]. After embolization of renal tumors air bubbles are frequently seen in embolized tumor in the early postembolization phase. The quantity of air is a valuable me-

asure for the breakdown of tumor. Although at this stage there is leukocytosis and an increase in temperature, there is no abscess. Air bubbles develope from protein destruction with the release of oxygen which can not be dissolved in the region of the coliquation necrosis. At the beginning of the era of embolization the exact cause of air bubbles in embolized tumors was unknown. Bacteria that produces gases was assumed to be responsible for this situation [23]. In later works it is stated that the sterile products of tumor cells decomposition are responsible for the development of gas bubbles [24].

It is well known from embolizing other organs that abscess formation can occur after embolization. Sometimes it is difficult to discern whether symptoms like temperature and leukocytosis develop due to a postembolization syndrome including sterile gas formation or due to abscess formation. The answer to this question is given by follow-up CT scans proving the considerable colliquation of the abscess close to air, as well by clinical and laboratory findings showing prolonged fever and an increase of the inflammatory parameters. Mostly large tumors are likely to form abscesses after embolization. Sometimes general condition of patient's is so bad that drainage must be performed after nephrectomy.

With our embolization technique we had no serious complications in terms of reflux and unwanted embolization into other vascular regions including the venous system.

Conclusion

Our results are consistent with data in the literature. Survival in patients without metastases was longer than in those with metastases, as confirmed by 14 of the patients from the study. An additional therapeutic safety in the treatment of small cancers is provided with a combination therapy of embolization and radiofrequency thermoablation.

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GENERAL REVIEW

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Structural features of arterial grafts important for surgical myocardial revascularization: Part II – Histology of the radial, inferior epigastric, and right gastroepiploic arteries

Strukturne karakteristike arterijskih graftova važnih za hiruršku revaskularizaciju miokarda: Deo II – Histologija radijalne, donje epigastrične i desne gastroepipliočne arterije

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Introduction

Clinical experience and numerous research indicate that arterial grafts are superior to venous grafts for surgical revascularization of myocardium. Arterial grafts in comparison to vein grafts of the great saphenous vein have higher patency rate in early and late postoperative period. After complete arterial revascularization, the in-hospital mortality is between 0.5% and 2.2%, with the survival rate of 99% and the major adverse events-free survival in 89% of patients after 12 months [1, 2]. In the period of 1 to 3 years postoperatively, the patency rate of the used arterial grafts is between 96.5% and 98.3% [1].

Besides the left and the right internal thoracic arteries, radial, inferior epigastric and the right gastroepiploic arteries are used for complete arterial revascularization and treatment of multivessel disease. The complex T- and Y- forms of composite grafts, the need for continual improvement of the surgical technique and harvesting procedure initiated studies focused on biological determinants of arterial grafts, their structure and functional remodeling. The final goal of morphological and morphometric analyses is to find the optimal combination of grafts ideally matched for the needs of patients.

Histology of the radial artery

The radial artery (RA) represents a classically structured muscular type artery (Figures 1A–C). The *tunica intima* of this artery is typically organized and made up of endothelium and subendothelial connective tissue. In experimental conditions, the RA is proved to have efficient supply of nitric oxide. Studies that analyzed the endothelium dependent vasodilatation induced by nitric oxide in the RA in comparison to the internal thoracic artery (ITA) brought opposite results. One group of results confirm a significantly reduced NO level in the RA [3], while the other group show that both endothelium-dependent and endothelium-independent vasodilatation are preserved and not different from the ITA [4]. The means of the intima thickness of the RA

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varies between 45.7 μm and 60.4 μm and steadily and significantly increases with aging [5].

The RA has the most prominent arteriosclerosis among arterial grafts, including both atherosclerosis and Mönckeberg medial calcific sclerosis [5]. Preoperative assessment of atherosclerosis of native arteries is an important parameter, since it determines the outcome of surgical revascularization and complications including recurrent *angina pectoris* and myocardial infarction [4, 6]. Graft patency is compromised by the progression of native atherosclerosis of the arterial graft.

Comparative analysis of the presence of atherosclerosis in different arterial grafts in general population indicated that only 7.09% of the RA segments were without changes and the rest (92.91%) had diffuse or focal intimal hyperplasia. Among the later, 24% were with Mönckeberg medial calcific sclerosis [5]. Ruengsakulrach et al. [7], in their vast series, described 94% of the RA with intimal hyperplasia along with 13.3% of arteries with medial calcific sclerosis. In their study, only 5% of arteries had atherosclerosis. Risk factors associated with intimal hyperplasia and atherosclerosis of the RA are aging, smoking, peripheral vascular disease and *diabetes mellitus*.

In a study that analyzed final 15% of the arterial length, 28% of arteries had stenosis of the lumen greater than 50% [8]. As shown with other studies 1–7% of the radial arteries were not suitable for the coronary artery by-pass grafting (CABG) procedure [9, 10]. However, early and mid-term graft patency rate were not affected by this relatively higher level of native atherosclerosis as compared to the ITA [11, 12]. The thickness of the intima did not increase even 37.6 ± 7.2 months after the surgery [12]. The long-term patency rate of the RA after 105 ± 9 months was 91.6% [4].

The internal elastic membrane is well-developed throughout the length of the radial artery. It contains almost twice the number of discontinuities (8.6/mm) than the same structure in the ITA (4.5/mm) [13]. The size of these fenestrations is also almost twice the size of fenestrations of the ITA in the same age group (2.73 ± 0.24 μm *vs* 4.27 ± 0.78 μm in forties and 2.72 ± 0.72 μm *vs* 4.10 ± 0.35 μm in sixties) [5]. The universal rule observed for the elastic lamellae of all arterial grafts is that their integrity diminishes with aging making the arteries prone to vascular smooth muscle cells migration and intimal hyperplasia development [14–16].

Tunica media of the RA is composed of circularly distributed smooth muscle cells, which is why this artery is prone to spasm, especially when it is implanted in the conditions of high pressure and flow, like the position of aorto-coronary by-pass conduit. The RA has the thickest media of all described arterial grafts with a mean value of 352.6 ± 10.2 μm, and variations of mean values between 302.4 μm and 450.8 μm [5]. The thickness of the media increases significantly with aging [5]. High resolution echo-tracking with Doppler and A-mode detection of the vascular structure confirmed hypertrophy of the RA media and hyperplasia of the intima associated with luminal narrowing in patients with coronary artery disease, candidates for the CABG surgery [17]. Despite that, the intima/media thickness was not increased, not even 3 to 4 years after implantation of the RA as CABG graft, as determined with similar, ultrasound studies [12].

The vasospasm that follows CABG surgery with the RA is surpassed with the usage of calcium channel blockers and papaverin-NaCl solutions. Nevertheless, the research studies have shown that papaverin-NaCl solution significantly damages endothelial layer of the RA [18].

This artery is characterized by the thickest wall among the applicable arterial grafts (about 550 μm). The outer layer (*tunica adventitia*) contains well-developed blood vessels (*vasa vasorum*), which have a significant stake in the perfusion of the artery wall, including its middle layer (*tunica media*) [5].

The radial artery is characterized by the presence of radially oriented fibers of type I collagen, which penetrate from the adventitial layer to the media [5] (Figures 1A and B). This finding suggests that a very developed and meticulous surgical technique is needed for the preparation of this artery, and that any damage of the wall during the preparation may affect the stability of the media and compromise the long-term graft patency. In support of this finding is a well-known fact in the literature, that intimal hyperplasia can be avoided with the preparation of the RA together with the surrounding periadventitial tissue and accompanying veins [8].

Gender specific differences. The radial arteries of females have significantly smaller lumen and perimeter, but there is no significant difference in the thickness of the intima and the media [19] among genders. Our results show no significant difference in the basic morphometric parameters of the males and females [5].

Left-to right specific differences. No significant difference in the basic morphometric parameters of left and right arteries were described [5].

Use in surgical myocardial revascularization

The RA was firstly used as an arterial graft in 1973 [20]. Shortly after that, the use of this artery was abandoned due to its strong tendency of spasm. However, during the last years of the twentieth century, thanks to the introduction of calcium channel blockers in the postoperative period, the use of this arterial graft was re-actualized [21, 22]. The patency rate with this new approach was 93.5% in the period of 9.2 months, i.e. 90–93% during the postoperative period of 1.5 ± 1.1 years [8, 9, 21]. This graft can be used in patients with impaired left ventricular function [23] and even after 10 years of CABG, the patency of the RA grafts goes up to 83% [24].

The RA got its special significance thanks to new surgical techniques of composite grafts, modern methods of ultrasound dissection and application of endoscopic dissection of the RA, which gives better cosmetic results, does not impair graft patency and postoperative outcome and gives rare neurological complications [25–27]. This artery has shown to be particularly suited for sequential anastomosis, as a part of composite grafts with good perioperative results, because of its bigger diameter compared to other arterial grafts [1, 28]. Comparison of the RA used as direct aorto-coronary conduit or as a part of T composite graft indicated that the outcome of the procedure depends primarily on the target vessel and its level of atherosclerosis and stenosis and not as much on the grafting strategy. Significantly worse prognosis was for the right coronary artery

as target vessel and vessels with stenosis ≤ 70% [29]. This conclusion potentiates again the fact, well-known for researchers in the arterial grafting domain, that the level of native atherosclerosis of coronary arteries, i.e. target vessels is important for the prediction of the CABG outcome [4, 30–32].

Good short-term postoperative results were reported when the RA was used with the left ITA for complete arterial revascularization of emergency patients with unstable angina and/or critical coronary stenosis with high risk for acute myocardial infarction [33]. This is also proved to be a safe procedure for elderly patients, older than 70 years [34].

However, the routine usage of the RA graft is still an issue for debate, in the search for the ideal second graft and a perfect match for the ITA [35]. There are supporters of revascularization with the ITA and saphenous vein (SV) graft and studies that suggest superior outcome of the ITA/SV grafting to the ITA/RA grafting [6]. Perioperative outcomes are similar in these two groups, but long-term outcomes in the period of 0–6 years showed that overall mortality was greater in the ITA/SV group. Incidence of repeated catheterization and the need for revascularization were similar for the two groups [36]. However, in this study, Zacharias et al. [36] did not confirm a significantly better patency rate of ITA/RA grafts in comparision to ITA/SV grafts. As confirmed angiographically, in the period of 1.8 ± 1.4 years postoperatively, 77 out of 242 (31.8%) RA grafts were occluded, while 216 out of 588 (36.7%) SV grafts were occluded. The observed difference was not statistically significant ($p = 0.11$). Zacharias et al. [36], insisted upon significant difference ($p = 0.039$) in patency rate between RA grafts (70.07%) and SV grafts (59%) in patients that received both conduits. However, these conclusions were obtained in a non-randomized study, where RA and SV grafts by-passed coronary arteries with diverse rates of stenoses and different irrigation areas of myocardium, i.e. with different run-off areas. If the SV or the RA grafts were used in randomized conditions as conduits for the second largest target artery, the patency was not significantly different. It was 90% for the SV graft after 5 years and 80% after 9 years [37].

Meta-analysis and systematic review of 35 studies using a random-effect model and odds ratio for statistical elaboration proved that mid-term (1–5 years) and long-term (> 5 years) patency was better in the RA grafts in comparision to SV grafts [38].

