Srp Arh Celok Lek ISSN 0370-8179 COBISS.SR-ID 3378434 UDC 61(497.1)=861

СРПСКИ АРХИВ

ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА ОСНОВАН 1872. ГОДИНЕ

ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

ГОДИШТЕ 144. | VOLUME 144
СЕПТЕМБАР–ОКТОБАР 2016. | SEPTEMBER–OCTOBER 2016
СВЕСКА 9-10 | NUMBER 9-10

SERBIAN ARCHIVES OF MEDICINE

OFFICIAL JOURNAL OF THE SERBIAN MEDICAL SOCIETY FOUNDED IN 1872

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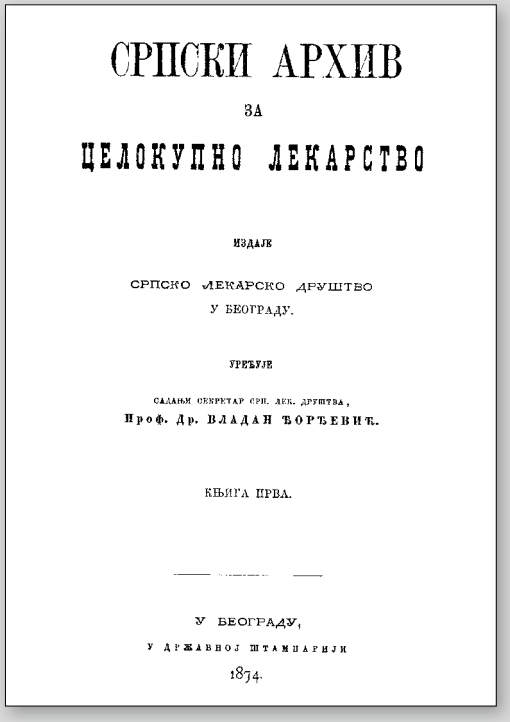

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

Проф. Др. ВЛАДАН ЂОРЂЕВИЋ.

КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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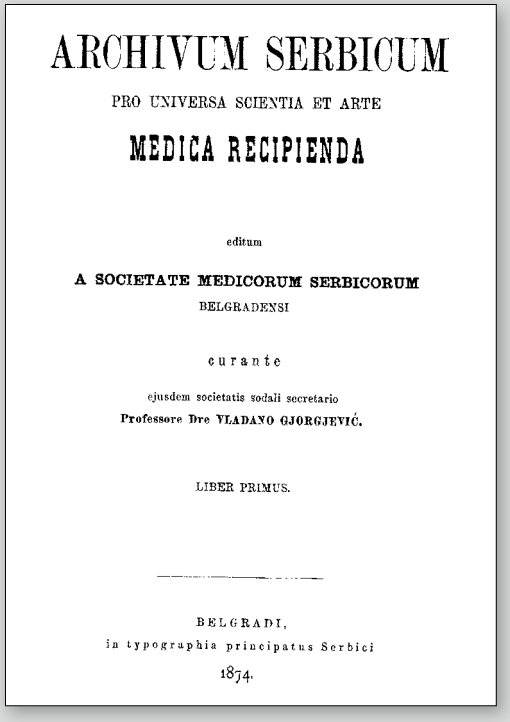

ARCHIVUM SERBICUM

PRO UNIVERSA SCIENTIA ET ARTE

MEDICA RECIPIENDA

editum

A SOCIETATE MEDICORUM SERBICORUM

BELGRADENSI

curante

ejusdem societatis sodali secretario

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LIBER PRIMUS.

BELGRADI,

in typographia principatus Serbici

1874.

Facsimile of the text in Latin language of the title page of the first Journal edition published in 1874 (two years after the Journal was founded)

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Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.1)=861
COBISS. SR-ID 3378434
Српски архив за целокупно лекарство је званична публикација Српског лекарског друштва и излази шест пута годишње.

СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

ГОДИШТЕ 144. СЕПТЕМБАР–ОКТОБАР 2016. СВЕСКА 9-10

Часопис „Српски архив за целокупно лекарство" је индексиран у базама: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

ИЗДАВАЧ
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
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Штампање „Српског архива за целокупно лекарство" током 2016. године помогло је Министарство просвете, науке и технолошког развоја Републике Србије.

ISSN 0370-8179; ISSN Suppl 0354-2793;
ISSN Online 2406-0895

Штампано у Србији
www.sld.org.rs
www.srpskiarhiv.rs

SERBIAN ARCHIVES OF MEDICINE

VOLUME 144 SEPTEMBER–OCTOBER 2016 NUMBER 9-10

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.1)=861
COBISS. SR-ID 3378434

Serbian Archives of Medicine is an official publication of the Serbian Medical Society, and is published six times per year.

The journal "Srpski arhiv za celokupno lekarstvo" (Serbian Archives of Medicine) is indexed in: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

PUBLISHER
Serbian Medical Society
Džordža Vašingtona 19, 11000 Belgrade, Serbia
President: Prof. Radoje Čolović, MD, PhD, MSASA
Chair of the Publishing Council:
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Bank accounts: 205-8041-21 and
355-1009094-22

Full-text articles are available at website: www.srpskiarhiv.rs

Calendar year subscriptions prices are: 3,000 dinars for individuals, 6,000 dinars for institutions, and 100 Euros for readers outside Serbia. The price of a current issue is 600 dinars, and of a back issue 300 dinars.

The publishing of the Serbian Archives of Medicine during 2016 is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ISSN 0370-8179; ISSN Suppl 0354-2793; ISSN Online 2406-0895

Printed in Serbia
www.sld.org.rs
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ОРИГИНАЛНИ РАДОВИ • ORIGINAL ARTICLES

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):470-473
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1610470T
UDC: 616.31-089.5

Anterior and middle superior alveolar block is efficient for maxillary premolar teeth extractions regardless of the injection system or anesthetic with adrenaline used

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SUMMARY

Introduction The anterior and middle superior alveolar nerve block was claimed to be unpredictably efficient for clinical application.

Objective The aim of this report was to establish the efficacy of the anterior and middle superior alveolar nerve block, applied with a computer-controlled injection system or a conventional syringe, for upper premolars extraction.

Methods Sixty healthy adults were divided into two groups regarding the device used as follows: the first group was injected by a computer-controlled injection system, and the second group by a conventional syringe. Pain ratings were obtained via a visual analog scale (VAS) and a verbal rating scale (VRS).

Results Anterior and middle superior alveolar injection enabled a painless extraction in all patients, regardless of the local anesthetic or injection system used. It was slightly less painful when administered by a computer-controlled injection system, but insignificantly when evaluated by VRS.

Conclusion The anterior and middle superior alveolar nerve block may be recommended if maxillary permanent premolars have to be extracted.

Keywords: nerve block; hard palate; analogue pain scale; tooth extraction

INTRODUCTION

Local anesthesia needed for tooth extraction in the maxilla is generally achieved by a supraperiosteal infiltration injection. However, this technique is sometimes inadequate for relieving pain during extraction in cases of teeth affected by periodontal infection; also, paresis of muscles of facial expression, which occurs to some degree, may interfere with esthetic dental work in the region. The anterior and middle superior alveolar (AMSA) nerve block, introduced in 1998 is an alternative technique that could solve the mentioned problems [1]. It derives its name from the fact that both the anterior and the middle (if existing) alveolar nerves are blocked, thus providing anesthesia of several maxillary teeth (including incisors, canines and premolars) [2].

Several studies have shown that AMSA nerve block provides for variable pulpal anesthesia of the mentioned teeth [3, 4, 5]. However, in some researches it was claimed to be too unpredictable in its efficiency to be recommended for clinical application as the first choice [3]; some others, however, ascertained quite efficient anesthesia when a computer-controlled injection system was used [4], or at least more successful than the AMSA nerve block achieved by use of a conventional syringe [5]. It was stressed that additional advantage of the use of computer-controlled injection system over conventional syringe is less pain during the injection, which is especially important for palatal injections [1, 4, 6]. Nevertheless, there are also reports on the same pain level, mainly of a low intensity, during injection regardless of the injection system used [7].

Interestingly, there are no available studies in literature concerning the efficacy of the AMSA injection in enabling painless permanent maxillary teeth extraction. However, there are results on the AMSA nerve block efficacy for the removal of maxillary primary molars, indicating approximately the same efficacy as that achieved by traditional supraperiosteal injection [8]. Using the AMSA nerve block in patients undergoing extraction of maxillary premolars, Nusstein et al. [9] found no statistical difference in comparison to routine supraperiosteal injection, and found that the incidence of postinjection pain and sequelae was low with both techniques. It was hypothesized that the AMSA nerve block will ensure a painless extraction of permanent maxillary premolars regardless of the local anesthetic or

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injection device used, but that the use of a computer-controlled injection system will enable a less painful delivery of local anesthesia.

OBJECTIVE

The aim of this study was to establish the efficacy of the AMSA nerve block for tooth extraction, applied with a computer-controlled injection system or a conventional syringe, when local anesthetics with different contents of adrenaline were used. An additional aim was to compare the pain experienced when a computer-controlled injection system was used to that of conventional syringes.

METHODS

The clinical trial was conducted at the Department of Oral Surgery, Medical Faculty in Foča, Bosnia and Herzegovina.

Patients

The study protocol was approved by the Ethical Committee of the Faculty of Medicine (registration number 01-8/8, issued 11/2/2009) and conducted in accordance with accepted ethical standards for research practice (guidelines of the Declaration of Helsinki of 1975, as revised in 1983). All participants signed an informed consent form.

Sixty healthy adults randomly selected from patients visiting the Department, requiring extraction of a single upper premolar, participated in the study. They were of both gender, otherwise healthy (determined by a written medical health form), ranging from 18 to 65 years of age, and not taking any medication that could alter their pain perception.

The patients were informed that computer-controlled and conventional injection techniques were being studied. All patients were divided into two groups regarding the device used for applying the AMSA nerve block: the first group received the AMSA nerve block by a computer-controlled injection system (Figure 1), using the Anaeject computer-controlled local anesthetic delivery system (Septodont, Saint-Maur-des-Fossés, France), and the second group received the AMSA block by a conventional syringe with carpules. All the patients had previously experienced a conventional syringe, but no one had previously received a computer-controlled injection.

Each group was subdivided into three subgroups depending on the content of adrenaline in the local anesthetic used – 0.9 mL of 3% mepivacaine plain (Septanest®, Septodont), 0.9 mL of 4% articaine with adrenaline 1:100,000 (Ubistesin™ forte, 3M Deutschland GmbH, Seefeld, Germany), and 0.9 mL of 2% lidocaine with adrenaline 1:80,000 (Xylestesin®, 3M Deutschland GmbH).

All the patients received the AMSA nerve block as previously described for local anesthesia preceding tooth extraction [1, 2]. They were positioned supine on the dental chair with slight hyperextension of the neck in order to provide good accessibility and visibility (Figure 2), and informed that the procedure will last slightly longer than usual, especially in the first group that received a computer-controlled injection (approximately 3 minutes).

Figure 1. Anaeject computer-controlled local anesthetic delivery system (Septodont, France)

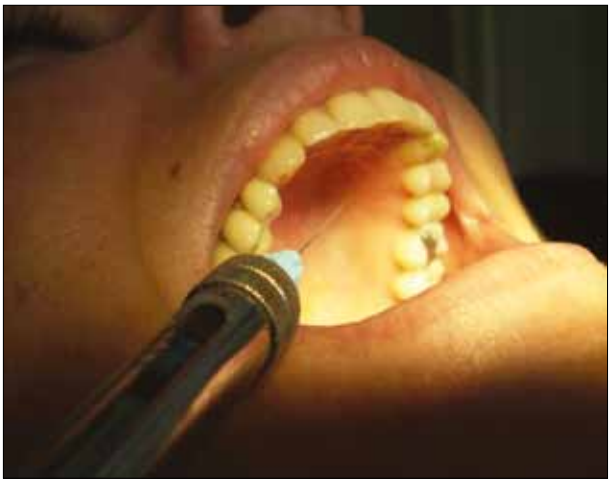

Figure 2. The AMSA nerve block done with slight hyperextension of the neck

The pain ratings were explained to the patients before the injection. Verbal pain level descriptions for the pain experienced during the injection were as follows: no pain (0), minimal pain (1), slight pain (2), moderate pain (3) and severe pain (4). The participants provided written and verbal pain ratings via a visual analog scale (VAS) – written, and a verbal rating scale (VRS) – verbal, immediately after the injection. The operator obtained the visual analogue scale filled in for evaluation of possible pain experienced during tooth extraction from each patient immediately after surgery.

Statistical analysis

Data was analyzed using descriptive statistical methods (frequency percentages, means and standard deviations) and Wilcoxon's test using SPSS ver. 13 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined at $p < 0.05$.

RESULTS

The AMSA injection enabled, plainly speaking, a painless extraction in all the selected patients, regardless of the local anesthetic or injection system used, and without statistical significance (Table 1). Descriptions of pain level varied between "no pain" and "minimal pain," not once being defined as worse or intolerable for tooth extraction (data not presented).

Experience of pain during the AMSA injection differed more, especially when reported by VAS (Table 2) – the greatest difference was noticed when local anesthetic without vasoconstrictor was used. However, when pain during the AMSA injection was evaluated by VRS, the differences were not significant (Table 3); the patients described the injection mostly by expressions "no pain" or "minimal pain," and only six out of 30 patients from the group who received the AMSA nerve block by conventional syringe experienced "slight pain" (Table 3). Regardless of the pain rating scale used, the pain was slightly stronger in patients who received the AMSA nerve block with conventional syringe (in comparison to those who received the AMSA nerve block with a computer-controlled injection system), but the differences were mostly non-significant (Tables 2 and 3).