The results of RAPS (Radial Artery Patency Study) confirmed that graft occlusion was lower in RA than in SV grafts at the mean period of 7.7 ± 1.5 years after surgery [39]. There were 12% of functionally occluded RA grafts and 19.7% functionally occluded SV grafts, defining functional occlusion as lack of thrombolysis in myocardial infarction flow grade 3. There were 8.9% of completely occluded RA grafts and 18.6% completely occluded SV grafts [39]. In both cases, differences are proved to be statistically significant ($p = 0.03$ and $p = 0.002$, respectively) [39].

Histology of the inferior epigastric artery

The inferior epigastric artery is a muscular type artery, with typical three-layered organization (Figure 1E-F). The *tunica intima* of this artery consists of endothelium and a very thin layer of subendothelial connective tissue. The thickness of the inner layer (*tunica intima*) of this artery is smaller than the thickness of the *tunica intima* of the ITA and of all other arterial grafts [5]. The mean intima thickness is 6.6 ± 4.4 μm and it does not change significantly with aging [5, 40].

The inferior epigastric artery is the artery with the lowest atherosclerosis level among the arterial grafts. In our study, 71.6% of the samples were completely unchanged, while the rest 28.4% were with low grade, focal intimal hyperplasia. Many studies confirmed the lack of atherosclerosis even in those patients with known risks for atherosclerosis [41–44].

The internal elastic membrane is well-developed and contains a small number of fenestrations (4/mm). According to some studies, it is less than the number of fenestrations of the ITA (4.62/mm) [44], while other data suggest that the average number of discontinuities of the internal elastic membrane of the inferior epigastric artery is approximately the same as the number of fenestrations in the elastic-muscular segments of the ITA [45]. According to our results the number of fenestrations is 3.99 ± 0.54 mm in the forties and 4.79 ± 1.45 mm after 61 years of age [5]. The size of fenestrations is similar to the RA fenestrations and equals 3.79 ± 0.67 μm in persons between the age of 40 and 60, and 3.53 ± 0.78 μm after 61 years [5]. Although much of the effort was invested in the morphometric studies of the inner elastic membrane parameters, they do not differ between the grafts proportionally to the intimal hyperplasia or atherosclerosis level. As for the number of elastic lamellae in the ITA, elastic skeleton alone is not of key importance to the delay of atherosclerosis or intimal hyperplasia, it is rather the complex interaction of vascular smooth muscle cells and the extracellular matrix that has this effect [14].

The structure of the *tunica media* corresponds to muscular type arteries with a predomination of smooth muscle cells and few formed elastic lamellas in the proximal part, immediately after the take off from the external iliac artery [45]. The media thickness is 164.4 μm ± 51.3 μm and it is not changed during aging.

The mean value of the artery wall thickness is 315.7 ± 75.2 μm and ideally corresponds to the wall thickness of the coronary arteries [5]. Because of the muscle phenotype of the medial layer, this artery has a strong tendency for spasm, so the late postoperative results are not satisfactory [45]. The presence of *vasa vasorum* is limited to the adventitial layer, so the perfusion of the media comes entirely from the lumen. Within this layer, the presence of longitudinally arranged contractile myocytes was observed. Although we defined this artery, at the beginning, as the muscular type artery, the presence of longitudinally arranged smooth muscle cells in the *tunica adventitia* gives this artery a character of a specialized type of artery [5, 40]. The presence of the smooth muscle cells in the adventitia could be important for physiological adaptability of this artery in a way similar to coronary arteries, which contain bundles of longitudinal smooth muscle fibers in the subendothelial tissue. The presence of these muscle cells enables adaptation of coronary arteries to changes of length and diameter during systole and diastole [14, 40, 46].

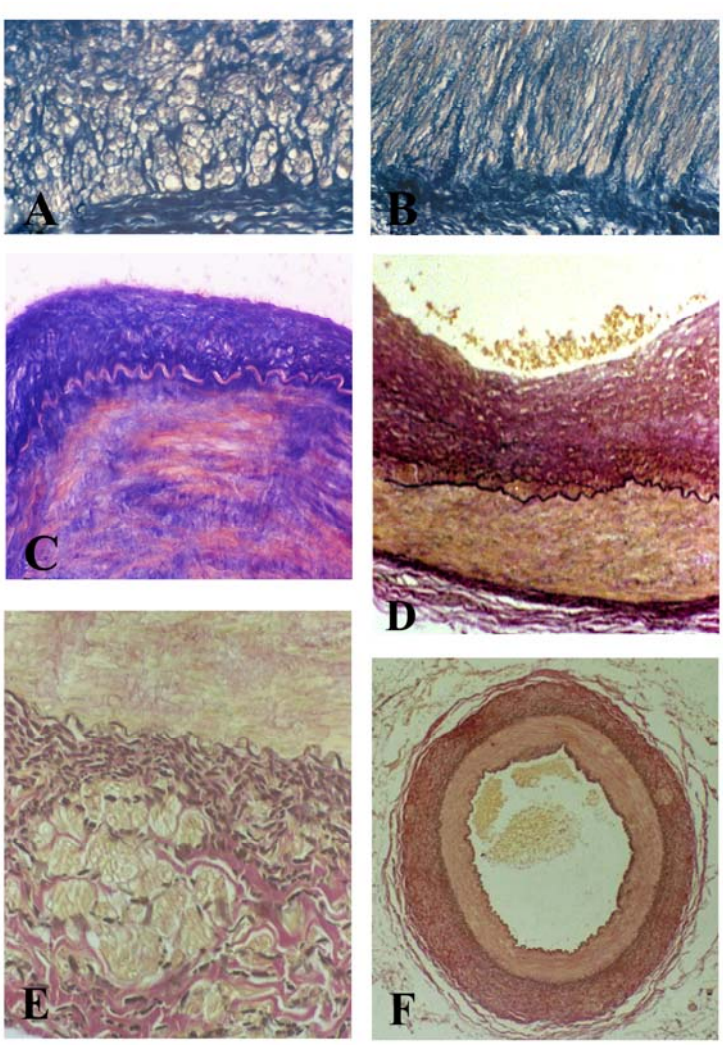

Fig. 1 – Arterial grafts – radial, inferior epigastric, right gastroepiploic arteries: A, B and C) the radial artery – Mallory staining for collagen fibers type I, [original magnification (A and B) ×64 and (C) ×100]; D) the right gastroepiploic artery – Weigert van Gieson staining for elastic fibers, [original magnification ×32]; E and F) the inferior epigastric artery – Weigert van Gieson staining for elastic fibers, [original magnification (E) ×256 and (F) ×8]. A and B – radial arrangement of collagen fibers in the media of the radial artery; C – the radial artery as the muscular type artery; D – the right gastroepiploic artery is the muscular type artery; intimal hyperplasia is present; E and F – the inferior epigastric artery is the muscular type artery; longitudinally arranged smooth muscle cells are present in the adventitia; there is no atherosclerosis.

In the adventitial layer of the inferior epigastric artery, numerous CD34 immunoreactive cells can be found. They are considered as highly active cells and a part of the pool of resident progenitor cells of the vascular wall. The presence of numerous CD34 immunoreactive endothelial cells was shown in the endothelium of the inferior epigastric artery as well, and in the ITA, whereas its expression in other analyzed grafts, as well as in the coronary arteries, is very scarce and reduced to rare, individual cells which are unevenly distributed along the circumference of a blood vessel [5].

If we look at the presence of CD34 positive endothelial cells as an indicator of blood vessel capacity for re-endothelization, there is a clear polarization, in terms of the distribution of this characteristic in the analyzed arterial grafts. The ITA and the inferior epigastric artery, which are characterized by the lowest degree of atherosclerosis, at the same time, have the biggest number of CD34 immunoreactive endothelial cells, as opposed to the coronary arteries, the radial artery and the right gastroepiploic arteries [5, 15].

Gender specific differences. The thickness of the media is higher in male patients [5].

Left-to right specific differences. Our results showed that left-sided arteries have thicker media than right-sided arteries in patients older than 61 year [5].

Use in surgical myocardial revascularization

The inferior epigastric artery was firstly used as free graft in 1988 [47]. Based on the results of preliminary studies, in the first two weeks after revascularization the graft patency rate was 97%, 12 months after the surgery the patency was 94%, and 14.8 months after the surgery the fatf patency was 79% [41, 48].

This artery is considered to be a suitable graft because of the extraperitoneal incision, avoidance of intra-abdominal dis-

section and the possibility of securing two grafts of sufficient length. Desirable characteristic of this graft is that the inferior epigastric artery *in situ*, represents an artery which rarely develops atherosclerosis. However, because of its position it represents a low pressure graft. The hypertensive stress to which it is exposed after implantation and anastomosis with the aorta, compromises the long-term patency of this graft [49].

Histology of the right gastroepiploic artery

The right gastroepiploic artery (GEA) is a typical muscular type artery (Figure 1D). The right GEA has a higher degree of intimal hyperplasia and atherosclerosis than the ITA or the inferior epigastric artery. Although the mean intima thickness is small (28.8 ± 33.1 μm), it grows dramatically after the age of 60 [5]. Studies show that 43% of the right gastroepiploic arteries have intimal hyperplasia with 25% of stenosis [50, 51]. In our series, 7.69% of sections had fibroatheroma, while 27.35% had no changes. The rest of 64.96% had intimal hyperplasia [5]. Among all analyzed grafts, the thickness of the intima is the lowest in the inferior epigastric artery, then comes the ITA and the right GEA, while the RA has the highest value of the intima thickness. These differences are statistically significant [5].

The right GEA contains a well formed internal elastic membrane, but with a higher number of fenestrations than the internal thoracic artery. The mean number of fenestrations is 5/mm. However, they are smaller than fenestrations of the RA and the inferior epigastric artery and equal between 2.6 ± 0.5 μm and 3.145 ± 0.145 μm [5].

Smooth muscle cells are the predominant component of the *tunica media*, and the amount of the elastic tissue is less than in the ITA [50, 51]. There are no clearly defined elastic lamellae, but rare and individual elastic fibers are sporadically distributed between muscle cells.

These histological features make the right gastroepiploic artery prone to spasm and to the development of early atherosclerotic changes. In contrast to the ITA, the right GEA does not possess the characteristic of positive remodeling during the development of atherosclerosis [5]. In general, the ITA is unique among the grafts by this ability. Although the RAs have statistically significant increase in the media and the wall thickness, which corresponds to the internal and the external elastic laminas area increase necessary for the definition of the positive remodeling, the progressive luminal narrowing clearly demonstrates the lack of positive remodeling in the RA. As for the inferior epigastric artery, it remains to be established.

The mean thickness of the media of the right GEA varies from 168.2 to 199.8 μm and it is not changed significantly with aging. The media thickness is the highest in the RA, while the ITA, the right GEA and the inferior epigastric artery have relatively similar values of this parameter [5].

The wall thickness of this artery is 321.3 ± 69.2 μm, which is significantly higher as compared to the same parameter of the ITA [52]. According to this parameter there is no statistical significance among the right GEA and the inferior epigastric artery. At the same time, they match ideally the coronary arteries. The exception is the RA with the highest values of the wall thickness (mean 536.6 ± 114.9 μm) [5, 53].