Table 1. Intensity of the achieved local anesthesia (painless tooth extraction) after the AMSA injection done by a computer-controlled injection system and conventional syringe

Local anesthetic	Intensity of anesthesia (VAS)		Statistical significance
	Computer-controlled injection system	Conventional syringe	
3% mepivacaine plain	99 ± 1.63	98.3 ± 2.50	ns
2% lidocaine/ adrenaline (1:80,000)	99.5 ± 1.27	99.5 ± 0.85	ns
4% articaine/ adrenaline (1:100,000)	99.2 ± 1.40	98.9 ± 1.73	ns

VAS – visual analogue scale: maximum intensity – 100 mm; no anesthesia – 0 mm
ns – not significant

Table 2. Pain experienced during the AMSA injection, expressed by the visual analogue scale (VAS)

Local anesthetic	Pain during the AMSA injection		Statistical significance (p)
	VAS (mm)*		
	Computer-controlled injection system	Conventional syringe	
3% mepivacaine plain	0.7 ± 1.16	3.1 ± 2.38	p = 0.01
2% lidocaine/ adrenaline (1:80,000)	0.7 ± 1.49	1.7 ± 1.64	ns
4% articaine/ adrenaline (1:100,000)	0.8 ± 1.32	2.9 ± 2.38	p = 0.03

*maximum intensity – 100 mm; no anesthesia – 0 mm
SD – standard deviation; ns – not significant

Table 3. Pain experienced during the AMSA injection, expressed by the verbal rate scale (VRS)

Local anesthetic	Pain during the AMSA injection*											
	Computer-controlled injection system						Conventional syringe					
	0	1	2	3	4	X ± SD	0	1	2	3	4	X ± SD
3% mepivacaine plain	7	3	-	-	-	0.3 ± 0.48	2	6	2	-	-	1.0 ± 0.67
2% lidocaine/adrenaline (1:80,000)	8	2	-	-	-	0.2 ± 0.42	4	5	1	-	-	0.7 ± 0.67
4% articaine/adrenaline (1:100,000)	7	3	-	-	-	0.3 ± 0.48	2	5	3	-	-	1.1 ± 0.74

*0 – no pain; 1 – minimal pain; 2 – slight pain; 3 – moderate pain; 4 – severe pain
X – mean value; SD – standard deviation

DISCUSSION

The main aim of this research was to evaluate efficacy of the AMSA nerve block in providing adequate local anesthesia for extraction of permanent upper premolars. Interestingly, it was difficult to find data concerning that particular matter in related literature. There are results on AMSA nerve block efficacy for removal of maxillary primary molars, indicating approximately the same efficacy as that achieved by traditional supraperiosteal injection [8]. However, two of 30 patients receiving the AMSA nerve block experienced severe pain during tooth extraction (it is not stated whether a supplemental anesthesia was needed and which teeth were extracted in these two patients).

In our study, regardless of the injection device and local anesthetic used for inducing the AMSA nerve block, the obtained anesthesia was sufficient for painless tooth extraction in all the patients. It is especially interesting that extraction of permanent maxillary premolars was painless or with minimal pain even in patients where local anesthetic without a vasoconstrictor (3% mepivacaine plain) was used. The differences between the devices used (a computer-controlled injection and a conventional syringe) were also insignificant. These results point out the predictable efficacy of the AMSA nerve block in achieving local anesthesia needed for permanent premolars extraction, regardless of the local anesthetic used.

It is well known that palatal injections are, generally speaking, more painful than injections at other sites in the oral cavity [2]. It is usually claimed that computer-controlled delivery systems enable less painful palatal injections compared to those with conventional syringes [1, 4, 6], although there are also different findings [7]. That is why we aimed to evaluate both delivery systems in terms of painlessness of the injection. Our results show that the AMSA nerve block is not as painful as it is usually claimed for palatal injections, possibly due to relatively slow anes-

thetic delivery regardless the device used. However, our results point to some differences in regard to the scale used for pain rating; although both scales (VAS and VRS) pointed to the fact that patients experienced less pain when a computer-controlled injection device was used; however, a conventional syringe was also tolerated very well and it can be said that patients did not experience even moderate pain during the AMSA nerve block, regardless of the injection device used. Comparing the pain experienced during the AMSA nerve block in regard to the injection device used, statistically significant differences were noted only with VAS ratings. The fact that VRS ratings, with the same patients and in the same situation, were similar and insignificantly different, might point to the fact that patients, especially in small and mainly rural milieus, understand VRS better than VAS.

CONCLUSION

Having in mind all the presented results, our experience with the AMSA nerve block used for local anesthesia needed for maxillary permanent premolars extraction is quite favorable. Therefore, the AMSA nerve block may be recommended if maxillary permanent premolars have to be extracted.

NOTE

This paper is a part of the PhD dissertation titled „The Influence of Adrenaline in Local Anaesthetic Solution on the Characteristics of Anterior and Middle Superior Alveolar Nerve Block Achieved by Palatal Approach" by Slavoljub Tomić.

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Блок предњих и средњих горњих алвеоларних нерава је ефикасан за вађење горњих сталних премолара без обзира на прибор за апликацију и врсту локалног анестетика

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КРАТАК САДРЖАЈ

Увод Ефикасност блока предњих и средњих горњих алвеоларних нерава у клиничкој примени је непредвидљива.

Циљ рада Циљ овог истраживања је био да се утврди ефикасност блока предњих и средњих горњих алвеоларних нерава, апликованог компјутерски потпомогнутим системом за континуирану апликацију анестетика или стандардном карпул бризгалицом, за вађење горњих премолара.

Методе рада Шездесет здравих одраслих особа подељено је у две групе у зависности од система за апликацију: прва група је инјекцију примила компјутерски потпомогнутим системом за континуирану апликацију анестетика и друга је анестетик примила стандардном карпул бризгалицом. Ниво бола је оцењиван путем визуелно аналогне скале (ВАС) и вербалне скале (ВС).

Резултати Блок предњих и средњих горњих алвеоларних нерава омогућио је безболно вађење зуба код свих пацијената, без обзира на коришћени локални анестетички раствор и начин апликације. Нешто је мање болно било код пацијената код којих је анестетик администриран компјутерски потпомогнутим системом за континуирану апликацију локалног анестетика, када је процењивана ВС, али је то безначајно.

Закључак Блок предњих и средњих горњих алвеоларних нерава се може препоручити за вађење горњих сталних премолара.

Кључне речи: нервни блок; тврдо непце; аналогна болна скала; вађење зуба

Примљен • Received: 29/09/2015 **Ревизија • Revision:** 11/02/2016 **Прихваћен • Accepted:** 17/02/2016

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):474-477
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1610474R
UDC: 616.314-089.87-056.24

Prosthetic treatment after teeth extractions in patients with type 2 diabetes mellitus

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SUMMARY
Introduction Good and well balanced diet provided by adequate mastication is part of therapy in patients with type 2 diabetes mellitus (DM). The critical period presents the time immediately after teeth extractions; hence, immediate denture is a rational therapeutical choice for diabetic patients. The presence of immediate denture and its compression might compromise wound healing process, affect chewing ability, food intake and consequently blood glucose level in type 2 DM patients.
Objective The objective of this study was to compare socket opening diameters (SOD), chewing ability, changes in blood glucose level and food intake in type 2 DM patients with and without maxillary immediate complete denture (MICD) during a three-week wound healing period.
Methods The study comprised 78 type 2 DM partially removable denture wearers (42 candidates for teeth extractions and 36 candidates for teeth extractions and insertion of MICDs). During the three-week period participants were followed for SOD, chewing ability and changes in blood glucose level and food intake.
Results Patients with MICD showed significantly lower reduction of SOD (seventh, 14th, 21st day) and higher chewing ability (seventh, 14th, 21st day) in comparison to patients without an MICD. Significantly lower number of patients with an MICD had changes in blood glucose level and food intake.
Conclusion Maxillary immediate complete denture presents a good therapeutic choice for type 2 DM patients, as it provides possibility of adequate mastication after teeth extractions and maintenance of nutritional status and blood glucose level.
Keywords: type 2 diabetes mellitus; immediate denture; chew ability; food intake

INTRODUCTION

Type 2 diabetes mellitus (DM), commonly found in dental patients, is one of the most prevalent diseases worldwide [1]. Oral disturbances associated with DM include more extensive periodontal disease, salivary gland dysfunction, various types of stomatitis and delayed wound healing [2–5]. Besides relationship between metabolic control of DM and oral health status [6], studies have also shown higher prevalence of missing teeth and teeth with deep pockets indicated for extractions in diabetic patients compared to nondiabetics [7]. Alterations in oral status and loss of teeth affect masticatory ability and food selection [8, 9, 10]. In connection with this, complete and partially edentulous patients showed nutrition disadvantage with reduced intake of dietary fiber and vitamins B6, C, carbohydrates, beta-carotene, and folate [11, 12]. Malnutrition and lack of some nutrients, such as carbohydrates, vitamin A, and omega-3 fatty acids, prolong wound healing process [13]. Clinical studies concerning the effects of type 2 DM on dental postextraction wound healing are rare, with lack of data about nutrition and chewing ability during the postextraction period. Aronovich et al. [14] reported unpredictable wound healing between well and poorly controlled diabetics after teeth extractions with no statistically significant difference in postextraction outcomes between observed groups. Estimating wound healing, four weeks after teeth extractions, Huang et al. [15] found similar outcomes in type 2 DM in oral hypoglycemics and nondiabetic patients. Since good and well balanced diet is important as a part of diabetes therapy and wound healing process generally, the rational choice for diabetic candidates for teeth extraction is to receive immediate dentures on the day of tooth extraction in order to obtain adequate chewing ability. However, the presence of immediate denture and its compression might change the conditions of postextraction wound healing process, representing another risk for impaired wound healing in diabetic patients.

OBJECTIVE

Having in mind the lack of data about nutrition and chewing ability in postextraction period in type 2 DM patients, the aim of our study was to determine whether the presence of immediate denture influences the reduction of postextraction sites during wound healing process, glycemic control, chewing ability and food intake of diabetic patients after tooth extractions.

METHODS

Study population

The study population comprised 78 type 2 DM patients aged 45 to 64 years (42 candidates for

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teeth extractions and 36 candidates for teeth extractions and insertion of complete maxillary immediate dentures – MICD). The inclusion criteria for all participants were the following: wearing maxillary partial removable dentures (with the presence of three remaining teeth in the anterior and premolar maxillary regions with signs of terminal periodontitis) and mandibular complete dentures for more than five years, and a disease history of at least two years with a glycosylate hemoglobin level less than nine (HbA1c < 9). The exclusion criteria were smoking and not wearing new dentures on a regular basis during the observation period (for the group of patients with MICD). Six individuals from the group of patients with MICD were excluded because they had not regularly worn new dentures; the final number of participants with MICD was 36.

Pre-prosthetic procedures included non-traumatic extractions of remaining three maxillary teeth without elevation of full-thickness flap to preserve the bone ridges and soft tissue. For patients who were indicated to receive MICD, new dentures were placed immediately after teeth extractions. Post-insertion denture adjustments necessary for removing difficulties that included pain and discomfort were performed during the regular follow-up. Informed consent was obtained from all participants in the study. The study protocol was approved by the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade (No. 32/36) and conducted in accordance with the Helsinki Declaration.

Clinical examinations

Dental records for all participants were provided at the Clinic of Prosthodontics and Clinic of Oral Surgery, Faculty of Dental Medicine, University of Belgrade. Regular follow-up of the postextraction wound healing in type 2 DM patients with and without MICD were scheduled after three and seven days, and weekly, during a three-week period in order to obtain the records of complete socket epithelization. In the course of observation period, the same investigator, who was unaware of patients' prosthetic status, (presence of MICD) measured bucco-lingual diameter (using dental caliper) from postextracted socket openings and expressed as mean value. The presence of any side effects or complications (symptoms of dry socket and infection) were also recorded. The patients were given both verbal and written instructions on how to control pain and discomfort in the first three weeks after the extractions and denture insertions. As analgesic, 400 mg per os of ibuprofen (Brufen®, Galenika, Belgrade, Serbia) was recommended. Using the same observation days, the patients self-reported the chewing ability of the food they usually eat. Chewing ability was categorized by the following six-point verbal rating scale (VRS): 1 = able to chew with no difficulties, 2 = just noticeable chew discomfort, 3 = weak discomfort during chewing, 4 = moderate discomfort during chewing, 5 = severe discomfort during chewing, 6 = not able to chew. Three weeks after wound healing, all the patients self-reported the usual blood glucose levels obtained through home testing with a glucose meter (in accordance with the study of Aronovich et al. [14]) and self-reported changes in food intake (in accordance with the study of Madhuri et al. [16]).

The results were presented as frequencies or as mean ± standard deviation. One-way ANOVA with repeated measures and post-hoc Bonferroni test was applied within the groups. Comparisons between appropriate group/time points were done by using Student's t-test for the independent samples. Frequencies of participants' characteristics in compared groups were analyzed by χ^2 test. The differences were considered to be significant for $p < 0.05$. The data were analyzed with Statistica (StatSoft Inc., Tulsa, OK, USA) for Windows (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Table 1 represents the socket opening diameter (SOD) in type 2 DM patients with/without an MICD. The reduction of SOD was progressive in both groups, being more prominent for type 2 DM patients without an MICD in comparison to patients with an MICD, with differences that were statistically significant on the seventh, 14th, and 21st day after teeth extractions. In addition, all postextraction sockets healed uneventfully without any signs of infection or dry socket conditions.

Table 1. Socket opening diameter in type 2 diabetes mellitus patients with/without maxillary immediate complete denture (MICD) during the wound healing process

Time of observation	Socket opening diameter (mm)	
	Patients without MICD (n = 42)	Patients with MICD (n = 36)
Day of extraction	6.57 ± 0.18	6.62 ± 0.16
3rd day	5.54 ± 0.24 [b]	6.05 ± 0.13 [b]
7th day	3.54 ± 0.27 [b]	4.45 ± 0.21 [b, a]
14th day	1.16 ± 0.10 [b]	2.47 ± 0.10 [b, a]
21st day	0.1 ± 0.04 [b]	0.4 ± 0.08 [b, a]

[a] t-test, p < 0.001, between patients with MICD and those without MICD; ANOVA test
[b] p < 0.001 within the group vs. corresponding day 0

Chewing ability between type 2 DM patients with MICD and type 2 DM patients without MICD measured during a three-week observation period is shown in Table 2. The results revealed that both groups reported chewing ability without difficulties in the period before teeth extractions.

Table 2. Chewing ability between type 2 diabetes mellitus patients with immediate complete denture (MICD) and type 2 diabetes mellitus patients without MICD, measured during a three-week observation period

Time of observation	Chewing ability (verbal rating scale)	
	Patients without MICD (n = 42)	Patients with MICD (n = 36)
Before extractions	1.1 ± 0.12	1.2 ± 0.02
3rd day	3.2 ± 0. 14	5.14 ± 0.16**
7th day	3.8 ± 0.17	2.8 ± 0.17*
14th day	3.8 ± 0.3	1.4 ± 0.2**
21st day	3.5 ± 0.12	1.2 ± 0.3**

χ^2 test between the groups – ** p < 0.01,* p < 0.05

During the wound healing process, the discomfort in chewing ability was the highest on the third day, and decreased in the group of patients with MICD, contrary to the patients without MICD, where the chewing ability did not change during the time. On the seventh, 14th, and 21st day, the group of patients with MICD had significantly higher chewing ability compared to the group without MICD, in contrast to to the third day, when chewing ability was significantly higher in the group of patients without MICD.

Table 3 shows significantly lower number of patients who had changes in blood glucose level following less intake of food than usual, in the group of patients wearing MICD, compared to the patients without MICD. The analysis did not include age and sex as the group consisted predominantly of male participants (76%) aged between 45 and 64 years.

Table 3. Changes of blood glucose levels (BGL) and food intake ability in patients with/without immediate complete denture (MICD) from period before teeth extractions to three weeks after wound healing

Parameters	Frequency	
	Patients without MICD (n = 42)	Patients with MICD (n = 36)
BGL: changed	61.9% (26)	38.9% (14)*
Food intake: less than usual	73.8% (31)	47.2% (17)*

χ^2 test – * $p < 0.05$

DISCUSSION

The obtained data indicate that during the wound healing period, type 2 DM patients with MICD had slower reduction of socket opening diameter, higher chewing ability and in higher percentage had maintained blood glucose level and food intake ability in comparison to type 2 DM patients without MICD.