Gender specific differences. The thickness of the intima and the media were significantly higher in the male gender [5].

Use in surgical myocardial revascularization

The use of the right GEA for coronary artery bypass surgery began with Bailey and his associates in 1966 [54]. The right gastroepiploic artery is used primarily for patients who require multiple coronary revascularizations, for grafting the left anterior descending, diagonal and circumflex coronary artery, as well as the right coronary artery. Application of this artery gained its importance with the introduction of new surgical techniques of composite grafts. According to a study, the mean rate of graft patency during the period of 7.6 months was 93% [51] or 84.5% during the 10 months follow-up [55], and according to some research, the patency of this graftin the early postoperative period is close to the patency of the ITA [45]. The results of modern research of graft patency are contradictory. Thus, it has been shown that the patency of this graft, three years after the operation, is slightly less than the patency of saphenous vein graft in the system of the right coronary artery [56], while other studies confirm a better outcome, and smaller number of complications, five years after the operation, in patients in whom the right GEA is used instead of the great saphenous vein [57].

Suma [58] has recently reported the results from the vast experience with over 1500 GEA grafts. The patency rate was 93.7% after 1 year, 86.2% after 5 years and 70.2% after 10 years. The operative mortality was 1.26%, and survival rates were 91.7% after 5 years, 81.4% after 10 years and 71.3% after 15 years.

From the standpoint of surgical anatomy, the benefits of using the right GEA is the ability to obtain graft of appropriate length and optimal diameter that fits the diameter of the coronary arteries, as well as the capacity for adequate flow. In addition, there are no gastric complications because of the artery dissection. However, a serious drawback is that the application of this artery requires intraperitoneal surgery, with its all known operative and postoperative risks, whereby only a single arterial graft can be provided [50, 51].

Conclusion

Besides the internal thoracic artery, the radial artery, the inferior epigastric artery and the right gastroepiploic artery are used for complete arterial revascularization and treatment of multivessel disease. The ultimate goal of continual basic research on arterial grafts is to find an optimal combination of grafts or new and improved grafts with morphological and functional qualities matched to provide the best therapeutic approach for each patient.

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CASE REPORTS

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Rectosigmoid prolapse – A case report

Rektosigmoidni prolaps

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Abstract

Introduction. Many factors have been indentified as a possible cause of rectal prolaps. Despite the fact that it is not a life-threating condition, its clinical presentation varies, and sometimes it can present as an emergency. We presented a patient with prolapse of an unusually large segment of the rectosigmoid colon caused by chronic constipation, as an incarcerated segment repaired surgically. **Case report.** A 62-year-old female patient was referred to the Emergency Department in bad condition with severe pain in the perianal region. On examination a complete rectal prolaps as well as a part of sigmoid colon were found. Macroscopically, the prolapsed segment appeared edematous, livid, with ulcerations. An attempt to manually reduce prolapse failed, therefore resection of 50 cm of sigmoid colon with rectopexy had to be performed. No complications occurred and the patient was without symptoms six months later. Colonoscopy did not reveal any abnormality. **Conclusion.** Although the preoperative management and preparation of the patient was limited, emergancy surgical intervention for such a case was the strategy of choice due to magnitude of the prolapsing segment. It provided a successful and permenant solution.

Key words:
rectal prolapse; colon, sigmoid; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod. Mnogi faktori mogu prouzrokovati prolaps rektuma. Mada se ne radi o životno ugrožavajućem stanju, klinička manifestacija može biti različita, a ponekad se manifestuje i kao urgentno stanje. Prikazali smo bolesnicu sa prolapsom većeg segmenta creva – prolapsom rektosigmoidalnog kolona, nastalog usled hronične konstipacije koji je bio u stanju uklještenja, uspešno rešenim hiruškim putem. **Prikaz bolesnika.** Bolesnica, stara 62 godine upućena je u urgentno odeljenje u lošem stanju, sa jakim bolovima u perianalnoj regiji. Pregledom je nađen kompletan rektalni prolaps kao i prolaps sigmoidnog kolona. Makroskopski, prolabirani segment je bio lividan, edematozan, sa ulceracijama. Kako je manuelna repozicija bila nemoguća, sprovedeno je urgentno hirurško lečenje transabdominalnim pristupom, repozicija i resekcija 50 cm izmenjenog rektosigmoidalnog segmenta, sa prednjom rektopeksijom. **Zaključak.** Iako je preoperativna dijagnostika i priprema bolesnice bila ograničena, stanje prolabiranog segmenta creva zahtevalo je urgentnu hirušku intervenciju. Primena navedene procedure dala je povoljan efekat.

Ključne reči:
rektum, prolaps; kolon, sigmoidni; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Complete rectal prolapse (*procidentia*) is the protrusion of the entire thickness of the rectal wall through the anal sphincter complex [1].

Its first description can be dated to the Ebers Papyrus of Ancient Egypt (approximately 1,500 BC) [1, 2].

In the twentieth centery two competing theories of rectal prolapse were evolved [3]. In 1912 Alexis Moschcowitz proposed that rectal prolapse was caused by sliding herniation of the pouch of Douglas through the pelvic floor fascia into the anterior aspect of the rectum. The pelvic floor of prolapse patients is mobile and unsupported and it was registred that other adjacent structures can occasionally be seen alongside the rectal component of the prolapse. Due to advent of defecography, Broden and Snellman in 1968 were able to show convincingly that *procidentia* is basically a full-thickness rectal intussusception starting approximately three inches above the dentate line and extending beyond the anal verge. According to these theories, it was concluded that the weakness of the pelvic floor in rectal prolapse cases, the concept of herniation, and the obser-

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vation that there are abnormal anatomic features characterize this condition [3].

Patients with rectal prolapse suffer from anal incontinence (50–75%), constipation (30–50%), mucus or blood discharge from the protruding tissue (25%) and pain during bowel movements. Rectal prolapse is most frequently seen in elderly multiparous women, but the etiology is not fully explained.

Although numerous possibilities have been proposed, the exact cause and mechanism of rectal prolapse is not completely understood [4, 5]. Conditions resulting in increased intraabdominal pressure such as pregnancy, obesity, perianal injury, chronic opstipation can cause rectal prolapse [4–6]. The anatomic abnormalities and variations associated with rectal prolapse are intussusception, deep *cul-de-sac* or pouch of Douglas, absent fixation of the rectum and sigmoid colon, weakness of the pelvic floor and sphincter muscles [4]. Functional abnormalities that can occur are fecal incontinence, constipation or incomplete evacuation [4].

The main clinical feature of rectal prolapse is a protruding mass following defecation or occurring spontaneously upon standing or coughing. It can be accompanied by rectal bleeding or mucoid discharge [4, 6].

When the prolapsed rectum becomes edematous, rectal blood supply can become compromised, creating life-threatening gut ischemia as a result of strangulation.

Rectal prolapse is diagnosed entirely clinically and the treatment is primarily surgical, including laparotomy and internal reduction when possible, or perineal resection.

Untill now there has not been reported prolapse of this magnitude in the medical literature, so we designated it as rectosigmoid prolapse.

Case report

A 62-year-old female patient was reffered to the Emergency Department in bad condition, with severe pain in the perianal region, because of an acute protruded rectal mass following defecation. The patient reported no rectal bleeding nor mucoid discharge, her medical history revealed no previous illnesses. She had two vaginal deliveries, and in the last 20 years she was constipated with occasional rectal bleeding and prolapsing, what she grasped as hemorrhoids, and seek no medical attention. The patient had pudendal damage caused by direct trauma (obstretical) during her deliveries, demonstrated in prolapse and responsible for denervation atrophy of external sphincter musculature, pelvic floor and anal sphincter weakening. Her physical examination revealed only mild tenderness in the left lower abdomen and an edematous, livid, visible mass with ulcerations (Figure 1). The rectal mucosae was thickened and ulcerated.

An attempt to manually reduce prolapse failed. Routine laboratory analysis showed a slightly elevated white cell number, and a higher sedimentation rate (32/h) without any other abnormalities in total blood count, nor biochemical tests.

Plain abdominal X-ray showed signs of aerocoly. Abdominal ultrasonography examination was performed and

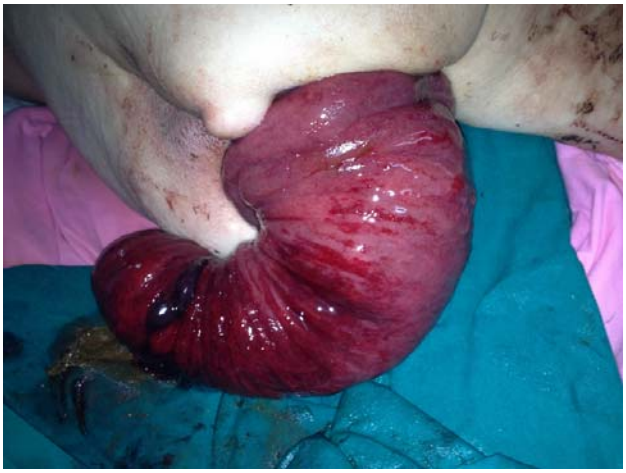

Fig. 1 – Complete rectal and partial sigmoid prolapse.

showed no abnormalities. Due to the magnitude of the prolapsed segment with signs of ischemia and necrosis, failure of an attempt to manually reduce it, indication for general anesthesia and urgent surgical treatment was appointed.

The main purpose of surgery in correcting rectal prolapse is to protect or restore fecal continence. The abdominal treatment approach was chosen due to its better results in terms of success in restoring anatomy and function. The incarceration of the prolapsed segment, as a complication of perianal rectosigmoidectomy, is sometimes manually reduced in general anesthesia.

Intraoperatively, dolichosigma was registred in the elongated mesorectum (midrectum?) of rectosigmoid. There was a mild dilatation of the proximal colon, with normal macroscopic morphology of the bowel wall and minimal residue particules of feces and gas in the lumen. The anal sphincter was weak and distended. After a succesful reposition of the prolapsed segment due to the intraoperative finding, rectosigmoid resection and suture rectopexy were done. After the low midline incision, the lateral peritoneal reflection of the sigmoid colon was incised by electrocautery. The gonadal vessels and the left ureter were identified and swept posteriorly. The peritoneal incision was continued to the left side of the rectum, 1 cm laterally to the rectal wall and curved anteriorly to the rectouterine sulcus. The peritoneum at the base of sigmoid mesentery was incised and continued to the right side of the rectum to unite with the incision on the left side of the sulcus. Posterior mobilization was continued to the pelvic floor muscles. The tissues laterally to the rectal wall were swept away laterally. The areolar tissue plane between the anterior wall of the rectum and the vagina was entered in the midline. Lateral peritoneal attachements in the midrectum were divided, while the rectum was pressed strongly to the opposite side. The lateral ligaments that were distal to this level were preserved. The rectosigmoid segment with changed bowel wall in the length of 50 cm was transected, reanastamosed and sutured to the presacral fascia at about the upper part of the third sacrum.

No complications occurred after the procedure and the patient was without symptoms and laboratory abnormalities on the control examination six months later. A colonoscopy

examination was performed as well, which revealed no abnormalities. Anorectal manometry and defecography were initially planned as a postoperative evaluation, but they were not conducted because the patient was not motivated to allow intervention.

Discussion

Despite the well-known etiology, pathophysiology and methods of surgical treatment, rectal prolapse remains a highly controversial disabling medical condition [2, 4].

The prevalence of external rectal prolapse is relatively low, estimated to occur in less than 0.5% of the general population overall [1]. It occurs more frequently in the elderly population and in females; estimation is that 3% of women in the United States have some form of pelvic organ prolapse, including rectal prolapse or uterine or vaginal prolapse, rectocele, cystocele, urethrocele, and enterocele [2, 7, 8].

Rectal prolaps can be caused by anatomical and functional abnormalities [8]. Anatomical features which can cause this condition include redundant sigmoid colon, diastasis of the *musculus levator ani*, loss of vertical position of the rectum and its sacral atacchment and/or an abnormally deep *cul-de-sac*. Any kind of pelvic dyssynergia, paradoxical puborectal contraction or sphincter abnormalities can leed the do development of *procidentia*. Pregnancy, obesity, perineal injury, chronic constipation, or other conditions resulting in increased intra-abdominal pressure are associated with rectal prolaps [4, 8].

The main clinical feature of rectal prolapse is a protruding mass following defecation [8]. In the beginnig the mass retracts in an upright position. With disease progression, protrusion is more often, even unrelated to stool discharge (any reason of intrabdominal pressure increase as sneezing and coughing can cause rectal prolaps). Finally, rectum prolapses with daily activities such as walking and may progress to continual prolapse. In the majority of cases, the patients can manually return the rectum, incarceration may occur but rarely. Pain is variable. Even 10–25% of patients also have uterine or bladder prolapse, and 35% may have an associated cystocele. Constipation occurs in 15–65% of cases. There may also be rectal bleeding, with or withoud mucous discharge [4, 5, 8, 9].

In the diagnostic workup, besides history and physical examination, colonoscopy is of great importance in order to exclude the presence of tumors and prolapsed internal hemorrhoids as the cause of patient's symptoms, as well as the presence of other colonic pathology. The rectal mucosa showes signs of congestion, and this finding should be discriminated from inflammatory bowel diseases. Anorectal manometry and defecography can be used in selected patients in order to choose better surgical tehniques that may improve the postoperative functional outcome, especially in patients with concomitant symptoms of abnormal bowel habits [8].

Many procedures have been reported that repair rectal prolapses, and the procedure used depends on the severity of prolapse [10]. Rectopexy combined with anterior sigmoid resection is currently the most popular operation in the United States for rectal prolapse. Recurrence rates 0–9% are expected. In choosing a surgical tecnhique, numerous factors must be considered, such as patient's age, comorbidities, gender, and importantly, preoperative constipation [5–7, 9]. Abdominal and perineal operations are the main surgical choices. Abdominal approaches such as rectopexy, resection and fixation and, recently, laparoscopic approaches are also widely performed in younger patients. However, these procedures tend to be time-consuming, they demand advanced surgical techniques, and the recurrence rate is not low [2]. In terms of perineal approaches, Delorme's and Gant-Miwa procedures might be feasible for mild prolapses in the elderly, whereas the Altemeier's procedure is the surgical option of choice for severe rectal prolapses [8].

Since 1993 the laparoscopic approach has been used, as well. A randomized controlled trial of perineal proctosigmoidectomy with pelvic floor reconstruction *vs* open resection rectopexy and pelvic floor reconstruction showed no difference in recurrence rates; however, incontinence was significantly improved in the resection rectopexy group [11].

We found only one case report in the literature describing a complete rectal prolaps in a young male patient, but contrary to our patient with no underlying colonic disease diagnosed, that patient suffered from rectal adenoma [7].

Conclusion

Although the preoperative management and preparation of the reported patient with unusually large rectosigmoid prolapse was limited, emergency surgical intervention was the strategy of choice.

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CASE REPORT

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Transplantation of infant kidneys – The surgical technique *en bloc* and transplant position variation: A case report

Transplantacija dečjih bubrega – hirurška tehnika *en bloc* i varijacija pozicije transplantata

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Abstract

Introduction. Due to the ever-present lack of kidney transplant grafts, more and more organs obtained from the so-called "marginal donors" group are accepted, which can provide suboptimal effect of transplantation, depending on their characteristics and/or implantation techniques. **Case report.** We presented a case with successful variation of kidney position with modified approach of kidney transplantation from an infant to an adult female patient with normal postoperative recovery. Urethral anastomosis was performed without antireflux procedure and this has not led to the development of reflux disease at an early stage. **Conclusion.** The position of a pair of kidneys proved to be satisfactory despite the growth of the kidney to the expected size and relatively small pelvis. There were no problems with venous stasis and kidney function from the very beginning was good.

Key words:
kidney transplantation; child; adult; surgical procedures, operative; postoperative period.

Apstrakt

Uvod. Zbog uvek prisutnog nedostatka graftova za transplantaciju bubrega, sve se više prihvataju organi iz grupe takozvanih „marginalnih donora". Ovo je grupa koja može da obezbedi optimalni efekat transplantacije, u zavisnosti od karakteristike organa i/ili tehnike implantacije. **Prikaz slučaja**. Pokazali smo uspešnu varijaciju pozicije para bubrega i modifikovanog pristupa transplantacije bubrega od deteta odrasloj ženskoj osobi. Ureteralna anastomoza rađena je bez antirefluksne procedure i to nije dovelo do razvoja refluksne bolesti u ranoj fazi. Postoperativni tok protekao je uredno. **Zaključak.** Položaj para bubrega pokazao se kao zadovoljavajući i pored rasta bubrega do očekivanih razmera i relativno male karlice. Nije bilo nikakvih problema sa venskom stazom i funkcijom bubrega.

Ključne reči:
transplantacija bubrega; deca; odrasle osobe; hirurgija, operativne procedure; postoperativni period.

Introduction

Growing disproportion between the number of patients waiting for kidney transplantation and adequate donors leads to expanding indications of cadaveric transplantation to so-called marginal donors [1], which have a certain degree of nephron deficit related to age and concomitant diseases, such as diabetes, hypertension etc. [2]. Besides the mentioned, this group comprises of very old or very young donors. Apart from problems with immunology and increased risk of early organ rejection, there are certain technical problems related to the choice and type of vascular and ureteral anastomosis and graft position in this group of patients [3].

As infant kidneys are small, it is common to use a pair of infant kidneys for transplantation to one patient of adequate body mass. Developmental discrepancy of such transplantation poses several problems, such as problem of venous run off from transplant and creation of urinary anastomosis complicated by strong urinary detrusor and weak ureters of donor [4].

We presented cadaveric transplantation of infant kidneys into a female adult, accompanied by the description of the surgical technique.

Case report

After confirmation of cerebral death due to malignant cerebral edema and hepatic insufficiency as a complication of

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primary disease – tyrosinemia, and in accordance to parents' decision on organ donation, the organ donor, male aged 2 years and 7 months, was indicated for kidney explantation. Explantation was performed at the Institute for Mother and Child Health Care of Serbia, Belgrade, using standard technique, based on the principles of multiorgan explantation. Both kidneys *en bloc* with aorta and vena cava were explanted. Irrigation with 1,000 mL Eurocollins® solution was performed *in situ* through venous confluent, with clamp of proximal and oversewing of distal parts of aorta and vena cava, practically without primary warm ischemia, and with cold storage during about 10 h.

Intraoperatively, dimensions of left and right kidney were approximately 6 × 4 × 1.3 cm, diameter of renal arteries was about 4 mm, while aorta and vena cava were about 10 mm.

After adequate recipient had not been found at the mentioned Institute, organs were offered to the Clinic for Nephrology and Clinical Immunology, Clinical Center of Vojvodina, Novi Sad, where the most convenient recipient was found on the waiting list (AB, Rh+, HLA compatibility 2/6, "cross-match" negative).

It was a female patient, age 29 (weight 47 kg, height 151 cm) in terminal stage of renal insufficiency, without diuresis. The disease was basically caused by bilateral vesicoureteral reflux with consecutive development of reflux nephropathy. It was diagnosed five years ago, since the patient underwent the hemodialysis program at the Institute for Internal Diseases, Clinic for Nephrology and Immunology, Clinical Center of Vojvodina. Dialysis was performed *via* distal arteriovenous (AV) fistula on the left arm. The patient also suffered from hypertension, chronic gastritis, and she underwent subtotal parathyroidectomy due to secondary hyperparathyroidism. Despite the dialysis (three times a week), preoperative laboratory work up indicated azotemia (urea 14.6 mmol/L, creatinine 406 mmol/L). Abdominal ultrasound verified bilateral renal atrophy, while findings on other abdominal organs were normal. The patient had been enrolled in the program for cadaveric kidney transplantation since 2003.

En bloc implantation of a pair of infant kidneys was performed at the Clinic for Vascular and Transplantation Surgery, Clinical Center of Vojvodina, Novi Sad. The kidneys were heterotopically positioned: the left kidney was placed in the right iliac fossa, while the right one in the pelvis, right below aortic bifurcation, medial to iliac vessels, "straddling" them. Arterial and venous anastomosis were created at the level of proximal segments of transplant aorta and vena cava with corresponding arterial and venous iliac blood vessels of recipient as lateroterminal (L-T) type, by single/anterior wall/ and extended /posterior wall/ sutures (Figure 1). The distal parts of the aorta and the vena cava were oversewn immediately below renal arteries and veins. After *in situ* fusion of the ureter by single sutures, we tried to create ureterocystoneostomy (UCN) on the posterior vesical wall, but we aborted it due to the occurrence of stasis within pyelocaliceal system. Implantation of fusioned ureters in the upper wall of the urinary bladder was performed by suture through all the layers of vesical and ureteral walls, without antireflux and with protection by a JJ stent for each ureter. The uniform kidney perfusion was detected, together with diuresis, which was 150 mL from after 30 minutes until the end of the operation (Figure 2).

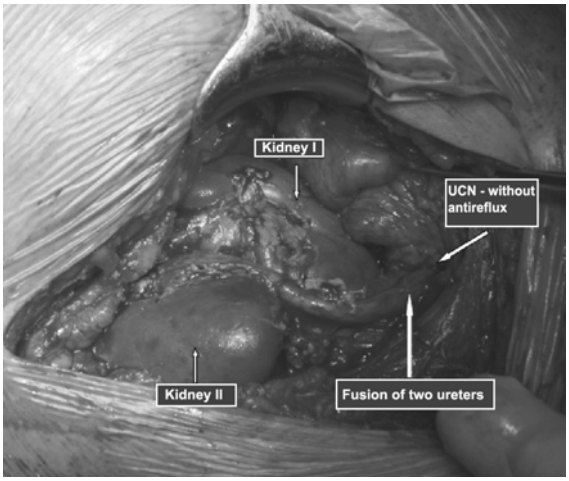

Fig. 1 – Arteriar and venous anastomoses of transplant aorta and *v. cava* with corresponding arterial and venous illiac blood vessels of the recipient (latero-terminal type).