One of the most debilitating complications in diabetes is impaired wound healing attributed to microvascular changes [17]. Microvascular abnormalities lead to reduced response to tissue injury causing underperfusion during tissue stress and hypoxia followed by peripheral neuropathy [18, 19]. Immediate denture over the postextraction sites induces the mechanical stress in underlying tissues and impairs hypoxic condition. Having in mind that deficiency of oxygen alters wound healing phases such as collagen deposition, the decreased reduction of socket opening diameter during wound healing in patients with MICD was expected. Furthermore, presence of denture might lead to mucosal and tissue ulcerations especially in diabetics, which is shown in studies reporting that denture stomatitis and tissue lesions are commonly found in diabetic denture wearers [20]. Denture-related lesions that commonly develop within one to two days after insertion of new dentures increase inflammatory or reactive hyperplasia, increase accumulation of plaque on dentures and cause pain with poor nutrition [21, 22]. Our result of severe chewing difficulty three days after teeth extractions in our denture wearing patients is most probably related to pain caused by teeth extractions and developed denture ulcers. The pain may adversely affect a patient's diet, glycemic control and consequently wound healing process. Therefore, during all appointments we performed all the usual procedures to reduce the pain and promote healing process in MICD denture wearers (denture adjustments of the margins, occlusal adaptation of dentures combined with instructions in home denture hygiene). The improvement in chewing ability in patients with MICD after seven days of wearing dentures is due to fact that the patients developed a pattern of functioning and a good denture fit important for mastication and denture wearing. Higher chewing ability in patients with MICD compared to the group without MICD is the reason of lower changes in food intake. In connection with this, recent studies showed statistically significant increase in nutrition during the study period after insertion of complete denture and associations between changes in dental status and dietary intake of specific nutrients [8, 9, 10]. Madhuri et al. [16] reported an increase of consumption of protein, fruits and vegetables in denture wearers after insertion of complete dentures in comparison to the period when patients were edentate. Well balanced diet obtained by adequate mastication preserves the blood glucose level, which is shown in the group of patients with MICD, contrary to the patients without MICD.

CONCLUSION

This study revealed that immediate dentures are a good therapeutic choice for type 2 DM patients, as they provided better ability of mastication after tooth extraction, thus maintaining nutritional status and blood glucose level. To better predict life quality of type 2 DM patients during the postextraction period, more participants and clinical factors should be analyzed in further investigations.

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Протетска рехабилитација након екстракција зуба код болесника са дијабетесом мелитусом типа 2

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КРАТАК САДРЖАЈ

Увод Редовна и балансирана исхрана обезбеђена адекватном мастикацијом је значајан део терапије пацијената са дијабетесом мелитусом типа 2 (ДМ тип 2). Критичан период за ове болеснике јесте време непосредно након екстракције зуба, па за њих имедијатна протеза представља терапијски избор. Присуство имедијатне протезе и компресија коју изазива могу компромитовати процес зарастања, утицати на способност жвакања, количину унете хране, а тиме и ниво гликемије код пацијената оболелих од овог типа дијабетеса.

Циљ рада Циљ истраживања је поређење дијаметра екстракционих алвеола (ДЕА), способности жвакања, промене количине унете хране и гликемије код ДМ типа 2 пацијената са горњом тоталном имедијатном протезом (ГТИП) и без ње.

Методе рада Истраживање је обухватило 78 ДМ типа 2 пацијената, носилаца парцијалних плочастих протеза (42 пацијента индикована за екстракције зуба и 36 пацијената индикованих за екстракције зуба и добијање ГТИП). Током постекстракционог периода од три недеље код пацијената су праћени ДЕА, способност жвакања, промена количине унете хране и гликемије.

Резултати Пацијенти са ГТИП показали су значајно мању редукцију ДЕА (7, 14, 21. дана), већу способност жвакања (7, 14, 21. дана) у односу на пацијенте без ГТИП. Значајно мањи број пацијената са ГТИП имао је промене у гликемији и уношењу хране у односу на групу без ГТИП.

Закључак Имедијатна протеза представља терапијски избор код пацијената са ДМ типа 2, будући да омогућава добру мастикацију након екстракција, уз одржање нутрицираног статуса и гликемије.

Кључне речи: дијабетес мелитус типа 2; имедијатна протеза; способност жвакања; уношење хране

Примљен • Received: 02/10/2015 **Ревизија • Revision:** 23/11/2015 **Прихваћен • Accepted:** 26/11/2015

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):478-484
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1610478R
UDC: 616.314-089-06; 615.46:616.314

Efficiency of photodynamic therapy in the treatment of peri-implantitis – A three-month randomized controlled clinical trial

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SUMMARY

Introduction Peri-implantitis is an inflammatory lesion of peri-implant tissues. Eradication of the causative bacteria and decontamination of the implant surface is essential in achieving predictable and stabile clinical results. Photodynamic therapy (PDT) is non-invasive adjuvant therapeutic method to surgery in the treatment of bacterial infection.

Objective The aim of this study was to evaluate early clinical and microbiological outcomes of peri-implantitis after surgical therapy with adjuvant PDT.

Methods Fifty-two diagnosed peri-implantitis sites were divided into two groups. PDT was used for decontamination of implant surface in the study group; in the control group, chlorhexidine gel (CHX) followed by saline irrigation was applied. Several clinical parameters were recorded before the treatment (baseline values) and three months after surgical treatment. Samples for microbiological identification were collected before therapy, during the surgical therapy (before and after decontamination of implant surface), and three months thereafter, and analyzed with identification systems using biochemical analysis.

Results The use of PDT resulted in significant decrease of bleeding on probing in comparison to CHX ($p < 0.001$). It showed significant decontamination of implant surfaces with complete elimination of anaerobic bacteria immediately after surgical procedure and three months later.

Conclusion The results indicate that PDT can be used as an adjuvant therapy to surgery for decontamination of implant surface and surrounding peri-implant tissues within the treatment of peri-implantitis.
Keywords: peri-implantitis; decontamination; photodynamic therapy

INTRODUCTION

Peri-implantitis has been defined as an inflammatory process that affects the supporting marginal bone around an implant in function and results in bone resorption [1, 2]. It has been shown that the biofilm formed around osseointegrated implant has an important role in initiation and progression of peri-implant diseases [3, 4]. Most common microorganisms that are related to peri-implantitis are anaerobic bacteria, such as *Prevotella intermedia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Bacteroides forsythia, Treponema denticola, Prevotella nigrescens, Peptostreptococcus* spp., *Fusobacterium nucleatum*. Some authors suggested that *Staphylococcus aureus* and *Candida albicans* may be connected with initiation of peri-implantitis [3, 5]. Excessive mechanical stress, residual cement, poor plaque control could be among risk factors in the onset and development of peri-implantitis [2].

Peri-implantitis is a complex disease; therefore, the therapy continues to be a challenge. Surgical therapy of peri-implantitis has been suggested to be superior to non-surgical therapy [6]. Decontamination of implant surface is one of the most important and difficult steps because of the screw-shaped design and roughness where microorganism and their products are incorporated. Many methods have been suggested, such as mechanical (dental curettes, ultrasonic scalers, air-powder abrasive), physical and/or chemical methods (citric acid, chlorhexidine, EDTA), usually combined with local or systematic antibiotics [7–12]. No single protocol has been suggested for solving this problem.

Photodynamic therapy (PDT) can be a new alternative approach for decontamination of implant surfaces combined with mechanical debridement during surgical therapy. It is a non-invasive therapeutic treatment of various infections caused by bacteria, fungi and viruses [13]. PDT has been defined as an oxygen-dependent reaction that occurs by action of low-energy single-frequency light (diode laser) and activation of the photoactive materials (photosensitizer). The photosensitizer is administered onto exposed tissue. It binds and dyes cells. Upon irradiation with light of specific wavelength of laser, photosensitizer undergoes a transition from a low energy ground state to

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an excited singlet state. Subsequently, it may decay back to its ground state, transition high-energy triplet state, which can react with biomolecules such as endogenous oxygen to produce very reactive products, like singlet oxygen. Singlet oxygen has cytotoxic effect through damage of cell membrane and cell wall. Using this therapy, the target bacteria can be destroyed without adverse effect on the implant surface and surrounding peri-implant tissue [14, 15].

Some articles showed that using chlorhexidine (CHX) as adjunctive chemical agent to decontamination, reduced inflammation and microorganisms on the implant surface. Bioadhesive gels with higher concentrations of CHX have shown greater effectiveness in various clinical situations [16, 17]. However, ideal treatment for peri-implantitis is not defined yet. There are no clinical trials showing effects of PDT during surgical therapy of peri-implantitis.

OBJECTIVE

The aim of this study was to compare early clinical and microbiological effects of the adjuvant use of PDT to surgical treatment of peri-implantitis, comparing the effects of decontamination of the implant surface to those when 1% CHX gel was used.

METHODS

Patients were selected from two dental clinics: Department of Periodontology, University of Belgrade, and Department of Implantology, Military Medical Academy, from January 2014 to February 2015. The research protocol was submitted to and approved by the Ethical Committee, University of Belgrade, Serbia (number 36/28). The study was carried out in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. Before the procedure, all participants were informed about the study and signed a written consent. All participants had to meet the following inclusion criteria: age >18 years, no periodontal or peri-implant treatment three months prior to the study, presence of minimum one implant in function, early or moderate type of peri-implantitis classified by Froum and Rosen [18]. Exclusion criteria were the following: uncontrolled medical conditions, use of systemic antibiotics in the previous three months, use of anti-inflammatory drugs in the previous six months, pregnancy and lactation, therapy of peri-implantitis in the last three months previously.

Measurements and recording of clinical parameters

The measurements were done and recorded before any treatment. All measurements were made at the following six sites of the implant with signs of peri-implantitis: mesiobuccal, mid-buccal, distobuccal, mesio-oral, mid-oral, disto-oral by one examiner (DR), using a graduated probe (PCPUNC 15, Hu Friedy, Chicago, IL, USA). The applied probing force was standardized force of 0.25 N. The implant shoulder was used as landmark for calculation of mucosal recession and clinical attachment level. The following clinical parameters were registered:

- Peri-implant probing depth (PPD) – measured in millimeters from the mucosal margin to the bottom of the peri-implant pocket;
- Clinical attachment level (CAL) – measured in millimeters from the implant shoulder to the bottom of the peri-implant pocket;
- Mucosal recession (MR) – calculated as the difference between the CAL and PPD;
- Bleeding on probing (BOP) – evaluated as being **present** if bleeding was evident within 30 seconds after probing, or **absent**, if no bleeding was observed;
- Suppuration (SUP) – present or absent.

Clinical parameters were measured and recorded before (baseline values) and three months after therapy. All clinical examinations were performed after removal of the abutments attached to the implants. Fifteen days after therapy the provisional restoration was performed on the treated implants.

Microbiological samples and analysis

The first samples for microbiological analysis were taken before any measurements from the deepest peri-implant pockets. After the removal of subgingival plaque, sample sites were isolated with cotton rolls and gently air dried to avoid contamination with saliva. Fine sterile paper points were inserted into the peri-implant pocket until mild resistance and left in place for 30 seconds. The paper points were immediately transferred to the substrate used as multipurpose transport system (ESWAB LQ Amies, COPAN Diagnostics Inc., Murrieta, CA, USA).

The second and third samples were taken separately during the surgical therapy from the implant surfaces. After opening the flap and removing granulation tissue, the second sample was obtained from the implant surface by a transport swab. The swab was immediately transferred to the substrate used as multipurpose transport system (ESWAB LQ Amies, COPAN Diagnostics Inc.). After decontamination using chemical/PDT therapy, the third swab was taken from the implant surface applying the aforementioned procedure.

The samples were inoculated by standard procedures for the diagnosis of anaerobic bacteria. Each sample was inoculated on two blood agars (Columbia agar). One blood agar was incubated at 36.5°C for the diagnosis of aerobic pathogens; the other was incubated at the same temperature in a container for anaerobic diagnostics. Anaerobic conditions in the container were provided by generation bags for anaerobic conditions GENbox anaer (bioMérieux, Marcy l'Etoile, France). Cultivated and isolated anaerobic strains underwent identification process. Identification of isolated strains was reaffirmed by automatic systems of two different manufacturers: BBL Crystal ID Kit for anaerobes (Becton Dickinson, Sparks Glencoe, MD, USA), and VITEK 2 ID ANC (bioMérieux, Marcy l'Etoile, France).

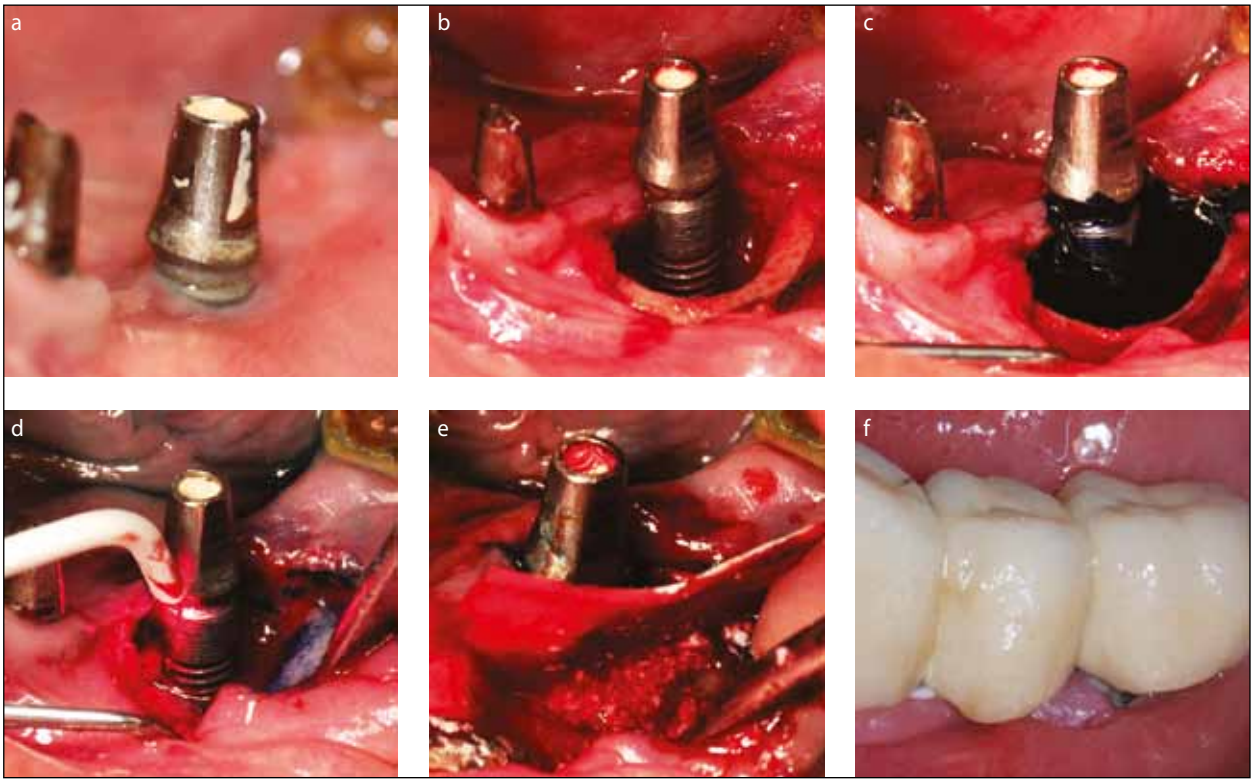

Figure 1. Surgery procedure using PDT for decontamination implant surface: a) peri-implantitis around the implant in function; b) peri-implant defect; c) application of photosensitizer (HELBO® Blue); d) activation of photosensitizer by using diode laser; e) application of bovine bone graft and resorbable membrane; f) result three months after the treatment

Treatment procedure

After clinical parameters were recorded and samples were taken, all the patients underwent a single episode of non-surgical therapy. It implied mechanical method for debridement of implants and remaining dentition in order to reduce signs of inflammation. Instructions for oral hygiene were proposed during the same visit.