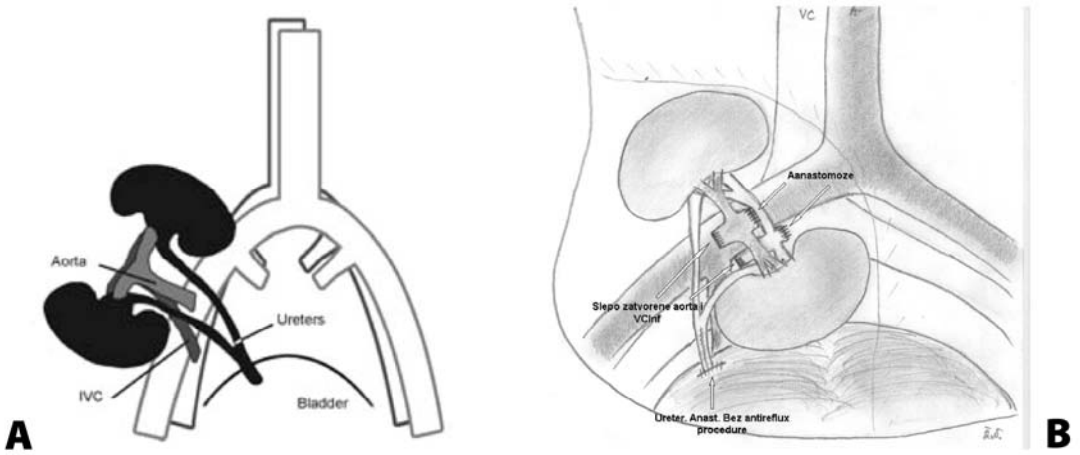

Fig. 2 – a) Placement of both kidneys in the reight iliac blood vessels and distal parts of the transplant aorta and *v. cava*; b) Implantation of fusioned ureters in the upper wall of urinary bladder with a JJ stent for each ureter.

The patient's postoperative recovery was normal. Daily diuresis during hospitalization was about 1,000 mL, and drop of blood urea nitrogen (BUN) and creatinine levels (BUN: 30 → 7 mmol/L, creatinine: 600 → 98 mmol/L) was detected. Control ultrasound and Doppler examinations revealed enlargement of both kidneys, whose new dimensions measured about 8 × 4 × 1.5 cm (both left and right), normal flow through arterial and venous anastomosis, without stasis in pyelocaliceal system, without significant fluid collections in retroperitoneum and drainage secretion. The kidney positions remained unchanged. Immunosuppressive therapy in accordance to the protocol (antithymocyte globulin – ATG, methylprednisolone 750 mg, mycophenolate mofetil) was administered. The patient was discharged from hospital to further home care on the day 9 of hospitalization.

Discussion

The presented successful transplantation of a pair of infant kidneys *en bloc* into the adult recipient is very rare in our region. The largest world transplantation centers perform such surgery several times per year, which is still not much compared to hundreds, even thousands of kidney transplantations performed at the same centers every year (less than 5%) [5]. This is quite reasonable as transplantation from marginal donor has much higher risks of complications, while expanding donorship expanded renal criteria (ECD) is caused by the lack of available organs [6].

When a transplantation team is faced with such a task, there is always a dilemma whether to separate the infant kidneys and give chance to two patients, or by transplanting a pair of kidneys *en bloc* and increase chances to a single one – the most appropriate patient. The common technique is a pair of kidneys placement into the iliac fossa on one side, thus creating anastomosis with distal parts of the aorta and the vena cava. According to the experience of other centers, 1- and 5-year graft survival in case of *en bloc* transplantation of two kidneys is retrospectively up to 90% and 70%, and is statistically higher compared to transplantation of a single infant kidney [7, 8].

Certain authors suggest scoring systems based on which the decisions would be made whether the kidneys of marginal donor should be transplanted separately, in pair or discarded [9]. Important criterion is recepient's body mass compared to kidney mass, and values of donor's diuresis, creatinine clearance and the number of functional nephrons determined by renal biopsy [10, 11]. Since a recipient is a person of lower body mass, more often female, it is considered that disproportion leads to hyperfiltration damage as a significant risk factor for early and late rejection reaction [12]. Other considered factors are diseases that preceded the death of donor, vital parameters at the moment and after confirmation of brain death, specificity of explantation procedure, duration of ischemia, anatomical variants of kidneys, ureters and vascular elements, anatomical characteristics of donor, earlier diseases and operations [13, 14].

In the presented case, we took into consideration metabolic disorder – hepatic insufficiency and threatening hepatorenal syndrome which preceded lethal outcome of cadaver, which could decrease the biological value of the kidneys. Also, it is well known that, due to a high incongruence and difference in compliance of infant cadaver and adult recipient vascular elements, in case of creating vascular anastomosis at the level of renal arteries graft faster neointimal hyperplasia at the level of anastomosis occurs, with consecutive development of renovascular hypertension and renal atrophy. This complication can be minimized if anastomosis is created at the level of largest blood vessels, i.e. cadaveric aorta and vena cava.

A valuable experience of surgical technique was obtained. The presented position variant according to our opinion yielded excellent results. According to the literature, the following procedures are most often performed: placement of both kidneys in the right iliac fossa thus creating anastomosis between recipient's iliac blood vessels and distal parts of transplant's aorta and vena cava interposition of transplant's aorta and vena cava on recipient's iliac blood vessels with two arterial and two venous anastomoses, where kidneys are positioned on both sides of right iliac blood vessels.

We recon that the advantages of the presented position are better venous drainage (due to the direction of blood flow in vena cava, decreased venous pressure and improved inflow of transplant's venous blood), better arrangement of vascular elements and ureters, without crossing and knee forming, and shortened time of secondary warm ischemia, as only one venous and one arterial anastomosis needed to be created.

While creating UCN the urinal retention and stasis in caliceal system of both kidneys were detected, assumably caused by disproportion between gracile muscles of infant ureters and thick and strong recipient's detrusor muscle. In this case, implantation of ureter into the upper vesical wall without antireflux is a better solution and recommendation for future similar transplantations, especially because vesicoureteral reflux was the main cause of renal insufficiency in patient (uretero-ureteral anastomosis was abandoned). The alternative is taking vesical fundus section. Carell's patch, with both ureters, and making anastomosis between the donor's part of vesical wall and the recipient's urinary bladder.

In the literature, complications of vascular stalk (perioperative venous and arterial anastomosis) and ureterocystic anastomosis (leak) can be observed in 15% and 25% cases, retrospectively [15, 16]. None of these complications was observed in our patient. Moreover, the postoperative period and one-month follow-up showed that kidney mass was sufficient to compensate azotemia in the presented patient, as well as the tendency of slight enlargement of kidneys even in the early postoperative phase.

Conclusion

En bloc transplantation of a pair of infant kidneys into the adult recipient can give good clinical results with few complications. This extends indications to certain number of marginal donors. The presented variation of renal position and creation of anastomosis (both vascular and urological) is a good solution, which can be used in similar future transplantations. Good results in the first 30 days in the presented patient are encouraging, but a longer follow-up will show the final effect.

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CASE REPORT

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Application of modern computer-aided technologies in the production of individual bone graft: A case report

Upotreba savremenih tehnologija podržanih računarom u izradi individualnog koštanog grafta

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Abstract

Introduction. An autologous bone (bone derived from the patient himself) is considered to be a "golden standard" in the treatment of bone defects and partial atrophic alveolar ridge. However, large defects and bone losses are difficult to restore in this manner, because extraction of large amounts of autologous tissue can cause donor-site problems. Alternatively, data from computed tomographic (CT) scan can be used to shape a precise 3D homologous bone block using a computer-aided design–computer-aided manufacturing (CAD-CAM) system. **Case report.** A 63-year old male patient referred to the Clinic of Dentistry of Vojvodina in Novi Sad, because of teeth loss in the right lateral region of the lower jaw. Clinical examination revealed a pronounced resorption of the residual ridge of the lower jaw in the aforementioned region, both horizontal and vertical. After clinical examination, the patient was referred for 3D cone beam (CB)CT scan that enables visualization of bony structures and accurate measurement of dimensions of the residual alveolar ridge. Considering the large extent of bone resorption, the required ridge augmentation was more than 3 mm in height and 2 mm in width along the length of some 2 cm, thus the use of granular material was excluded. After consulting prosthodontists and engineers from the Faculty of Technical Sciences in Novi Sad we decided to fabricate an individual (custom) bovine-derived bone graft designed according to the obtained 3D CBCT scan. **Conclusion.** Application of 3D CBCT images, computer-aided systems and software in manufacturing custom bone grafts represents the most recent method of guided bone regeneration. This method substantially reduces time of recovery and carries minimum risk of postoperative complications, yet the results fully satisfy the requirements of both the patient and the therapist.

Key words:
computer-aided design; cone-beam computed tomography; bone regeneration; alveolar bone loss; patient satisfaction.

Apstrakt

Uvod. Autologna kost (kost koja potiče od samog pacijenta) smatra se zlatnim standardom u obnavljanju koštanih defekata i delimično atrofičnog alveolarnog grebena. Međutim, velike defekte i gubitke kosti teško je restaurirati na ovaj način jer uzimanje veće količine autologne kosti može stvoriti ozbiljne komplikacije na donorskim mestima. Kao alternativna metoda mogu se koristiti podaci dobijeni kompjuterizovanom tomografijom, pomoću kojih je moguće izraditi precizan 3D homologni koštani graft. **Prikaz bolesnika.** Na Kliniku za stomatologiju Vojvodine u Novom Sadu upućen je 63-godišnji muškarac zbog nedostatka zuba u donjoj vilici, bočno. Kliničkim pregledom utvrđena je izražena resorpcija ostatka grebena donje vilice u navedenom delu, kako horizontalno, tako i vertikalno. Pacijent je potom upućen na 3-dimenzijalnu kompjutersku tomografiju konusnim snopom (3D CBCT) da bi se snimila koštana struktura i tačno izmerile dimenzije preostalog alveolarnog grebena. Usled velike resorpcije kosti, bilo je potrebno uvećanje grebena više od 3 mm i šire od 2 mm duž 2 cm, zbog čega je isključena upotreba zrnastog materijala. Posle savetovanja sa prostadontistima i inženjerima sa Fakulteta tehničkih nauka, odlučeno je da se napravi individualni (po meri) graft od goveđe kosti prema modelu dobijenom 3D CBCT snimanjem. **Zaključak.** Primena 3D CBCT računarskih sistema i softvera za proizvodnju individualnih koštanih graftova predstavlja najnoviju metodu vođene koštane regeneracije. Ova metoda značajno skraćuje vreme oporavka i nosi minimalni rizik od postoperativnih komplikacija, uz potpuno zadovoljenje zahteva kako pacijenata tako i terapeuta.

Ključne reči:
kompjuterski podržan dizajn; kompjuterizovana tomografija konusnog zraka; kost, regeneracija; alveolna kost, gubitak; bolesnik, zadovoljstvo.