Surgical treatment

During the surgical procedure, all the patients were randomly divided into two groups: the study group (PDT group) and the control group (CHX group). Similar methodology procedure was described in a study by De Waal et al. [17].

Surgical treatment of peri-implantitis was performed by one experienced oral surgeon (ZL) two weeks after non-surgical therapy (in accordance with Schwarz et al. [19]). Mucoperiosteal buccal and lingual incisions were made with surgical blade No. 15 under local anesthesia (2% lidocaine with epinephrine, 1:100,000). Flaps were designed to permit optimal access to the peri-implant bone defect for granulation tissue removal and decontamination of the implant surface. Full thickness mucoperiosteal flaps were elevated buccally and lingually. Removal of granulation tissue and mechanical implant surface cleaning were done using graphite curettes (Straumann® Dental Implant System; Straumann AG, Basel, Switzerland).

Decontamination of implant surfaces

In the study group, after careful removal of granulation tissue and mechanical debridement of implant surface, decontamination of implant surfaces and peri-implant tissues was performed using PDT (HELBO, Photodynamic Systems GmbH, Wels, Austria). Photosensitizer, phenothiazine chloride (HELBO® Blue Photosensitizer, bredent medical GmbH & Co. KG), was applied onto implant surface, bone and peri-implant soft tissue, for 3 minutes. Irrigation of photosensitizer was performed with saline, according to instructions of the manufacturer. Implant surface and the surrounding tissue were exposed to the laser light by means of fibers (HELBO® TheraLite Laser HELBO® 2D Spot Probe; Bredent medical GmbH & Co. KG) for 30 seconds/spot, which operates on wave length of 660 nm and irradiance of 100 mW (Figure 1a–f).

In the control group, after removal of granulation tissue, 1% gel of chlorhexidine (Chlorhexamed® – Direkt; GlaxoSmithKline, GmbH & Co. KG, München, Germany) was put on implant surface. One minute after exposing implant surface with CHX, it was irrigated for 1 minute by saline.

Bone augmentation and bio-resorbable membrane were applied in peri-implant defects using artificial bone of bovine origin (Bio-Oss and Bio-Gide; Geistlich Pharma, Wolhusen, Switzerland). The mucoperiosteal flaps were repositioned and sutured [17, 19].

All the patients from both groups were prescribed antibiotics over a five-day period (amoxicillin caps. 500 mg,

doi: 10.2298/SARH1610478R

Table 1. Demographic and clinical description of the study population

Characteristics		Study group	Control group	p-value
Number of subjects		21 (52.5%)	19 (47.5%)	
Gender	Male	12 (57.14%)	12 (63.15%)	0.703
Mean age (years) (mean ±SD)		57.59	60.00	0.408
Mean time (years) after implant placement (mean ±SD)		7.68 ± 3.76	6.21 ± 3.064	0.236
Subjects with a history of treated periodontitis; n (%)		8 (29.6%)	10 (40%)	0.432
Localization of implants, n (%)	Maxilla	8 (29.6%)	6 (24)	0.647
	Mandible	19 (70.4%)	19 (76%)	
Type of restoration, n (%)	Cement retained fixed partial denature	13 (46.4%)	19 (79.2%)	0.010[a]
	Screw retained fixed partial denture	6 (21.4%)	2 (8.3%)	
	Cement retained single crown	4 (14.3%)	3 (12.5%)	
	Overdenture on implants	5 (17.9%)	0 (0)	

[a] Significant statistical difference in type of prosthetic restauration among the groups at baseline by Pearson's χ^2 test ($p < 0.01$)
SD – standard deviation
No significant differences were observed among the groups at baseline by Pearson's χ^2 or Independent Samples Test (T-test).

Table 2. Mean pocket probing depth (PPD) ±SD, mean clinical attachment level (CAL) ±SD, mean number of marginal recession (%), mean bleeding on probing (BOP) – positive sites (%) at each implant at baseline and three months later

Parameter	Baseline		3 months	
	PDT	CHX	PDT	CHX
Peri-implant PPD (mm)	5.74 ± 1.55[a]	4.48 ± 1.08[b]	3.26 ± 0.79[a]	2.86 ± 0.755[b]
CAL (mm)	5.32 ± 1.36[a]	4.63 ± 1.28[b]	3.35 ± 1.67[a]	3.16 ± 1.25[b]
BOP	28 (100%)	24 (100%)	5 (17.9%)[c]	12 (50%)[c]

[a] Significant statistical difference measured before and three months after PDT by T-test ($p < 0.001$);
[b] Significant statistical difference measured before and three months after CHX by T-test ($p < 0.001$);
[c] Significant statistical difference between the groups by Pearson's χ^2 test ($p < 0.001$)
CHL – chlorohexidine gel

three per day). It was recommended that patients don't use mouthwash during the postoperative period.

Followed up period

Three months after therapy samples for microbiological analysis were taken from the reduced peri-implant pockets in the same way described above. In addition, measurements of clinical parameters were recorded as well.

Statistical analysis

Data are presented as mean ± standard deviation or n (%) depending on data type. Chi-squared test, Mann–Whitney U-test, and Student's t-test were used to assess the differences between the groups. Cochran's Q-test and Wilcoxon signed-rank test were used to assess significant differences within the groups. All analyses were performed in SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). All p-values less than 0.05 were considered statistically significant.

RESULTS

There were 52 peri-implantitis sites diagnosed in 40 systemically healthy patients, which were treated and re-examined in a three-month period. Demography and clinical description of the study population are shown in Table 1. No adverse events and side effects were reported during and after therapy.

Clinical parameters and outcomes

From a total of 52 treated peri-implantitis sites, 12 peri-implantitis sites belonged to the category of moderate, and 31 to the category of early peri-implantitis, classified by Froum and Rosen [18]. The rest of peri-implantitis instances had depths of ≥ 5 mm in only one peri-implant pocket, as one of the inclusion criteria.

The mean value and standard deviation of PPD and CAL are shown in Table 2. Both groups showed statistically significant reduction of peri-implant pockets ($p < 0.001$). It has been shown that the greater (but not statistically significant) reduction of PPD was in the study group compared to the controls ($p = 0.07$). The results of our study showed that there was no statistically significant difference in CAL between the two tested groups three months after the therapy ($p = 0.883$). Also, there were no statistically significant differences in marginal recession.

In the study group, there was a statistically significant reduction of BOP at all six points compared with the control group three months after the therapy ($p < 0.001$). Changes in percentage of BOP are shown in Table 2. PDT has shown statistically significant reduction of suppuration compared to CHX treatment ($p < 0.002$).

Microbiological outcomes

All cultivated anaerobic microorganisms which were isolated from deepest peri-implant pockets and implant surfaces are shown in Table 3. It was shown that some of these bacteria were isolated only from implant surfaces.

Table 3. Number (n) of culture-positive implants at baseline and culture-negative after decontamination of selected anaerobes; mean number (%) of total anaerobic bacteria load on culture-positive implants; before any treatment (T_{pre}); before decontamination – during surgical therapy (S_{pre}); after decontamination – during surgical therapy (S_{post}); three months later (T_{post})

Anaerobes	Experimental group (PDT, n = 27)				Control group (1% CHX gel, n = 25)			
	T_{pre}	S_{pre}	S_{post}	T_{post}	T_{pre}	S_{pre}	S_{post}	T_{post}
Porphyromonas gingivalis	4 (14.3)	8 (28.6)	0 (0)	0 (0)¥	4 (16.7)	2 (8.3)	0 (0)	0 (0)
Prevotella intermedia	5 (17.9)	6 (21.4)	0 (0)	0 (0)¥	1 (4.2)	3 (12.5)	0 (0)	0 (0)
Peptostreptococcus spp.	5 (17.9)*	3 (10.7)	0 (0)	0 (0)¥	0 (0)	5 (20.8)	0 (0)	0 (0)¥
Fusobacterium nucleatum	1 (3.6)	4 (14.3)	0 (0)	0 (0)¥	2 (8.3)	5 (20.8)	0 (0)	0 (0)¥
Peptostreptococcus asaccharolyticus	4 (14.3)	1 (3.6)	0 (0)	0 (0) ¥	2 (8.3)	0 (0)	0 (0)	0 (0)
Actinomyces naeslundii	2 (7.1)	8 (28.6)	0 (0)	3 (10.7)¥	4 (16.7)	6 (25)	0 (0)	2 (8.3)
Veillonella spp.	9 (32.1)	9 (32.1)	2 (7.1)*	2 (7.1) ¥	3 (12.7)	9 (37.5)	7 (29.2)*	3 (12.5) ¥
Staphylococcus aureus	0 (0)	3 (10.7)	0 (0)	0 (0)¥	0 (0)	5 (20.8)	0 (0)	0 (0)¥
Staphylococcus saccharolyticus	0 (0)	2 (7.1)	0 (0)	0 (0)	0 (0)	2 (8.3)	0 (0)	0 (0)
Actinomyces meyeri	0 (0)	4 (14.3)	0 (0)	0 (0)¥	2 (8.3)	0 (0)	0 (0)	0 (0)
Actinomyces odontolyticus	1 (3.6)	0 (0)	0 (0)	3 (10.7)	4 (16.7)	0 (0)	0 (0)	4 (16.7)¥

N = 52;
* Statistically significant change from baseline to three months after therapy, as well as before and after decontamination of implant surface between the two groups according to Pearson's χ^2 test, $p < 0.05$;
¥ Statistically significant change from baseline to three months after therapy, as well as before and after decontamination of implant surface during surgical procedure within the two tested groups according to Cochran's Q-test, $p < 0.05$

Candida spp. was also isolated from the deepest peri-implant pockets.

The results showed that the amount of anaerobic microorganisms was significantly reduced and in most cases eliminated, after using both anti-infection therapeutic methods during surgery therapy as well as three months after the therapy procedure. It showed significant reduction of anaerobic microorganisms in the study group in comparison with the control group, immediately after decontamination of the implant surface and also three months after (*Veillonella* spp., $p < 0.010$; *Staphylococcus aureus*, $p < 0.002$; *Peptostreptococcus* spp., $p < 0.002$; *Peptostreptococcus asaccharolyticus*, $p < 0.035$; *Actinomyces naeslundii*, $p < 0.014$; *Prevotella intermedia*, $p < 0.011$; *Porphyromonas gingivalis*, $p < 0.001$; *Actinomyces meyeri*, $p < 0.007$). Three months after therapy, *Actinomyces naeslundii* was only isolated in peri-implant pockets in the control group.

The results of the study show that using chlorhexidine leads to significant reduction of *Actinomyces odontolyticus* ($p < 0.046$), *Fusobacterium nucleatum* ($p = 0.23$) compared with using photodynamic therapy. The presence of *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia,* and *Treponema denticola* was not identified.

DISCUSSION

The short-term results of the present study show that both examined methods for debridement and decontamination of implant surfaces during surgical therapy (PDT and 1% chlorhexidine gel), significantly improve clinical and microbiological outcomes.

In the current literature, most attention is given to systemic and/or local use of antibiotics or implantoplasty for the decontamination of implant surfaces during surgical procedures [3, 10, 20]. However, these methods showed side effects, as well as bacterial resistance to some drugs and allergic reactions during and after their consumption [10]. It has been recorded that implantoplasty leads to marginal recession, which is disadvantageous in terms of function and aesthetics [2, 20].

Some authors consider PDT to be less harmful and effective solution for the decontamination of the implant surface [13, 14]. This therapy was widely used in non-surgical therapy of peri-implantitis [15, 21, 22]. It has been suggested that using only photodynamic therapy several times may lead to total recovery [22]. Schwarz et al. [6] suggested that surgical therapy of peri-implantitis has been superior to non-surgical therapy. The explanation lies in the open access and controlled removal of granulation tissue and decontamination of the exposed implant surfaces.

The results of the present study show that there are statistically significant changes in peri-implant probing depth and clinical attachment level measured at baseline and three months after therapy. The depth of peri-implant pockets in both tested groups was reduced approximately 2 mm. Higher reduction of peri-implant pocket depth was in the study group, but without statistical significance. This could be explained by larger baseline levels of peri-implant pockets depth recorded in the study group. It seems that PDT can promote re-osseointegration more rapidly than CHX, without side effects on the bone. The results show significant reduction of BOP and suppuration three months after using PDT. It is known that BOP is one of the most essential and important signs of peri-implantitis; therefore, the use of PDT can achieve elimination of BOP and promote better healing. In the study by De Waal et al. [17], similar methodology was used, but they didn't achieve the significant difference in clinical parameters using two concentration of chlorhexidine solutions (0.12% and 2%) in the examined follow-up periods. These results differ from ours due to improvements of clinical parameters in our study. In the study by Heitz-Mayfield et al. [10], after surgical debridement and implant surface decontamination following by saline irrigation, systemic amoxicillin

and metronidazole were prescribed. Although clinical results of their study were similar to ours, using adjunctive PDT could be efficient, as it leads to lower consumption of antibiotics and antiseptics, without side effects.

Cultivation of anaerobic microorganisms showed the presence of various anaerobes isolated from peri-implant pocket before any treatment, as well as on implant surface before decontamination. Most of these bacteria belonged to the pathogenic microorganisms from red and orange complex described by Socransky et al. [23]. In our study, bacteria of yellow, purple, and green complex were also found, which is assumed to be the first phase of colonization of implant surface. This can also represent a possible bridge for the adherence of bacteria from orange and red complexes in a later phase of colonization and maturation of dental biofilm. The results also showed the presence of *Staphylococcus aureus*. It was mentioned that it can be one of the possible pathogenic microorganisms that promote initiation and progression of peri-implantitis. This bacteria was isolated only from the implant surface, so it can be assumed that this can be a very virulent strain, which can enable the progression of peri-implantitis [24]. The presence of *Candida albicans* could be due to inappropriate oral hygiene. Therefore, *Candida* couldn't have had an influence on initiation of peri-implantitis, as mentioned in some early papers [2].