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Introduction

Prosthetic rehabilitation of the posterior atrophic edentulous mandible presents a common clinical problem [1–7]. Fixed implant supported prosthesis is an ideal therapeutic solution. However, this can be impeded by the deficiency in height and width of the residual alveolar bone, associated with the consequent superficial position of the inferior alveolar nerve. In these circumstances, the placement of an implant of adequate length and appropriate subsequent prosthetic rehabilitation is difficult, almost impossible [2]. The knife-edge configuration of the residual bone crest does not provide sufficient base to contain particulate grafting material. Therefore, a strong rigid graft is required to allow fixation to the recipient site and 3-dimensional (3D) stability to withstand muscular forces [8]. For all these reasons, when we require a graft in the posterior mandible, which exceeds 3 mm in either width, height or both, a bone block graft is recommended [9, 10]. An autologous bone (bone derived from the patient himself) is considered to be a "golden standard" in the treatment of bone defects and partial atrophic alveolar ridge. It exhibits excellent bioabsorption capabilities and is never rejected by the body. However, large defects and bone losses are difficult to restore in this manner, because the extraction of large amounts of autologous tissue can cause donor-site problems. Alternatively, data from a cone-beam computed tomographic (CBCT) scan can be used to shape a precise 3D homologous bone block using a computer-aided design–computer-aided manufacturing (CAD-CAM) system. In this way, the bone block can be transferred directly from its sterile packaging to the receiving site without the need to be shaped [1].

Case report

A 63-year old male patient referred to the Clinic of Dentistry of Vojvodina, Novi Sad, because of teeth loss in the right lateral region of the lower jaw. Clinical examination revealed a pronounced resorption of the residual ridge of the lower jaw in the aforementioned region, both horizontal and vertical (Figure 1). After clinical examination, the patient was referred for 3D CBCT scan that enables visualization of bony structures and accurate measurement of dimensions of the residual alveolar ridge (Figure 2).

After a thorough measurement of bony structures, 3D CBCT scan indicated that an adequate implant-prosthetic rehabilitation is impossible without prior augmentation of the residual alveolar ridge in the mandibular region. Considering the large extent of bone resorption, the required ridge augmentation was more than 3 mm in height and 2 mm in width along the length of some 2 cm, thus the use of granular material was excluded. After consulting prosthodontists and engineers from the Faculty of Technical Sciences in Novi Sad we decided to fabricate an individual (custom) bovine-derived bone graft designed according to the obtained 3D CBCT scan.

3D design of a graft model

Generating 3D model of the jaw is the first step in the graft modelling procedure. The procedure is performed according to Cone Beam CT images provided in DICOM format that enables further generation of the 3D model of patient's lower jaw. This procedure is essential since the 3D model of the lower jaw is the basis for graft modelling. The procedure also enables visual and functional inspection of the 3D graft model. The 3D model of the patient's lower jaw generated on the basis of CBCT scans made with 3D-DOCTOR software is presented in Figure 3. After generating the 3D model of the lower jaw, input parameters, such as shape and size of the graft and its position in the jaw, were defined. At this stage, the highest level of cooperation within a multidisciplinary team involving oral surgeons and engineers was accomplished to obtain a 3D graft model that would satisfy both medico-esthetic and technical and functional requirements.

Upon defining the input parameters, the 3D graft modelling procedure was performed. Since the modelling procedure involved the complex free-form surfaces, the application of conventional CAD-software for modelling of standard geometric shapes was impossible [11] and the use of specialized 3D-modelling software was suggested. The 3D model of the lower jaw was used as the basis for modelling of lower graft surface (Figure 4). In this way, an adequate con-

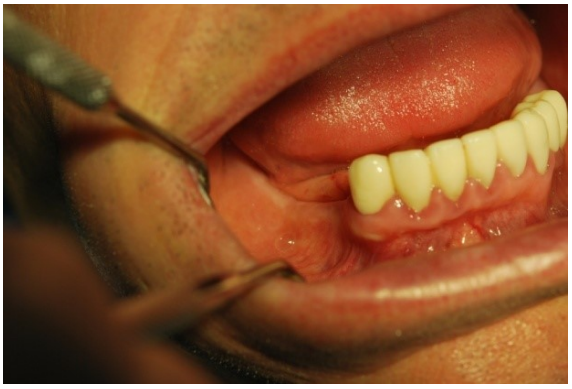

Fig. 1 – Intraoral image of the patient.

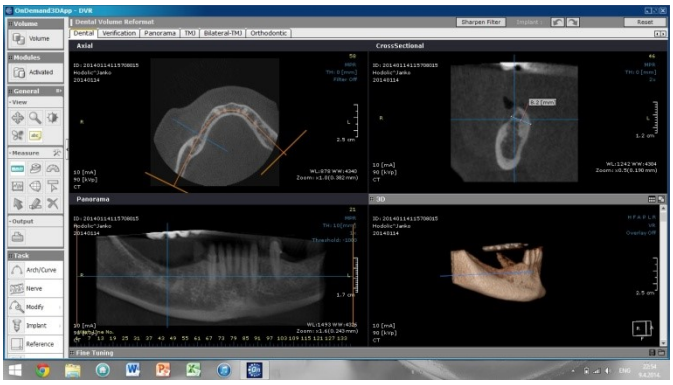

Fig. 2 – Preoperative 3D cone-beam computed tomography (CBCT).

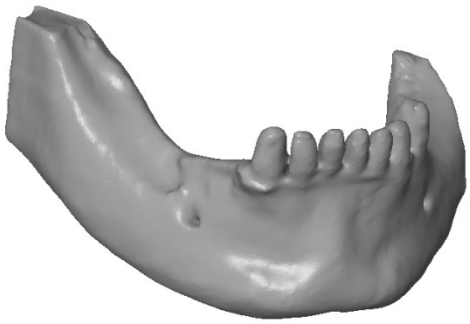

Fig. 3 – 3D model of patient's lower jaw generated according to cone-beam computed tomography (CBCT) scans.

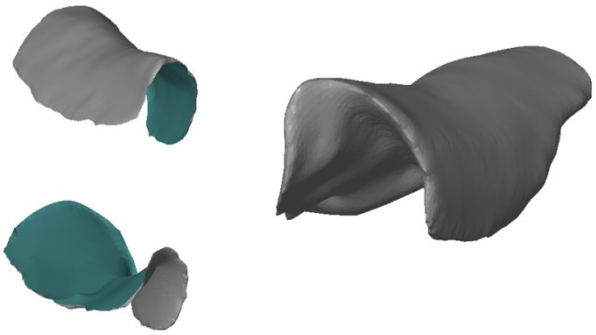

Fig. 4 – The procedure of 3D graft modelling.

formation of the bone graft was provided. Subsequently, the upper graft surface was modelled using complex-surface manipulation tools taking into consideration the shape, thickness and size of the graft. The size of the graft was determined according to the number of required implants. Finalized 3D model of the graft was saved in stereolitography (STL) file format that enables easy manipulation and data exchange between software programs.

In the next step, the 3D graft model was fitted together with the 3D model of the lower jaw and implant models using OnDemand3D computer software. During this stage, geometric characteristics of the graft were analyzed, as well as its position in the jaw in relation to the implants (Figures 5 and 6). This step also involved a multidisciplinary team composed of surgeons and engineers and encompassed the final check-up of the following parameters: cross-sections of the grafts; maximum dimensions (length, width, height); minimum graft wall thickness; negative angles of the graft; sharp edges.

The process of designing the 3D graft model was carried out taking into consideration the performance of the equipment for graft manufacturing and the minimum required cross-sectional graft thickness of 3 mm. Cross-sectional graft thickness less than 3mm would seriously disturb its mechanical properties. Reduction of mechanical properties is directly associated with the porosity of graft material. Thus, graft placement into the jaw might be compromised by potential breakage of the graft while positioning and fixing it with appropriate screws. After satisfying all virtual esthetic and functional requirements, the

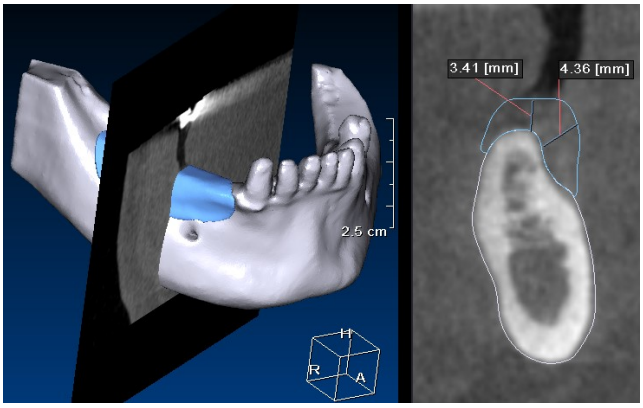

Fig. 5 – Cross-sectional view of the graft thickness obtained using OnDemand 3D software.

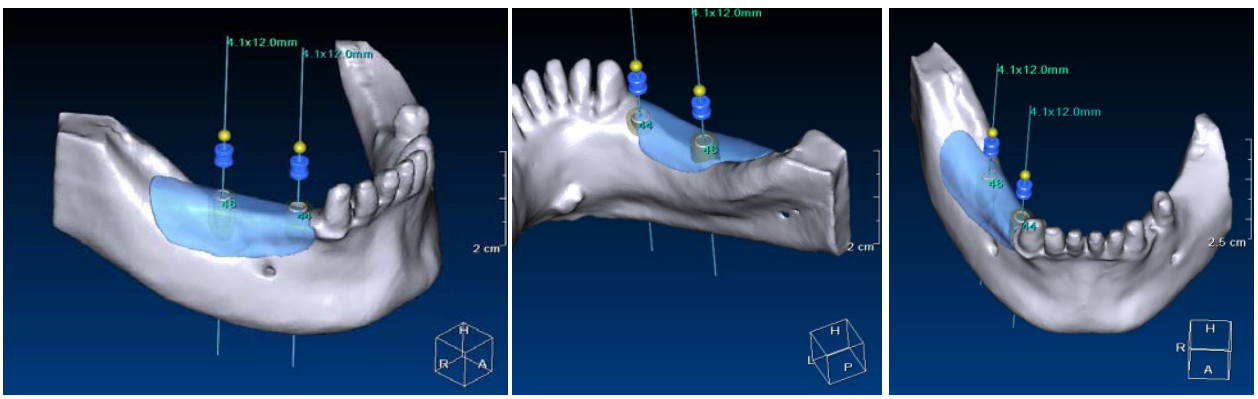

Fig. 6 – Virtual positioning of the implant and graft into the lower jaw using OnDemand 3D computer software.

graft model was manufactured along with the jaw by applying the rapid prototyping (RP) technology. 3D printing of the graft and jaw enabled oral surgeons to "hold the result", i.e. the solid object in their hands and to analyze the physical model (Figure 7). This step represented the last inspection prior to final fabrication, which enabled identification and elimination of some potential problems that were not visible in the virtual 3D model. After completing the required modifications and corrections of the 3D model, the final version of the graft was sent for fabrication using computer numerical control (CNC) milling machine-tool. Having in mind the complex shape of the graft, the fabrication was performed using a CNC machine-tool with the 5 degrees of freedom. The graft was manufactured from a monoblock of biocompatible material of bovine origin. Upon finishing, it was sterilized using ethylene oxide. Besides the prism-shaped block as the initial shape, a variety of shapes such as plates, cylinders, rods, etc, are available for manufacturing grafts with desired shape and dimensions. Virtual planning image of the composite block used for graft fabrication is presented in Figure 8. Block dimensions were (36.7 × 14.2 × 12 mm) length × height × width with the total block volume of 6.44 cm^3.