In both examined groups, statistically significant reduction of anaerobic microorganisms from the peri-implant pockets was evident in the follow-up period and also from implant surface immediately after decontamination. The results show that application of PDT significantly reduced the amount of bacteria, especially those from the red and orange complex. De Waal et al. [17] achieved reduction of microorganisms from implant surface between groups during a surgical procedure, but results were not as statistically significant as in our study. Our study showed significant reduction of microorganisms after using both anti-infection therapeutic methods of decontamination during surgical therapy. *In vitro* study of Marotti et al. [14] showed significant reduction of anaerobic microorganisms after application of PDT and 0.12% solution of chlorhexidine on anodized implants, which are results similar to ours. Another study showed that using PDT with toluidine blue as a dye can reduce but not eliminate pathogenic microorganisms from implant surfaces (*Prevotella intermedia*, *Porphyromonas gingivalis*) [15]. Presence of these bacteria is one of the most important factors in peri-implantitis progression. In contrast, our study has shown elimination of these bacteria. The reason for these differences could be the composition of photosensitizer. Phenothiazine chloride might have greater ability to bind different microorganisms compared to toluidine blue.

In the control group, chlorhexidine gel was chosen for decontamination because of its widely spread use as an antiseptic in different therapeutic procedures. It might be capable of adhering to implant surface. The results show statistically significant reduction of *Fusobacterium nucleatum* and *Actinomyces odontolyticus* after using 1% of CHX in adhesive gel, which can be explained by high concentration and viscosity of CHX in the gel. Although earlier studies described that CHX has a bactericidal effect on microorganisms regardless of the concentration [17], it seems that CHX cannot remove dental biofilm, which can lead to renewed adherence of microorganism and reappearance of inflammation. Similarly, in the control group, the results of the present study showed the reduction of BOP, as one of the signs of inflammation, of only 50%, which confirmed the earlier assumption.

CONCLUSION

Even with limitations of the present study, such as short observation period, it can be concluded that photodynamic therapy achieves significant reduction of microorganisms from implant surface and peri-implant tissue when compared with chlorhexidine application, without adverse and side effects on implants and surrounding tissue. Therefore, PDT could be suggested as a new strategy for decontamination of implant surface during surgical treatment of peri-implantitis.

ACKNOWLEDGEMENT

This study was partly supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project No. 41008).

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Процена ефикасности фотодинамске терапије у терапији периимплантитиса после три месеца: рандомизирана контролисана клиничка студија

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КРАТАК САДРЖАЈ

Увод Периимплантитис је инфламаторни процес који захвата мека ткива и потпорну кост око осеоинтегрисаног имплантата. Елиминација патогених микроорганизама и деконтаминација имплантне површине представљу најбитнији корак у постизању стабилних клиничких резултата. Фотодинамска терапија (ФДТ) представља додатни неинвазивни метод у терапији бактеријских инфекција.

Циљ рада Циљ рада била је процена клиничких и микробиолошких параметара након хируршке терапије периимплантитиса уз додатну примену ФДТ.

Методе рада Сва дијагностикована места периимплантитиса (n = 52) била су подељена у две групе: у студијској групи, за деконтаминацију имплантне површине током хируршке процедуре коришћена је ФДТ; у контролној групи, за деконтаминацију имплантне површине коришћен је хлорхексидин у гелу (*CHX*). Клинички параметри праћени су пре терапијске процедуре и три месеца после терапије. Узорци за микробиолошку анализу узимани су пре и три месеца после терапије, као и током хируршке процедуре, пре и после деконтаминације имплантне површине. За идентификацију изолованих анаероба коришћен је систем који ради по принципу биохемијске анализе изолованих микробиолошких сојева.

Резултати Резултати студије су показали да применом ФДТ долази до знатне редукције крварења на провокацију у поређењу са применом *CHX* (p < 0,001). Примена ФДТ, као помоћног терапијског средства, омогућава потпуну елиминацију анаеробних бактерија са имплантне површине.

Закључак Резултати показују да ФДТ може да се користи као помоћно терапијско средство за деконтаминацију имплантне површине и периимплантног ткива у оквиру терапије периимплантитиса.

Кључне речи: периимплантитис; деконтаминација; фотодинамска терапија

Примљен • Received: 09/12/2015 **Ревизија • Revision:** 20/06/2016 **Прихваћен • Accepted:** 30/06/2016

Location of out-of-hospital cardiac arrest as a determinant in the survival of patients

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SUMMARY

Introduction Cardiac arrest (CA) is defined as a sudden cessation of normal circulation of blood due to failure of the heart to contract effectively during systole.

Objective The aim of this study was to determine the difference in outcome among patients, depending on the location of out-of-hospital CA; to determine the influence of observed determinants on the survival rate.

Methods Observational and retrospective study was conducted in the Institute for Emergency Medical Service Novi Sad (IEMS NS). It included patients who underwent cardiopulmonary resuscitation (CPR) by medical ambulance squads. Patients were divided into three groups, based on the location of CA: private place, public place, and medical institution.

Results CA occurred in private places in 151 cases (76.26%). The shortest duration of a phone call with the dispatcher and Reaction Time I was in the group of patients with CA in a public place (59.1 ± 36.4 seconds and 137.1 ± 89.8 seconds, respectively). CA was recognized in more than 80% of cases, but CPR was initiated in only 9.09% of patients in private places and in 19.35% of patients in public places. Though they initially presented with shockable rhythm in 57.14% of cases in public places, this group has the worst immediate outcome (11.43%), in contrast to the patients with CA in medical institutions (58.33%). Factors determining the survival of patients with CA were CPR attempted immediately after collapse, initial rhythm and eyewitnesses of CA.

Conclusion In order to improve survival of patients with out-of-hospital CA, both education of laymen and introduction of standard questioning protocol in the IEMS Call Centre are necessary.

Keywords: out-of-hospital cardiac arrest; cardiopulmonary resuscitation; survival rate

INTRODUCTION

Cardiac arrest (CA) (lat. *institio cordis*) is defined as a sudden cessation of normal circulation of blood due to failure of the heart to contract effectively during systole [1].

This condition is experienced by 800,000 people in Europe and the USA every year [2, 3]. Cardiopulmonary resuscitation (CPR) is attempted in most cases [4]. Data collected in 37 European countries demonstrate that sudden CA incidence in which CPR is initiated by ambulance is 38 per 100,000 inhabitants yearly. Hospital survival rate (defined as survival up to the discharge) was 10.7% for all initial rhythms and 21.2% for shockable rhythms (ventricular fibrillation and pulseless ventricular tachycardia) [5]. Prehospital emergency medical services in four cities in Serbia (Belgrade, Novi Sad, Niš, Kragujevac) registered 2,827 patients with CA during 12 month follow-up (2005/2006). CPR was initiated by ambulance in 591 patients (incidence: 27/100,000). Hospital survival rate was 12.5% [6].

Out-of-hospital CA most often occurred in patients' homes [6, 7]. Location of CA is considered non-relevant to the outcome, provided that CPR is initiated immediately [8].

OBJECTIVE

The aim of the study is to determine frequency of CA in relation to the location, difference in immediate survival of patients with CA in relation to the location (private/public space/medical institution) and effect of these determinants to the immediate survival rate in out-of-hospital CA.

METHODS

This observational and retrospective study was conducted in the Institute for Emergency Medical Service of Novi Sad (IEMS NS) in the period between January 1st, 2010 and December 31st, 2010.

IEMS NS covers territories of the municipalities of Novi Sad and Sremski Karlovci, with 389,130 inhabitants, according to the statistical data from public institutions. IEMS NS is the only medical institution capable of providing prehospital emergency life support in the territory of these municipalities. In the studied period, there was a single Institute for Emergency Medical Service (IEMS) Call Centre collecting the calls for medical emergencies and

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non-urgent patient transport, with five telephone lines and one radio channel for the communication with the ambulance squads. A doctor and an emergency medical technician work in the IEMS Call Centre in twelve-hour shifts. There were eight ambulance squads in each working shift consisting of a doctor, an emergency medical technician and an ambulance driver (non-medical personnel trained to provide basic life support (BLS)). All ambulances are equipped identically and are capable of providing advanced life support. Non-urgent patient transport squads consist of a medical technician and a driver, or only a driver, in some instances. There are 10 non-urgent patient transport squads on workdays, five on Saturdays and two on Sundays and in night shifts.

All patients with attempted CPR by the IEMS NS during the studied period were enrolled into the study. The patients were divided into the following three groups, based on the location of sudden CA (SCA): private place (Group I – homes, apartments, nursing homes), public place (Group II – streets, agricultural fields, offices, shopping malls, sport centers, courts, prison, police stations), and medical institution (Group III – health centers and hospitals without emergency medical squads). Determinants taken into consideration for each patient included the following:

- Gender;
- Average age;
- Duration of the phone call to the IEMS Call Centre (from the moment of taking the call to the moment of ending the call);
- Reaction Time I (RT I) – the period between the moments when the call is answered and when the ambulance squad is dispatched;
- Arrival Time – the period between the moments of receiving the order from the IEMS Call Centre and arriving to the location;
- Witnesses of SCA: laymen, medical professionals or ambulance squads;
- Early CPR initiation, before the arrival of an ambulance;
- Phone-assisted CPR – instructions provided by IEMS Call Centre operator on how to initiate CPR before the arrival of an ambulance;
- Initial rhythm – shockable (ventricular fibrillation / pulseless ventricular tachycardia) or non-shockable (asystole / pulseless electrical activity);
- Return of spontaneous circulation after initiated CPR, until hospital admission.

Collected data were coded and stored into specially created computer database. All data were calculated by using statistic packages SPSS for Windows, version 11 (SPSS Inc., Chicago, IL, USA). The three studied groups were compared and numeric data were represented by mean arithmetic values and standard deviations, while the significance of a particular parameter was determined using Student's t-test and χ^2-test. Impact of particular factors on survival was determined by using univariate binary logistic regression analysis. The results are presented in tables.

RESULTS

In 2010, ambulance squads of IEMS Novi Sad had 35,083 patients in total. CPR was initiated in 198 patients. Number of patients with CA in Group I was significantly higher than in the other two groups (Table 1). Among patients with CA, there were more men and they were on average younger than women, especially in Group II. There is a statistically significant correlation in age and gender distribution of CA in the first two groups ($p < 0.05$).

Duration of conversation with IEMS operator was longer than one minute in all three groups. The shortest duration of conversation was with eyewitnesses of CA in Group II. IEMS dispatcher sent ambulance squad before the termination of phone call in four cases of CA which occurred in Group I, and only in one case of CA in each of the other two groups. RT I less than 60 seconds was observed in 19 patients (11.26%) in Group I, in two patients (2.86%) in Group II, and in three patients (16.67%) in Group III. The shortest arrival time was recorded in patients in Group III (Table 1).

In most cases in Groups I and II, eyewitness of CA were laymen. However, BLS was initiated before the arrival of ambulance in small number of cases. Moreover, BLS was not even initiated in some cases in all three Groups where health professionals were present. IEMS Call Centre operator gave instructions for phone-assisted CPR in only three cases in Group I (Table 1).

Shockable initial rhythm most often occurred in patients in Group II, but the immediate survival rate was the highest among the patients in Group III (Table 1).

It is possible to calculate individual impact for each observed determinants on the immediate survival rate if univariate binary logistic regression analysis is applied (Table 2). Early CPR initiation, before the arrival of an ambulance, had the highest impact on the survival rate (Table 2) and these patients were 2.4 times more likely to survive initially. Initial rhythm was also an important determinant in survival – patients with shockable rhythm were 3.2 times more likely to survive than patients with non-shockable rhythms. Presence of witnesses was comparably a less important determinant. If SCA was witnessed by the ambulance squad, patients were 2.3 times more likely to survive compared to the presence of laymen. Witnessed by other health professionals, patients were 1.3 times more likely to survive.

DISCUSSION

Out-of-hospital CA most often takes place in private houses, apartments and nursing homes (84.7%), while in small number of cases it occurs in public places (14.3%) and in health institutions (1%), according to literature [7]. A study conducted in four IEMSs in Serbia in 2004 and 2005 showed that out-of-hospital CA was also most often in private houses, apartments and nursing homes (70.2%), while small number of cases were recorded in public places (24.8%) and health institutions (5%) [6]. Out-of-hospital

doi: 10.2298/SARH1610485M

Table 1. Analysis of individual determinants in all three groups of patients

Predictors		Group I	Group II	Group III	p
Number of CPRs	Total	151 (76.26%)	35 (17.68%)	12 (6.06%)	<0.001
	Male	97	28	8	<0.001†
	Female	54	7	4	0.248††
Average age (years)	All patients	65.8 ± 13.7	59.9 ± 15.1	61.1 ± 15.8	0.029‡
	Male	63.6 ± 14.1	57.5 ± 15.4	59.1 ± 14.7	0.012‡‡
	Female	69.5 ± 12.1	71.8 ± 6.0	65.7 ± 21.0	
Duration of conversation (sec.)		72.8 ± 39.0	591 ± 364	62.7 ± 48.5	>0.05
RT I (sec.)		223.9 ± 347.6	137.1 ± 89.8	80.4 ± 196.3	>0.05
Arrival time (min.)		8.4 ± 4.4	6.6 ± 4.5	4.1 ± 1.6	<0.05
CA recognized by callers		102 (84.29%)	33 (94.29%)	8 (100.00%)	<0.05
Witnesses	Laymen	116 (76.82%)	31 (88.57%)	0	
	Health professionals	5 (3.31%)	4 (11.43%)	8 (66.67%)	
	Ambulance	30 (19.87%)	0	4 (33.33%)	
CPR initiated before arrival	Total	11 (9.09%)	6 (19.35%)	6 (75.00%)	<0.001
	By laymen	9 (7.76%)	4 (12.90%)	0	
	By health professionals	2 (40.00%)	2 (50.00%)	6 (75.00%)	
Phone-assisted CPR		3 (1.99%)	0	0	
VF/VT		47 (31.13%)	20 (57.14%)	4 (33.33%)	<0.05ی
Asystolia/PEA		104 (68.87%)	15 (42.86%)	8 (66.67%)	
Immediate		31 (20.53%)	4 (11.43%)	7 (58.33%)	<0.001

CPR – cardiopulmonary resuscitation; RT I – reaction time I; CA – cardiac arrest; VF – ventricular fibrillation; VT – ventricular tachycardia; PEA – pulseless electrical activity; p – statistical significance

† statistical significance of CA frequency between males and females in Group I
†† statistical significance of CA frequency between males and females in Group II
‡ statistical significance of average age between Groups I and II
‡‡ statistical significance of average age between males and females in Group I
ی statistical significance of shockable and non-shockable rhythm frequency between Groups I and II and between Groups II and III

Table 2. Univariate binary logistic regression analysis of factors determining return of spontaneous circulation in all groups of patients

Predictors	CA at all locations		
	OR	95% CI	p
Sex	0.654	0.323–1.321	0.236
Age	1.008	0.974–1.032	0.521
Duration of conversation	1.006	0.996–1.016	0.260
RT I	0.999	0.998–1.000	0.050
Arrival time	1.087	0.991–1.192	0.076
Witnesses	0.563	0.346–0.917	0.021
Immediately initiated CPR	3.803	1.864–7.757	0.000
Phone assisted CPR	0.539	0.048–6.098	0.618
Initial rhythm	2.421	1.379–4.252	0.002
Location	0.638	0.372–1.094	0.102

CA – cardiac arrest; OR – odds ratio; CI – confidence interval; RT I – reaction time I; CPR – cardiopulmonary resuscitation; p – statistical significance

CA was more often in men, while average age was higher in women [6, 9]. The highest average age was among patients in private places [9]. Our study showed the same distribution of patients in relation to location, sex and age. Average age of patients in Group II was lower than that in Groups I and III.