Surgical procedure

After delivering the custom bone graft in an original sterile package, the surgical procedure was performed (Figure 9a). Under block anesthesia of the inferior alveolar nerve, the full-thickness mucoperiosteal flap was lifted to expose the residual alveolar ridge. Using a 1-mm steel micro drill, perforations in the mandibular cortex were made to enhance blood supply to the graft. Bone graft was carefully positioned and fixed using two 12 mm titanium screws (Figure 9b). With the aim of preventing proliferation of fibrous tissue and infection, the graft was covered with two bio-absorbable collagen membranes that are essential for a successful augmentation procedure (Figure 9c). To eliminate tension force and reduce pressure onto the bone graft, periosteal releasing incision was made at the base of the flap and surgical region was closed with non-resorptive surgical suture 5-0 applying the horizontal mattress stitch technique. After completing surgery, control dental panoramic tomography (OPT) radiograph was made for checking the position of bone graft (Figure 9d).

During the postoperative course, antibiotic therapy was introduced (clindamycin + metronidazole) along with cold compresses and analgesics, and the patient was instructed on an appropriate hygienic-dietary regime.

Discussion

Deficit of the residual bone required to provide optimal conditions for an ideal placement of dental implant is a common problem in daily clinical practice. In such situation, adequate bone regeneration can provide the structural support. More than 60% of the population in highly industriali-

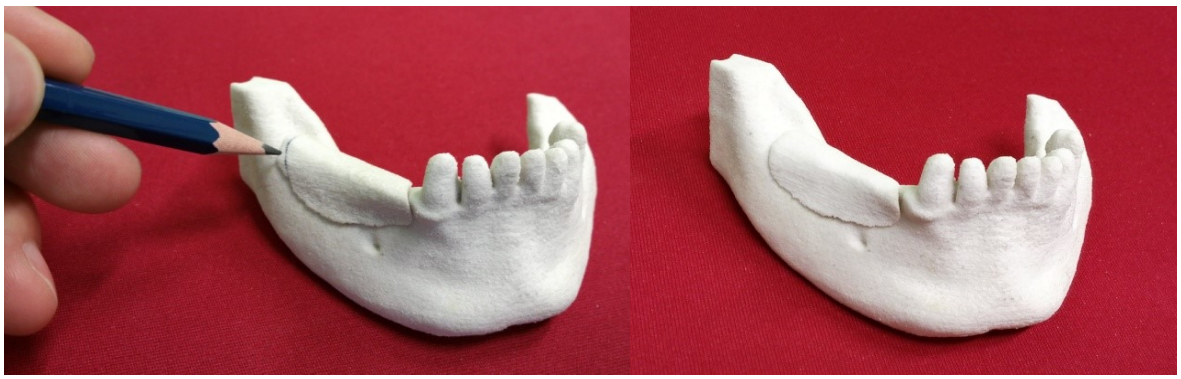

Fig. 7 – Physical models of the lower jaw generated by the rapid prototyping RP technology.

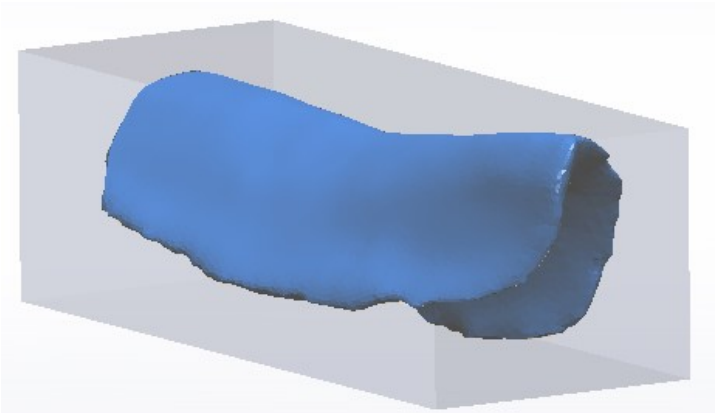

Fig. 8 – Virtual block used for graft fabrication using a computer numerical control (CNC) processing center.

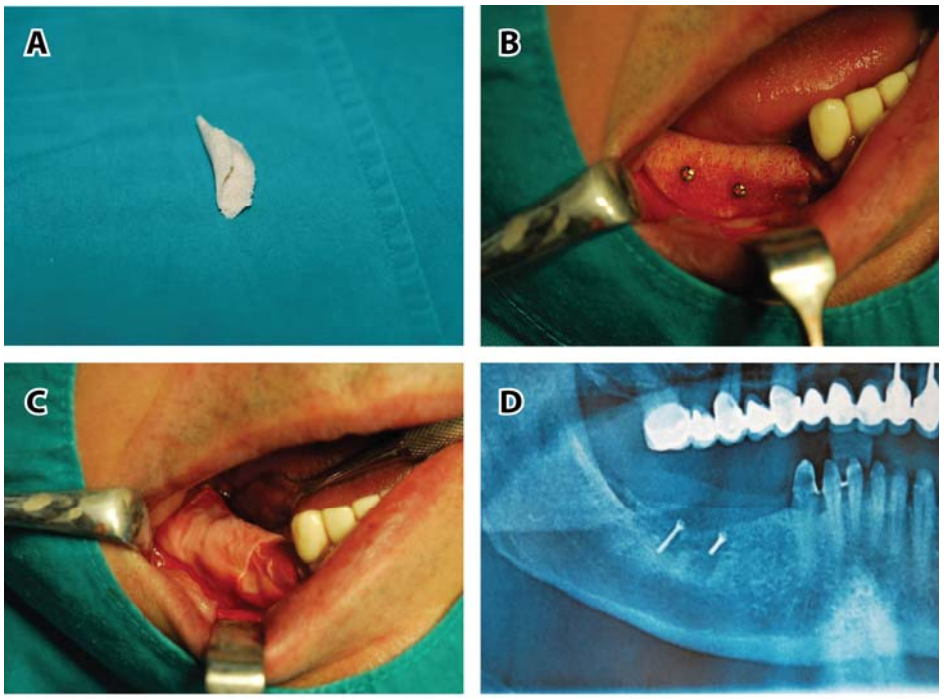

Fig. 9 – Surgical procedure step-by-step: a) custom bone graft ready for implantation; b) bone graft positioning and fixing in the lower jaw; c) graft covering with two bio-absorbable collagen membranes; d) control dental panoramic tomography radiograph showing correct graft position in the jaw.

zed countries requires implant-prosthetic rehabilitation, which results in a yearly increase of implant market for some 15% [12]. Nowadays, autologous bone grafts are considered the "golden standard" in bone regeneration and augmentation of substantial volume of the lost alveolar ridge. However, their major drawbacks include limited number of donor sites, the necessity of additional surgical procedure in a hardly accessible region of the oral cavity, need for general anesthesia, substantial trauma for the patient, as well as a prolonged postoperative recovery [13]. Thus, development and application of "artificial" custom (individual) grafts, which are characterized by relatively simple preparation, good predictability of the outcome and "comfort" for the patient himself, has been gaining increased attention. Demineralized freeze-dried allogeneic bone transplants can stimulate new bone formation and are a viable alternative to bone autograft material [14]. The custom-made grafts well matched the shape of the bone defects and could be easily implanted during surgery. This matching of the shape helped to reduce the time for the operation and contributed to the good healing of the defects [15].

The presented clinical report confirms that bone grafts could be created in the automated manner, starting from CT, and customized to each patient and for each type of clinical situation by applying modern X-ray techniques (3D CBCT) and advanced computer aided software systems. This enables diagnostic and surgical procedures, reduces time and improves the precision in adapting the graft, which is critical to its integration with the surrounding bone [16].

Conclusion

In our everyday clinical practice, we face a relatively large number of patients indicated for implant-prosthetic treatment. The loss of single or multiple teeth may result in substantial deficit of the residual alveolar ridge. Such situations require augmentation of lost bony structures in order to provide optimal conditions for dental implant placement and subsequent prosthetic rehabilitation. Application of 3D CBCT images, computer-aided systems and software in manufacturing custom bone grafts represents the most recent example of guided bone regeneration. This method substantially reduces time of recovery and carries minimum risk of postoperative complications, yet the results fully satisfy the requirements of both the patient and the therapist. The results presented in this article confirm the importance and effectiveness of computer-aided systems for 3D digitization, design and fabrication of custom bone grafts.

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CASE REPORT

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Unusual case of mixed form of femoroacetabular impingement combined with nonspecific synovitis of the hip joint in a young adult – A case report

Neobičan slučaj mešovite forme femoroacetabularnog impingementa kombinovanog sa nespecifičnim sinovitisom zgloba kuka kod mlade osobe

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Abstract

Introduction. Minimal bone changes in the acetabulum and/or proximal femur, through mechanism known as femoroacetabular impingement, during flexion, adduction and internal rotation lead to early contact between femoral head-neck junction and acetabular brim, in anterosuperior region. Each additional pathological substrate which further decreases specified clearance provokes earlier onset of femoroacetabular impingement symptoms. **Case report.** We presented a 20-year-old male patient with groin pain, limping, positive impingement test, radiological signs of mixed form of femoroacetabular impingement and unrecognized chronic hypertrophic synovitis with earlier development of clinical hip symptoms than it has been expected. Open surgery of the left hip was done. Two years after the surgery, patient was asymptomatic, painless, and free of motion, with stable x-rays. **Conclusion.** Hypertrophic synovial tissue further reduces the distance between the femoral head-neck junction and the acetabulum, leading to the earlier onset of femoroacetabular impingement symptoms. Surgical treatment is the method of choice.

Key words:
femoracetabular impingement; synovitis; hip joint; orthopedic procedures; treatment outcome.

Apstrakt

Uvod. Minimalne koštane promene na acetabulumu i/ili proksimalnom femuru, mehanizmom poznatim kao femoroacetabularni impingement u toku fleksije, addukcije i unutrašnje rotacije vode u raniji kontakt između spoja femoralne glave i vrata sa ivicom acetabuluma u anterosuperiornoj regiji. Svaki dodatni patološki supstrat koji dalje smanjuje rastojanje između ivice acetabuluma i vrata femura provocira raniju pojavu simptoma femoroacetabularnog impingementa. **Prikaz bolesnika.** Prikazali smo bolesnika, starog 20 godina, sa bolom u preponi, hramanjem, pozitivnim testom impingementa, radiografskim snimcima mešovite forme femoroacetabularnog impingementa i neprepoznatim hroničnim hipertrofičnim sinovitisom kuka. Urađena je otvorena hirurška procedura levog kuka. Dve godine nakon operacije, bolesnik je bez tegoba, punog obima pokreta, sa nepromenjenim radiografskim nalazom zgloba kuka. **Zaključak.** Hipertrofični sinovitis kuka dodatno smanjuje rastojanje između spoja femoralne glave i vrata i acetabuluma i dovodi do ranije pojave simptoma femoroacetabularnog impingementa. Hirurško lečenje je metoda izbora.

Ključne reči:
femoroacetabularni sudar; sinovitis; kuk, zglob; ortopedske procedure; lečenje, ishod.