RT I, for Priority I calls, should be less than one minute [10, 11, 12]. IEMS call center operators should send an ambulance without interrupting a phone call. They are supposed to provide pre-arrival instructions for CPR, so that callers (usually laymen) could initiate BLS before the arrival of the ambulance [13]. Therefore, duration of a phone call should be longer than RT I. However, due to the lack of personnel and absence of questioning protocols, RT I is longer than one minute in most cases, while the duration of the phone call is shorter than RT I. After a caller provides the location of the CA, the phone call is interrupted and an ambulance is sent, while the caller is not given pre-arrival instructions for CPR. The shortest RT I was in Group II, although it should be the shortest in Group III, because CA is being reported by health professionals.

On the other hand, the shortest arrival time was in Group III, which is similar to the arrival time in most European countries, which is 5.5 minutes [9]. Arrival time in Group II, and especially in Group I, was above average. The aim of CPR is to receive early defibrillation in the period between three and five minutes, which increases chances for survival 49–75% [14].

CA is most often witnessed by laymen. If BLS is initiated immediately, delay of early defibrillation decreases the chances for survival for only 3–4% for every minute of delay (compared to 10–12% if BLS is not initiated before the arrival of an ambulance) [15]. Unfortunately, laymen rarely decide to initiate BLS, in the range of 3–45%, or 16–33% on average [16–19]. BLS was more often initiated in Group II – in 60% of cases [9]. Increase of BLS initiated by laymen has been recorded in the last 15 years, due to trainings of laymen and introduction of a standard questionnaire and guidelines for CPR in emergency call centers [20, 21]. In our study, CA was most frequently witnessed by laymen in Groups I and II. In more than 80% of cases, CA was recognized, but laymen failed to initiate CPR in most cases. The lowest number of attempted CPR before the arrival of an ambulance was in Group I, which is three times lower than

in Western European and Scandinavian countries. What is worse, not all health professionals attempted CPR before the arrival of the ambulance. This could be explained by inadequate training of both health professionals and laymen. In Serbia, BLS is taught in the final year of primary education. High school and university students are not trained to apply BLS. In driving schools, training in BLS and principles of first medical aid is still not obligatory. Until recently, first aid and emergency medicine were not taught at medical faculties, while students were informed of the principles of CPR during the courses in Surgery.

Patients in Group II more often presented with shockable rhythms, in 60%, which is equal to those in Western European and Scandinavian countries. In Group I, however, shockable rhythm was considerably lower, especially in comparison to Western European and Scandinavian countries, which is 38% [9].

Even though arrival time and RT I in Group I were the longest and most patients were in non-shockable rhythm, immediate survival was better than in Group II. This could be attributed to the higher number of CA witnessed by the ambulance. The highest immediate survival rate was in Group III, with most number of patients with initiated CPR.

Univariate binary logistic regression analysis showed that survival rate depends on the early CPR initiation, initial rhythm and presence of witnesses. In the last thirty years, more and more non-health professionals are trained to recognize CA and initiate BLS, and operators in many emergency call centers are introduced to protocols for phone-assisted CPR, as immediately attempted CPR increases chances for survival two- or three-fold [22, 23]. In addition to this, quality of life after the hospital discharge is better thanks to the lower degree of neurological damage during hypoxia [24]. If callers are not trained in providing CPR, an emergency call center operator should give instructions to initiate BLS after an ambulance is dispatched [21, 25, 26]. Until the arrival of the ambulance, chest compressions by untrained laymen could be sufficient [27]. Quality of phone-assisted CPR could be equal to the quality of CPR provided by trained persons. However, it was established that the quality of ventilation differs between the two [28]. In our study, presence of witnesses determined the survival rate, because laymen rarely initiated CPR.

Better outcome of patients in Group II could also be attributed to the availability of automated external defibrillators (AEDs) in public places [7, 14]. In our circumstances, survival rate in Group II is low, even though most patients are relatively younger and with shockable rhythm. Because our ambulances are not always able to reach patients in less than five minutes, available AEDs and training of laymen could be part of the solution to this problem.

This study was limited to the prehospital level of health system, so we could not calculate short-term survival rate (to the hospital discharge), evaluate neurological damage at the moment of discharge, and take into consideration the application of therapeutic hypothermia.

First defibrillation, endotracheal intubation and venous access were applied in the first two minutes after the arrival of the ambulance, which is in accordance with the European Resuscitation Council Guidelines 2010 [29].

CONCLUSION

Prehospital CAs most frequently occur in private places, but the highest survival rate was recorded in patients who experienced CA in health institutions. This could be explained by the immediately initiated BLS, before the arrival of the ambulance. Laymen usually do not attempt to initiate BLS. On the other hand, IEMS call center operators rarely initiate phone-assisted CPR due to the lack of time. RT I is still over 1 minute, while the arrival time is more than 5 minutes, so early defibrillation (within 3–5 minutes) could be applied only if SCA is witnessed by the ambulance personnel.

In order to improve prehospital survival of patients with SCA, it is necessary to introduce BLS training of laymen and obligatory protocols for phone-assisted CPR in emergency call centers, and place AEDs in public places and instruct laymen on how to apply them.

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Утицај места догађаја на преживљавање болесника са ванболничким срчаним застојем

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КРАТАК САДРЖАЈ

Увод Срчани застој се дефинише као изненадни прекид нормалне циркулације крви због немогућности срца да се контрахује током систоле.

Циљ рада Циљ рада је био да се прикаже разлика у непосредном преживљавању пацијената у ванболничком срчаном застоју у зависности од места догађаја и утицај појединачних посматраних фактора на преживљавање.

Методе рада Спроведено је опсервационо и ретроспективно истраживање у Заводу за хитну медицинску помоћ Нови Сад (ЗЗХМП НС). Обухваћени су пацијенти код којих су ургентне екипе радиле кардиопулмоналну реанимацију (КПР). Пацијенти су подељени у три групе на основу места догађаја: приватна локација, јавно место и здравствена установа.

Резултати Највећи број пацијената срчани застој је доживело на приватној локацији – 151 (76,26%). Најкраће време разговора диспечера са позиваоцем и реакционо време I је било код пацијената реанимираних на јавном месту – 59,1 ± 36,4 и 137,1 ± 89,8 секунди. Срчани застој је препознат у преко 80% случајева, али су мере реанимације предузете у 9,09% случајева на приватној локацији и 19,35% на јавном месту. Иако је иницијални ритам на монитору дефибрилатора био шокабилан код 57,14% реанимираних пацијената на јавном месту, најлошије непосредно преживљавање је било у овој групи – 11,43%, док је најбоље било код пацијената реанимираних у здравственим установама – 58,33%. Фактори од којих је зависило непосредно преживљавање су: одмах започете мере КПР, иницијални ритам и ко је био очевидац срчаног застоја.

Закључак Лаици се ретко одлучују на пружање мера КПР, зато је преживљавање много боље у здравственој установи. Да би се побољшало преживљавање пацијената у ванболничком срчаном застоју, потребна је обука лаика, али и увођење протокола за телефонски асистирани КПР у диспечерске центре.

Кључне речи: ванболнички срчани застој; кардиопулмонална реанимација; стопа преживљавања

Примљен • Received: 12/08/2015 **Прихваћен • Accepted:** 30/11/2015

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):490-496
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1610490D
UDC: 616.6-002; 616-022.1:616.6

Risk factors for healthcare-acquired urinary tract infections caused by multi-drug resistant microorganisms

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SUMMARY

Introduction Healthcare-acquired urinary tract infections (HAUTI) make up to 40% of all healthcare-acquired infections and contribute significantly to hospital morbidity, mortality, and overall cost of treatment.

Objective The aim of our study was to investigate possible risk factors for development of HAUTI caused by multi-drug resistant pathogens.

Methods The prospective case-control study in a large tertiary-care hospital was conducted during a five-year period. The cases were patients with HAUTI caused by multi-drug resistant (MDR) pathogens, and the controls were patients with HAUTI caused by non-MDR pathogens.

Results There were 562 (62.6%) patients with MDR isolates and 336 (37.4%) patients with non-MDR isolates in the study. There were four significant predictors of HAUTI caused by MDR pathogens: hospitalization before insertion of urinary catheter for more than eight days ($OR_{adjusted}$ = 2.763; 95% CI = 1.352–5.647; p = 0.005), hospitalization for more than 15 days ($OR_{adjusted}$ = 2.144; 95% CI = 1.547–2.970; p < 0.001), previous stay in another department (intensive care units, other wards or hospitals) ($OR_{adjusted}$ = 2.147; 95% CI = 1.585–2.908; p < 0.001), and cancer of various localizations ($OR_{adjusted}$ = 2.313; 95% CI = 1.255–4.262; p = 0.007).

Conclusion Early removal of urinary catheter and reduction of time spent in a hospital or in an ICU could contribute to a decrease in the rate of HAUTI caused by MDR pathogens.

Keywords: urinary tract infections; nosocomial infections; multiple antibacterial drug resistance; risk factors

INTRODUCTION

Healthcare-acquired urinary tract infections (HAUTI) make up to 40% of all healthcare-acquired infections and contribute significantly to hospital morbidity, mortality and overall cost of treatment [1]. Major risk factor for HAUTI is an indwelling urinary catheter. Bacteriuria develops in up to 25% of patients who carry urinary catheter for one week or more, with a daily risk of 5–7% [2, 3]. It was estimated that there are about one million cases of HAUTI in hospitals and nursing homes annually associated with bladder catheter [4].

Bacteria are the primary organisms that cause HAUTI. Among gram-negative microorganisms, Enterobacteriaceae are predominant pathogens (*Escherichia coli*, *Klebsiella*, *Proteus* and *Enterobacter*). However, non-fermenting organisms (e.g. *Pseudomonas aeruginosa*) and gram-positive cocci (e.g. *Staphylococci* and *Enterococci*) may also play an important role, depending on the underlying conditions [5].

There are numerous recent reports coming from various hospitals around the world about increasing incidence of HAUTI caused by multi-drug resistant (MDR) microorganisms [6, 7]. Emergence of resistant strains is becoming a serious health problem because it limits the number of available antibiotics with potential to successfully treat these infections, and increases the costs of treatment. Knowledge of local and national antimicrobial resistance trends is of utmost importance for translation of evidence based recommendations to empiric antibiotic treatment of HAUTI.

Previous epidemiological studies identified indwelling urinary catheters, prior exposure to broad-spectrum antimicrobial therapy, advanced age of patients and male sex as risk factors for the development of HAUTI caused by MDR pathogens [8, 9, 10]. However, there is a whole spectrum of other characteristics of patients or hospital environments that were not investigated, and yet are candidates for risk factors for emergence of these infections.

OBJECTIVE

The aim of our study was to investigate possible risk factors for development of HAUTI caused by MDR pathogens, and to reveal their resistance patterns to various antibiotics. Good knowledge of the risk factors is the prerequisite for devising effective infection control strategies

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for HAUTI relevant to local settings, which may reduce the burden of healthcare-acquired infections.

METHODS

Study design

We conducted a prospective case-control study in a large tertiary-care hospital in Kragujevac, Serbia (1,183 beds and 50,000 inpatients per year) from January 2009 to December 2013 (five years).

The patient population

The study enrolled all patients with HAUTI according to standard definition established by the Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States, who were hospitalized during the study period for more than five days [11]. The exclusion criteria were isolation of a causative agent within the first 48 hours from the admission, and patients younger than 18 years. In patients who had several episodes of HAUTI during hospitalization, only the first episode was included in the analysis. The study was approved by the local Ethics Committee.

The cases consisted of patients with HAUTI caused by MDR agents. The controls were patients with HAUTI caused by non-MDR isolates.

For each participant a special epidemiological questionnaire was completed containing the following information: age, sex, hospital ward, dates of the patient's admission and discharge from the hospital, date when the HAUTI was diagnosed, dates of insertion and removal of urinary catheter, stay in another hospital ward before emergence of the HAUTI, and co-morbidities (diabetes mellitus, injuries, cancer of various locations). The data were obtained from both the patients' files and interviews with the patients and their physicians.

Each study participant was then analyzed by the Expert Group, comprising an epidemiologist, an infectologist, and a clinical pharmacologist, formed with the purpose of this study, and patients with colonization of the urinary tract were excluded from further analysis.

Antibiotic sensitivity

The isolation and identification of causative agents was performed in the hospital microbiology laboratory, using conventional biochemical methods [12]. An antimicrobial susceptibility test (AST) was performed using disk-diffusion method on Mueller-Hinton agar (bioMerieux, Marcy l'Etoile, France), by measuring the diameter of the zones of inhibition. The results were interpreted in accordance with the guidelines of The Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory [13]. The susceptibility of isolates to the following antibiotics was analyzed: ampicillin (up to 25 μg/mL), cefotaxime (up to 30 μg/mL), ceftriaxone (up to 30 μg/mL), ceftazidime (up to 30 μg/mL), cefepime (up to 30 μg/mL), imipenem (up to 10 μg/mL), meropenem (up to 10 μg/mL), gentamicin (up to 10 μg/mL), amikacin (up to 30 μg/mL), ciprofloxacin (up to 5 μg/mL), trimethoprim-sulfamethoxazole (up to 2.5 μg/mL). MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Statistical analysis

Primary analysis of collected data was made by descriptive statistics (arithmetic mean, standard deviation, and percentages), by testing statistical hypotheses, and by analysis of relations between outcomes and potential predictors. The differences among the study groups were tested by the Student's t-test for continual variables (after confirming normal distribution of data) and by the χ^2 test for categorical variables. The variables which turned out to be significant predictors of HAUTI caused by MDR pathogens after univariate logistic analysis were included in a multivariate binary logistic regression analysis. The hypotheses were tested at 0.05 level of statistical significance. The statistical software SPSS version 18 for Windows (SPSS Inc., Chicago, IL, USA) was used for all calculations.

RESULTS

During the study period, 775 patients met all eligibility criteria for HAUTI. The average age of the patients was 67.6 ± 13.4 years. Participation of male (n = 389; 50.2%) and female subjects (n = 386; 49.8%) was similar.

The majority of patients (n = 664; 85.7%) suffered from HAUTI caused by a single organism, whereas the rest were with two or three isolated pathogens. A total of 841 isolates were gram-negative agents (93.7%) (Table 1). Generally, the most common pathogen was *Klebsiella* spp. (32.4%), followed by *Proteus mirabilis* (16.8%), *E. coli* (12.8%), *Enterobacter* spp. (12.7%), and *Pseudomonas aeruginosa* (11.4%), all accounting for over 85% of total isolates. A statistically significant difference in the frequency of isolation between the patients with a bladder catheter and those without one was found for only one of the top five most common agents – *Pseudomonas aeruginosa* (p = 0.034).

The percentage of MDR isolates by types of bacteria varied between 0% and 82.4%, and showed higher values in gram-negative than among gram-positive isolates (64.2% and 38.6%, respectively). *Pseudomonas aeruginosa* and *Acinetobacter* spp. were the most common MDR uropathogens (Table 1).

There were 562 (62.6%) patients with MDR isolates (cases) and 336 (37.4%) patients with non-MDR isolates (controls) in the study. The results of univariate analysis of risk factors for HAUTI caused by MDR pathogens are shown in Table 2. According to the univariate analysis, age 65 years and above (p = 0.019), insertion of a urinary catheter (p = 0.005), hospitalization before the insertion

Table 1. Causes of nosocomial urinary tract infections at the Clinical Center in Kragujevac, 2009–2013

Microorganism	Catheterized n (%)	Non-catheterized n (%)	Total n (%)	χ^2 test	p-value	MDR n (%)
All gram negative	679 (94.0)	162 (92.0)	841 (93.7)	1.543	0.214	540 (64.2)
Klebsiella spp.	237 (32.8)	54 (30.7)	291 (32.4)	0.294	0.586	199 (68.4)
Proteus mirabilis	117 (16.2)	34 (19.3)	151 (16.8)	0.980	0.322	98 (64.9)
Escherichia coli	88 (12.2)	27 (15.3)	115 (12.8)	1.259	0.262	51 (44.3)
Enterobacter spp.	95 (13.2)	19 (10.8)	114 (12.7)	0.713	0.399	71 (62.3)
Pseudomonas aeruginosa	90 (12.5)	12 (6.8)	102 (11.4)	4.482	0.034	80 (78.4)
Proteus vulgaris	37 (5.1)	10 (5.7)	47 (5.2)	0.089	0.766	27 (57.4)
Acinetobacter spp.	13 (1.8)	4 (2.3)	17 (1.9)	0.170	0.680	14 (82.4)
Providencia spp.	2 (0.3)	2 (1.1)	4 (0.4)	2.357	0.125	0 (0.0)
All gram positive	43 (6.0)	14 (8.0)	57 (6.3)	1.543	0.214	22 (38.6)
Enterococcus spp.	42 (5.8)	12 (6.8)	54 (6.0)	0.251	0.616	21 (38.9)
Staphylococcus spp.	1 (0.1)	2 (1.1)	3 (0.3)	4.232	0.040	1 (33.3)
Total	722 (100.0)	176 (100.0)	898 (100.0)			562 (62.6)

Table 2. Risk factors for healthcare-acquired urinary tract infections caused by multi-drug resistant (MDR) pathogens (univariate analysis)

Variable	MDR (n = 562)	Non-MDR (n = 336)	t-test/ χ^2 test	p-value
Age (years)	66.65 ± 13.71	68.49 ± 13.46	t = -1.956	0.051
Age ≥65 years	349 (62.1)	226 (67.3)	χ^2 = 2.433	0.019
Male sex	298 (53.0)	157 (46.7)	χ^2 = 3.338	0.068
Urinary catheter	468 (83.3)	254 (75.6)	χ^2 = 7.868	0.005
Hospitalization before urinary catheter ≥8 days	61 (10.9)	10 (3.0)	χ^2 = 17.923	<0.001
Length of hospitalization ≥15 days	463 (82.4)	222 (66.1)	χ^2 = 30.927	<0.001
Previous stay in another department	278 (49.5)	97 (28.9)	χ^2 = 6.680	<0.001
Surgical departments	230 (40.9)	115 (34.2)	χ^2 = 3.989	0.046
Diabetes mellitus	95 (16.9)	41 (12.2)	χ^2 = 3.317	0.057
Injuries on admission	79 (14.1)	57 (17.0)	χ^2 = 1.383	0.240
Cancer of various localizations	63 (11.2)	15 (4.5)	χ^2 = 12.064	0.001

The results are presented as mean value ± standard deviation, or n (%)

Table 3. Risk factors for healthcare-acquired urinary tract infections caused by multi-drug resistant pathogens (multivariate analysis*)

Variable	B	$OR_{adjusted}$	95% CI	p-value
Hospitalization before urinary catheter ≥8 days	1.016	2.763	1.352–5.647	0.005
Length of hospitalization ≥15 days	0.763	2.144	1.547–2.970	<0.001
Previous stay in another department	0.764	2.147	1.585–2.908	<0.001
Cancer of various localizations	0.839	2.313	1.255–4.262	0.007

Only significant factors are presented for the sake of clarity.
B – coefficient of logistic regression analysis; OR – odds ratio; CI – confidence interval
*The model includes the following covariates: age 65 years and above, insertion of urinary catheter, hospitalization before the insertion of a urinary catheter longer than eight days, hospitalization longer than 15 days, previous stay in another department, patients in the surgical department, cancer of various localizations

of a urinary catheter longer than eight days ($p < 0.001$), hospitalization longer than 15 days ($p < 0.001$), previous stay in another department (other wards, intensive care units or other hospitals) ($p < 0.001$), patients in the surgical department ($p = 0.046$), and cancer of various localizations ($p = 0.001$) were significant risk factors for HAUTI caused by MDR pathogens.

The results of multivariate binary logistic regression are shown in Table 3. There are four significant predictors of HAUTI caused by MDR pathogens: hospitalization before the insertion of a urinary catheter for more than eight days ($OR_{adjusted} = 2.763$; 95% CI = 1.352–5.647; $p = 0.005$), hospitalization for more than 15 days ($OR_{adjusted} = 2.144$; 95% CI = 1.547–2.970; $p < 0.001$), previous stay in another department (intensive care units, other wards or hospitals) ($OR_{adjusted} = 2.147$; 95% CI = 1.585–2.908; $p < 0.001$) and cancer of various localizations ($OR_{adjusted} = 2.313$; 95% CI = 1.255–4.262; $p = 0.007$). The Hosmer–Lemeshow goodness-of-fit test for this logistic regression model was $\chi^2 = 6.032$; df = 7; $p = 0.536$.

Resistance of the isolates from the patients with HAUTI is shown in Table 4. The isolates of *Klebsiella* spp. were highly resistant to trimethoprim-sulfamethoxazole, cephalosporins of the third and fourth generation, and ciprofloxacin (over 90%), whereas 199 (68.4%) isolates were multiresistant. The highest level of sensitivity was retained toward carbapenems (around 13% of isolates were resistant). *Proteus mirabilis* isolates showed the highest resistance to ampicillin (96.4%), followed by resistance to trimethoprim-sulfamethoxazole (91.3%), gentamicin (90.8%), third-generation cephalosporins (89.5–91.0%), and ciprofloxacin (84.1%). Isolates of *E. coli* showed high degree of resistance to trimethoprim-sulfamethoxazole (84.1%) and ciprofloxacin (80.8%), while the resistance to aminoglycosides was somewhat lower (77.5% to gentamicin, and 48.1% to amikacin). The percentage of isolates

Table 4. Degree of antimicrobial resistance (%) of the most important pathogens causing HAUTIs

Antibiotic	Microorganism				
	Klebsiella spp.	*Proteus mirabilis*	*Escherichia coli*	*Enterobacter* spp.	*Pseudomonas aeruginosa*
Ampicillin	99.0	96.4	98.1	96.7	100.0
Cefotaxime	97.5	90.4	83.3	97.4	95.9
Ceftriaxone	97.6	89.5	83.2	95.5	96.5
Ceftazidime	98.1	91.0	86.2	95.4	87.4
Cefepime	89.9	49.6	64.8	86.7	85.8
Imipenem	13.3	13.7	9.6	11.9	46.3
Meropenem	13.8	13.3	9.7	13.1	43.6
Gentamicin	92.3	90.8	77.5	93.3	100.0
Amikacin	64.7	83.1	48.1	63.0	84.2
Ciprofloxacin	90.8	84.1	80.8	90.8	90.0
Trimethoprim-sulfamethoxazole	96.6	91.3	84.1	96.2	90.0

resistant to carbapenems was rather low (10%). The other isolated gram-negative bacteria showed high degree of resistance to cephalosporins (85–98%), aminoglycosides (65–100%), fluoroquinolones (90%), while resistance to carbapenems was lower (10–45%).

The difference in hospital mortality between the patients with MDR infection [94 (19.6%) patients died from 479 in total] and those without it [56 (18.9%) died from 296 in total] was not significant ($\chi^2 = 0.058$, $p = 0.809$).

DISCUSSION

There are various reports on HAUTI causative agents in medical literature. In a study similar to ours nearly 95% of all isolates were gram-negative pathogens: *Klebsiella* spp. making one third (32.4%), followed by *Proteus mirabilis* (16.8%) and *E. coli* (12.8%) [14]. In a prospective study of HAUTI which included 29 European countries it was found that six most common causative agents are *E. coli, Enterococcus, Candida* spp., *Klebsiella, Proteus* and *Pseudomonas aeruginosa* [15]. This result is not surprising since these bacteria belong to normal flora of the human intestine and therefore easily colonize urinary tract. This study showed significant difference in prevalence of only one of the gram-negative uropathogens in relation to presence of urinary catheter: *Pseudomonas aeruginosa* was isolated more frequently from patients with a catheter, which is consistent with the results of other authors [16]. Previous studies have also indicated that patients with HAUTI caused by *Pseudomonas aeruginosa* were more likely to have history of urinary tract procedures, to have a neurogenic bladder, to be male, and to have received recent antibiotic therapy [17].

There are many different definitions of MDR in medical literature which characterize different patterns of resistance found in healthcare-associated, antimicrobial-resistant bacteria. However, a group of international experts came together through a joint initiative by the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention, to create a standardized international terminology for describing acquired resistance profiles of all bacteria often responsible for healthcare-associated infections and prone to multidrug resistance. Epidemiologically significant antimicrobial categories were determined for each bacterium. Lists of antimicrobial categories proposed for antimicrobial susceptibility testing were created using documents and breakpoints from the Clinical Laboratory Standards Institute, the European Committee on Antimicrobial Susceptibility Testing, and the United States Food and Drug Administration. The experts reached consensus that MDR organisms are those which acquired non-susceptibility to at least one agent in three or more antimicrobial categories [18]. This definition has practical value because it allows differentiation of sensitive and MDR strains in clinical settings.

In accordance with this definition, we separated patients with MDR isolates and observed that there was a high percentage of MDR particularly among gram-negative isolates (64.2%). Increase in the prevalence of MDR isolates is being registered all over the world, and these microorganisms have become a global public health problem. Extremely rapid development of antimicrobial resistance is probably the result of the ability of uropathogens to quickly adapt to antibiotics, together with the widespread overuse of antibiotics in the hospital environment. In addition, broad-spectrum antibiotics cause suppression and eradication of competing microorganisms and facilitate selection of the MDR strains [19]. Infections caused by MDR pathogens are difficult to treat and control, leading to prolonged hospital stay, increased mortality, and higher hospitalization costs [20].

Our study showed that hospitalization before the insertion of a urinary catheter for more than eight hours increases the risk of developing HAUTI caused by MDR pathogens 2.7 times. Some other recent studies have shown that unnecessary catheterization is widely prevalent (30–50%), even in tertiary care referral centers. Large proportion of these patients who did not need a catheter in accordance with accepted indications subsequently went on to develop HAUTI, especially if the catheter was kept for longer time period [21]. These findings also emphasize the need for more stringent implementation of aseptic techniques while inserting a catheter and therefore better infection control.

Catheters and other foreign bodies in the urinary tract disrupt natural protective barriers (urethral sphincter) and provide a nidus for infections by offering surface for formation of biofilm [22]. Several studies have shown that

most uropathogens are able to form biofilm over urinary catheter shortly after its placement. Biofilms are resistant to antimicrobial agents as well as to host defense mechanisms and hence are difficult to eradicate. Biofilms contribute to virulence of the pathogens as these often cause persistent and recurrent infections [23, 24]. In our study, carrying a urinary catheter was frequent in both groups (cases 83.3% vs. 75.6% of control). However, multivariate analysis excluded wearing of urinary catheter as a risk factor for HAUTI caused by MDR pathogens, although in univariate analysis it was significant ($p = 0.005$).

It has been known for some time that longer hospitalization of patients increases the risk of nosocomial infections [25], and our study has also shown that the risk of HAUTI caused by MDR pathogens is 2.1 times higher if patients were hospitalized for longer than 15 days. Longer stay in a hospital is associated with invasive medical procedures, and with increased contact with the bacteria from hospital environment, which are often multi drug-resistant.

Our study shows that a previous stay in another department (intensive care unit, other wards or hospitals) increased the risk of HAUTI caused by MDR pathogens by 2.1 times. Our patients usually stayed in intensive and semi-intensive care units (65%) where MDR pathogens are common causes of nosocomial infections [26, 27]. The obtained result is not surprising if one considers that the majority of patients in these departments are those with severe underlying diseases, the elderly, the immunocompromised, and patients with many comorbidities, who require large number of medical procedures (e.g. placement of urinary catheters), which further violate epithelial barriers.

In addition to horizontal transmission of pathogens, there was a vertical one, because our study site is an institution of tertiary care which receives patients from secondary care hospitals within the region. In hospitals without consistent antibiotic policy and with practice of injudicious utilization of antibiotics, patients rapidly become potential sources of infection, particularly of MDR bacteria. A recent study of Falagas and Kopterides [28] found higher rate of MDR bacterial isolates in patients with a history of previous hospitalizations. Increased vigilance and complete implementation of infection control policies and practices in these hospitals could be one part of the solution.

After taking into account individual characteristics of the patients, multivariate analysis showed that only cancer of various localizations increases the risk of HAUTI caused by MDR pathogens by 2.3 times. Generally speaking, patients with cancer frequently have HAUTIs, due to immunosuppression caused by the malignancy itself or by cytotoxic therapy. Also, information on previous catheterization, and previous hospitalization, which are often found in the history of patients with malignant tumors, may explain increased prevalence of perineal colonization with potential MDR pathogens in oncology patients, which may be important for the development of HAUTI [29].