Introduction

Acetabular labrum damage leads to loss of its function, early appearance of groin pain, loss of hip function and early hip osteoarthritis development [1, 2]. Numerous pathomechanism have been proposed to explain the labrum damage, including the femoroacetabular impingement (FAI) [3, 4]. Minimal bone changes of the acetabulum and/or femoral head, reduce the distance between them, leading to premature contact-impact of the femoral head-neck junction on the edge of the acetabulum, damaging labrum and labrum adjacent cartilage, wich are early signs of hip arthritis [5–8]. Three types of

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FAI have been described: the first one is cam type with a morphological changes at the femoral head-neck junction in the form of cam deformmity [9]. The secoond one is pincer type, in which the patological changes are localized exclusively to the acetabulum as a global (*coxa profunda*, *protrusio acetabuli*) or local overcoverage (*retroversio acetabuli*) of the femoral head, where the motion of the hip leads to the impact of the femoral neck on the edge of the acetabulum and consequent damage to the acetabular labrum [4]. The third, mixed and the most common type of FAI is the combination of the previous two [7, 10–13]. Synovitis of the hip is a reaction of the articular synovium on the systemic or local pathological substrate in the form of synovial joint fluid production [14–16] or specific hypertrofic synovium reaction that is seen in pathological conditions such as pigmented villonodular synovitis, tuberculosis or rheumatoid pannus [17–20]. In the literature we found no case of hypertrofic non-specific synovitis combined with any kind of FAI.

We presented a patient with nonspecific chronic hypertrofic synovitis of the hip, combined with a mixed form of FAI, who was surgically treated.

Case report

A 20-year-old male patient suffered from pain in the left groin, which lasted two years before the surgery. The patient was treated in several orthopedic institutions with non-steroid anti-inflammatory drugs, physiotherapy, skin traction, and suggested total hip replacement. Six months before the surgery, during the ultrasound examination of the hip joint, synovial fluid in the hip was asserted for which injection of corticosteroids into the joint was administered. After that, the symptoms became more pronounced, the patient began to limp visibly, walking on flat surfaces was difficult, and climbing up the stairs was practically impossible. At clinical examination the patient walked with the left leg in external rotation, with highlighted limps on that leg, Trendeleburg sign was positive on the left leg, highlighted weakness of muscles of the thigh and the left gluteal region, active flexion of the hip was possible up to 70°, internal rotation up to 5°, adduction up to 10° in the hip flexion of 70°. Impingement test was positive in the hip flexion of 30–70° [3]. The patient brought computed tomography (CT) image of the hips and nuclear magnetic resonance (NMR) that were made before the application of corticosteroids into the joint and which showed that the bone and soft tissue structures of the hips were normal. Biochemical and laboratory tests were also within normal limits. Standardized anteroposterior radiography of the hips showed characteristics findigns for femoroacetabular impingement reported earlic in literature: (Figure 1) a strong mutual retroversion of the acetabulum 12° on the left and 11° on the right hip [21], mutual positive sign of ischial spine projection [22], the angle α was 46° on the right and 44° on the left [23], acetabular index, mutual 10° [24], mutual Wiberg angle 36° [25], a kolodyaphyseal angle of 126° that was asserted bilaterally.

In profile, Dunn-Ripstein-Müller's radiographs of the hips [26], the angle α on the left was 64° (normal value of this angle is below 50°), and on the right 42° (Figure 2).

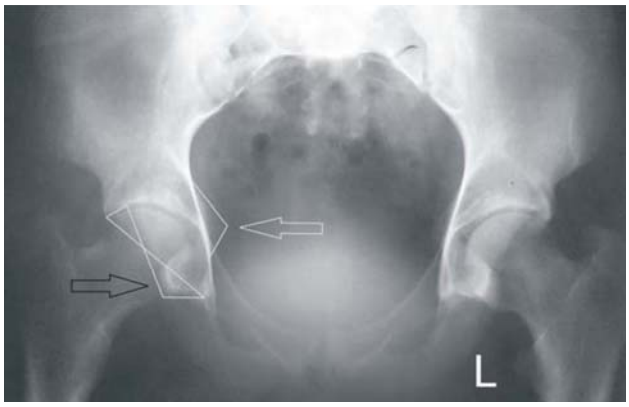

Fig. 1 – Standardized anteroposterior x-ray of the hips showing bilaterally positive sign of the ischial spine (white arrow), and acetabular retroversion (black arrow).

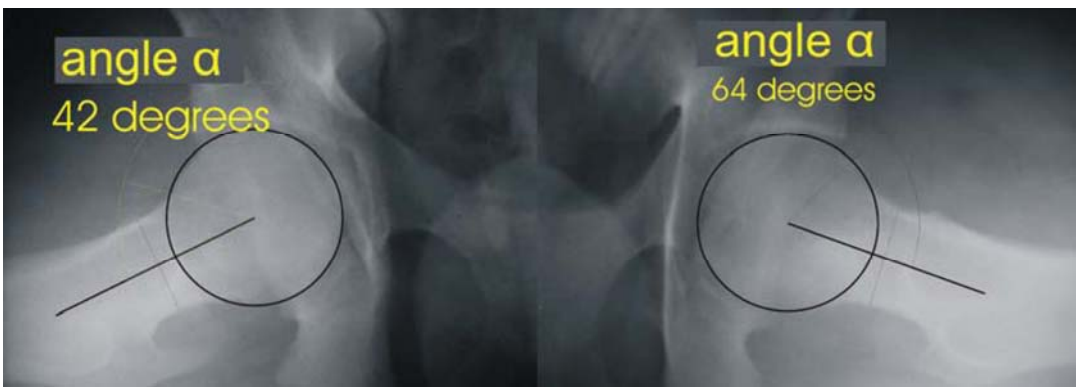

Fig. 2 – Standardized profile Dunn-Rippstein-Müller x-ray images of the hips: the left hip (right picture) – angle α 64°; the right hip (left picture) – angle α 42°.

Such clinical and radiographic findings revealed the existence of mixed-type of FAI on the left hip, due to which we proposed surgical treatment to the patient. The patient was operated on. It was planned to lift the acetabular labrum from its base, to osteotomize overcovered anterior and superior edge of the acetabulum, then, reinsert acetabular labrum in a new slot, and osteotomize cam deformity at the femoral head-neck junction in its antero-superior region. Lateral hip incision and transtrohanteric flip osteotomy of the hip were done [27], and after anterior "Z" hip capsulotomy unexpected hypertrophic synovial hip reaction was aserted (Figure 3).

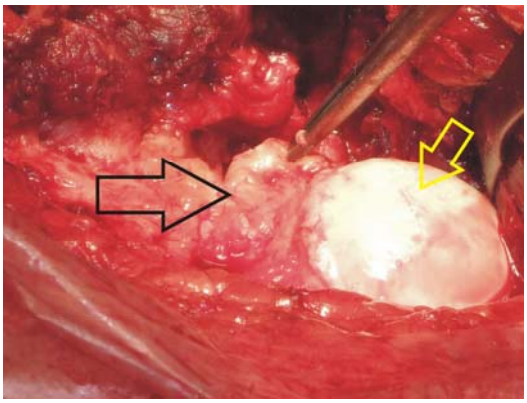

Fig. 3 – Intraoperative image: hypertrophied synovitis of the joint capsule on the anterior and superior femoral neck and head (black arrow), and femoral head (yelow arrow).

Entire extra-acetabular part of the joint was filled with synovial tissue which had fully and seriously damaged the anterior and the superior part of the acetabular labrum. Partial hip synoviectomy was performed, tissue samples were sent to histopathological analysis, the damaged parts of the labrum was resected, without possibility to be reinserted, anterior and superior part of the acetabular edge were osteotomized to reduce acetabular retroversion. Osteochondroplasty of the cam prominence at the femoral head-neck junction was done (Figure 4), and thus, intraoperatively, hip flexion of 90° and internal rotation of 30° was obtained [28]. After joint capsule reconstruction, osteotomized part of the greater trochanter was re-fixed with two cortical screws and the operational wound was closed.

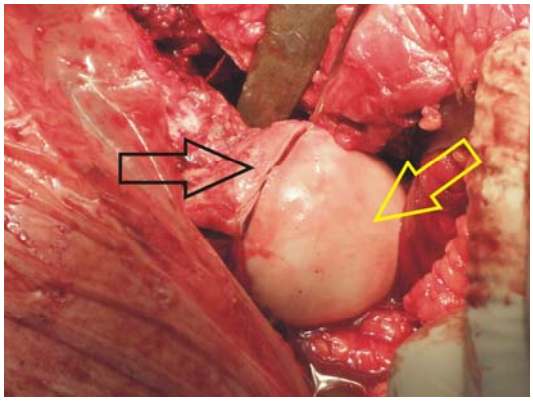

Fig. 4 – Intraoperative picture: osteochondroplasty of the cam deformity of the femoral head-neck junction (black arrow), and femoral head (yellow arrow).

Postoperatively, the patient was reimbursed with 400 mL of blood; low molecular weight heparin thromboprophylaxis for seven days was introduced, followed by oral anticoagulant therapy for up to six weeks after the surgery. On the first postoperative day active exercises in bed started and walking on the crutches was allowed from the second day with touching the tip of toes of the operated leg for six weeks after the surgery. Histopathological findings confirmed a nonspecific chronic hypertrophic synovitis of the hip. Two years after the surgery, the patient was asymptomatic, without limping on the left leg and with normal life activities. Flexion of the operated hip was 90°, internal rotation 25°, adduction 30°, impingement test was negative. On the anteroposterior radiographs of the hips, the acetabular angle of retrovesion was reduced up to 4°, the angle α was 40° and 49° on the profile of Dunn-Rippsetin-Müller radiographs of the left hip.

Discussion

Damages of the acetabular labrum cause pain and partial loss of function of the hip in young adults. There is a number of reasons for labrum lesions, such as mechanical trauma of the hip (hip dyslocations, acetabular fractures) and in patients with FAI [1–7, 10–13], otherwise, it is the result of biological specific or nonspecific synovial hypertrophic pannus production [17–20]. Morphological bone changes in the area of the acetabulum and /or proximal femur in patients with FAI reduce physiological distance between these anatomical structures of the hip, lead to premature contact-impact of the femoral neck on the edge of the acetabulum, causing damage of the acetabular labrum and acetabular cartilage adjacent to it, but never lead to mechanical irritation of the articular capsule and its hypertrophic response.

We presented a patient with a clear clinical and radiographic picture of mixed form of FAI with normal biochemical, CT and NMR findings in which the subjective symptoms suddenly worsened after inta-articular application of corticosteroids in the hip joint. We found intraoperatively and verified histologically, a nonspecific reaction of the hypertrophic synovium, an unexplained etiology, that filled the hip joint, further decreasing the space between the edge of the acetabulum and the anterior and superor part of the femoral neck, with additional deterioration of the patient's subjective complaints, hip movements and irreversible damage of the acetabular labrum without the possibility to be reinserted but only partially resected. There were no reports in the literature to compare with our experience. We hypothesized, as a possible explanation, that the intraarticular administration of corticosteroids was the reason for the development of nonspecified hypertrophy of the hip synovium, but it needs furhter research.

FAI has been, in the last 20 years, well-defined pathological and pathophysiological entity which is treated only surgically [1–15, 29].

Conclusion

We believe that young adults with pain in the groin and with normal biochemical, CT and NMR parameters should not be exposed to unnecessary and inappropriate treatment (skin traction, total hip replacement or intra-articular corticosteroid application), but to be appropriately operated on.

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

3. ***Text***

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

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

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

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

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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

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

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

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

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Primeri referenci:

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

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

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

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

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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.

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