A disturbing result of our study was that isolates of *Klebsiella* spp. showed high resistance (over 90%) to trimethoprim-sulfamethoxazole, third- and fourth-generation cephalosporins, and ciprofloxacin, antibiotics that are commonly used to treat infections caused by these microorganisms in hospitals. Such high rates of resistance limit their use in empirical therapy. The results were significantly worse than in other countries [30, 31], and are even more significant given the high proportion of *Klebsiella* spp. causing HAUTIs in our study (32.4%). This could be explained by a wide use of these antibiotics for treatment of HAUTIs and community-acquired urinary tract infections over the past decade in this region. Further efforts of the entire community are necessary in order to maintain sensitivity of urinary pathogens to these antibiotics in the future.

The degree of *Proteus mirabilis* resistance to antibiotics in our study was generally high for all tested antimicrobials. Thus, the resistance to trimethoprim-sulfamethoxazole was 91.3%, to cephalosporins of the third generation 89.5–91.0% and to ciprofloxacin 84.1%, which are much higher rates than those observed in other studies [29, 30].

Isolates of *E. coli* in our study showed high degree of resistance to trimethoprim-sulfamethoxazole (84.1%) and ciprofloxacin (80.8%), while the resistance to aminoglycosides was somewhat lower (77.5% to gentamicin, and 48.1% to amikacin). Such high resistance rates are two to three times higher than in other recent studies [31, 32], but it was already reported in the study conducted in the same hospital during the previous period [9]. Particularly worrying is resistance of *E. coli* isolates to broad-spectrum antibiotics such as fluoroquinolones and cephalosporins due to overutilization of these two groups and parallel development of co-resistance to other antibiotics (collateral damage) [33].

Carbapenems were increasingly used during the 1980s for treatment of serious nosocomial infections. However, the emergence of resistant gram-negative bacilli to these antibiotics is nowadays depriving doctors of these very active antibiotics against nosocomial infections. However, in our study, resistance to carbapenems was relatively low (around 10%, except that of *Pseudomonas aeruginosa*, which was around 45%), which is encouraging. According to the recommendations of European Association of Urology, carbapenems should be used as therapy for complicated cases of HAUTIs [5]. Our results emphasize the importance of optimizing the use of carbapenems, in order to preserve their activity in the future. Hospitals that achieved at least some control over the use of carbapenems halted further increase in resistance to these antibiotics [34].

Having taken into account the incidence of each bacterium (nearly 90% of all isolates were gram negative) and rate of resistance in our study, it can be concluded that the role of fluoroquinolones and aminoglycosides in the empiric treatment of HAUTIs is becoming more and more limited. Available antimicrobials with good activity against majority of pathogens include cefepime and carbapenems. The therapy should be adjusted according to local data on bacterial susceptibility to antibiotics. Whenever possible, empirical therapy should be replaced by a therapy targeted to the specific infective organisms identified in the urine culture. Appropriate antimicrobial selection, surveillance systems, and effective infection control procedures are key measures for limiting antimicrobial-resistant pathogen occurrence and spread.

doi: 10.2298/SARH1610490D

The overall mortality rate in our study was similar in the groups with and without MDR infections, but we were not able to compare the rates of urinary tract infections – attributed mortality, which is reported to be low [35]. Only 5% of patients with bacteriuria develop bacteriemia, but mortality rate in these patients is almost 10% [35].

There are certain limitations of our study. First, the study was conducted in a single center, reflecting the possibility of institutional bias either in the selection of patients or routine medical practices. Second, we were not able to conduct molecular epidemiological research in order to discover the mechanisms by which the drug resistance developed. In addition, we did not determine the minimum inhibitory concentration of the studied antibiotics.

CONCLUSION

The results of our study point to hospitalization for more than eight days before insertion of urinary catheter, to prolonged hospitalization, to previous stay in an another department, and to cancer of various localizations as important risk factors associated with HAUTI caused by MDR pathogens. Early removal of urinary catheter and reduction of time spent in a hospital or an ICU could contribute to a decrease in the rate of these infections. Moreover, good knowledge of the susceptibility profile of isolated pathogens should help physicians when prescribing empiric therapy. Our study emphasizes the need for aggressive infection control strategies to prevent urinary tract infections with MDR pathogens.

ACKNOWLEDGEMENT

This study was partially financed by research grant No. 175007 awarded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, and through grant No. 404 from the Ministry of Science, Montenegro.

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Фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним микроорганизмима

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КРАТАК САДРЖАЈ

Увод Болничке инфекције уринарног тракта чине и до 40% свих болничких инфекција и значајно доприносе болничком морбидитету, морталитету и расту укупних трошкова лечења.

Циљ рада Циљ нашег истраживања био је да се утврде потенцијални фактори ризика за развој болничких инфекција уринарног тракта узрокованих мултирезистентним (МР) патогенима.

Методе рада Спроведена је клиничка студија типа случај-контрола у периоду од пет година у здравственој установи терцијарног нивоа здравствене делатности. Групу случајева чинили су болесници са болничким инфекцијама уринарног тракта проузрокованим МР бактеријама, док су контролну групу чинили пацијенти са болничким инфекцијама уринарног тракта узрокованим бактеријама које нису припадале претходно наведеној групи узрочника инфекција.

Резултати Укупно је било 562 (62,6%) болесника са верификованим МР изолатима, односно 336 (37,4%) пацијената са изолатима који нису припадали МР групи патогена. Идентификована су четири значајна предиктора која могу допринети развоју болничких инфекција уринарног тракта проузрокованих мултирезистентним патогенима: хоспитализација пре пласирања уринарног катетера дужа од осам дана ($OR_{adjusted}$ = 2,763; 95% *CI* = 1,352–5,647, p = 0,005), дужина хоспитализација од 15 и више дана ($OR_{adjusted}$ = 2,144; 95% *CI* = 1,547–2,970, p < 0,001), претходни боравак на другом одељењу (интензивна нега, друга одељења или болнице) ($OR_{adjusted}$ = 2,147; 95% *CI* = 1,585–2,908, p < 0,001) и карциноми различитих локализација ($OR_{adjusted}$ = 2,313; 95% *CI* = 1,255–4,262; p = 0,007).

Закључак Правовремено (рано) уклањање уринарног катетера и смањење времена проведеног у болници или у интензивној нези могли би значајно да допринесу редукцији стопе болничких инфекција уринарног тракта проузрокованих мултирезистентним патогенима.

Кључне речи: инфекције уринарног тракта; болничке инфекције; мултипла резистенција на антибиотике; фактори ризика

Примљен • Received: 12/08/2015 **Ревизија • Revision:** 23/03/2016 **Прихваћен • Accepted:** 01/04/2016

The effect of combination therapy of insulin glargine, metformin, and sitagliptin on insulin secretion, insulin resistance, and metabolic parameters in obese subjects with type 2 diabetes

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SUMMARY

Introduction A combination of drugs is required for treatment of obese subjects with diabetes, due to multiple pathogenic mechanisms implicated in the development of both diabetes and obesity.

Objective Assessment of the effect of sitagliptin added to insulin glargine and metformin, in obese subjects with type 2 diabetes.

Methods A total of 23 obese subjects on metformin and insulin glargine participated in the study. Titration of insulin glargine during a one-month period preceded the addition of 100 mg of sitagliptin daily. Body mass index, waist circumference, fasting, and prandial glucose were measured monthly, lipids and hemoglobin A1c (HbA1c) every three months, insulin, c-peptide and glucagon at the start and after six months of treatment. Homeostatic models for insulin secretion (HOMA B) and insulin resistance (HOMA IR) were calculated.

Results Participants were 58.65 ± 7.62 years of age with a body mass index of 35.06 ± 5.15 kg/m^2, waist circumference of 115.04 ± 15.5 cm, and the duration of diabetes of 4.11 ± 2.57 years. With the titration of insulin glargine, target fasting glucose levels were not achieved. Waist circumference and body mass index decreased during three months of sitagliptin treatment, thereafter remaining stable. HbA1c decreased significantly after three and six months of therapy. C-peptide increased significantly, while glucagon level fell. HOMA indexes were unchanged.

Conclusion Sitagliptin can improve diabetes control and induce modest weight loss in obese subjects poorly controlled on insulin glargine and metformin. Titration of insulin glargine to optimal fasting glucose values is a prerequisite of success of this combination therapy.

Keywords: sitagliptin; glargine; obesity; diabetes

INTRODUCTION

Multiple pathogenic mechanisms are implicated in the development of diabetes mellitus type 2, a progressive condition characterized by hyperglycemia, often dyslipidemia, and hypertension. Decreased insulin secretion and incretin response, increased insulin resistance, peripheral outflow of free fatty acids, and increased reabsorption of glucose from the proximal tubules of the kidney may complicate metabolic control of diabetes [1].

Basal insulin secretion is insufficient to stop hepatic glucose production, due to hepatic insulin resistance, leading to high fasting glucose values. This is usually the first secretory insulin abnormality to develop in type 2 diabetes. However, loss of first phase of insulin prandial secretion may develop early in the course of the disease. The second phase of insulin secretion is slow in onset and is insufficient, leading to prandial hyperglycemia. Prandial secretion of proinsulin is increased. At the same time, hyperglycemia does not stop glucagon production, which further stimulates gluconeogenesis and glycogenolysis in the liver [2]. Incretin effects are controlled by gut hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide, secreted after food consumption [3]. Diminished incretin effects lead to inadequate insulin secretion, glucagon hypersecretion, low satiety, and fast transition of food through the stomach.

Obese people with diabetes present a clinical challenge. Insulin resistance occurs when insulin levels are sufficiently high over a prolonged period of time causing the reduction of body's own sensitivity to insulin. Insulin resistance in type 2 diabetes, brought on by obesity, is closely linked to inflammation [4]. Hepatic insulin resistance precedes peripheral insulin resistance in the muscle and adipose tissue. Glucose utilization in the muscle is diminished, whereas outflow of free fatty acids from the adipose tissue is uninhibited. Free fatty acids have a lipotoxic effect on beta cell.

The National Serbian Guideline for the Treatment of Diabetes Mellitus [5], as well as

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recent American Diabetes Association and European Association for the Study of Diabetes position statement [6], defines four optional steps in diabetes therapy. A combination of metformin, basal insulin and DPP-4 inhibitors corrects insulin resistance, secretory defects and diminished incretin effects. This three-step optional combination may be useful in treating diabetes in obese subjects. Sitagliptin is an orally active inhibitor of DPP-4, with a fully reversible action [7]. It increases glucose-dependent insulin secretion, while decreasing glucagon secretion and hepatic glucose production. Sitagliptin has been associated with an approximate two-fold increase in postprandial GLP-1 plasma concentrations, compared to placebo, in healthy human study participants and in patients with type 2 diabetes mellitus [8].

OBJECTIVE

The objective of this study was to assess the effect of combination therapy of insulin glargine, metformin and sitagliptin on insulin secretion, insulin resistance, and metabolic parameters in obese subjects with type 2 diabetes.

METHODS

Twenty-three obese subjects with diabetes were included in the study. They were recruited from the outpatient clinic of the Department of Endocrinology, Diabetes and Metabolic Disorders of Zvezdara University Hospital. After obtaining a signed informed consent, subjects were scheduled an appointment in the daily outpatient unit. This study was approved by the Ethics Committee of Zvezdara University Hospital.

Inclusion criteria for the participation in the study were as follows: unfavorable control of diabetes with glycosylated hemoglobin (HbA1c) of 7–12%, on combined insulin glargine and metformin therapy lasting at least 12 months, age between 35 and 75 years, and body mass index (BMI) ≥25 kg/m². At first visit, the subjects were educated to titrate insulin glargine to fasting glucose levels between 3.9 and 5.5 mmol/l, using a simple algorithm [9]. They were instructed to perform fasting glucose measurements every morning. An average of three consecutive fasting glucose measurements was calculated. If it exceeded 5.5 mmol/l, two units of insulin glargine were added to the previous dose. If the measurement was equal to or lower than ≤3.9 mmol/l, two units were subtracted from the previous dose. If the average fasting glucose reading was 3.9–5.5 mmol/l, the dose of insulin glargine remained unchanged. The subjects were instructed to write down the measurements with dose titration changes in the diary and to titrate the dose of insulin glargine every three days.

After one month of insulin glargine titration, 100 mg of sitagliptin was added to metformin and insulin glargine for six months, to improve prandial control. The subjects were invited for monthly visits for blood glucose, blood pressure and body weight measurements. BMI was calculated as body weight in kilograms divided by square of height expressed in meters. At start, after three and six months of triple combination therapy, blood samples were taken for the measurement of HbA1c, lipids, fasting and two hours prandial glucose levels. Low density lipoprotein (LDL) cholesterol was calculated from total cholesterol, high density lipoprotein cholesterol (HDL) and triglycerides using Friedwald's formula: LDL (mmol/l) = total cholesterol - HDL - (triglycerides / 2.17). Insulin, c-peptide and glucagon levels were assessed before and after six months of sitagliptin therapy. The time between the last dose of insulin glargine and blood sampling had to be longer than 24 hours. Insulin and c-peptide were evaluated by the electrochemiluminescence (ECLIA) method, on the Roche analyzer at the Konzilijum Laboratory. The referent range for insulin was 6.0–27.0 μIU/ml, and for c-peptide 0.9–7.1 ng/ml. Glucagon was analyzed using the radioimmunoassay (RIA) method. The referent values for glucagon were 60–177 ng/L. Insulin secretion was evaluated using the HOMA-B model. It was calculated from the formula by Matthews [10]: HOMA B = Insulin (mU/l) × 2 / fasting glucose level (mmol/l) - 3,5. Insulin resistance was evaluated with the HOMA-IR model. It was calculated as HOMA IR = Insulin (mU/l) × fasting glucose (mmol/l) / 22.5.

Data are presented as mean ± standard deviation or n (%) depending on the data type. Paired samples tests (t-test and Wilcoxon signed-rank test) were used to assess significant differences within measurements. All the data were analyzed using SPSS 20.0 (IBM corp., Armonk, NY, USA) statistical software. All p-values less than 0.05 were considered statistically significant.

RESULTS

Twenty-three obese subjects, 13 men and 10 women, with type 2 diabetes inadequately controlled by insulin glargine and metformin participated in the study. Their mean age was 58.65 ± 7.62 years, while mean diabetes duration was 4.11 ± 2.57 years. The mean weight of the studied subjects was105.30 ± 19.29 kg, BMI 35.06 ± 5.15 kg/m², and the mean waist circumference 115.04 ± 15.47 cm.

After three months of treatment with 100 mg of sitagliptin added to insulin glargine and metformin, a significant reduction of weight, waist circumference, and BMI was observed. The difference in baseline weight and weight at six months was also significant. However, there was no significant weight change between three and six months of therapy, indicating that the loss of weight achieved in the first three months was maintained through the whole follow-up period. Similar changes were observed in waist circumference and BMI (Table 1). The triple combination therapy had no effects on lipids and blood pressure.

During one month of insulin glargine titration, its dose was significantly increased from an average of 38.97 ± 15.28 units to 48.64 ± 20.62 units, $p < 0.01$. However, target fasting blood glucose values were not achieved. The average fasting blood glucose value at the end of insulin

doi: 10.2298/SARH1610497Z

Table 1. The effect of sitagliptin added to insulin glargine and metformin on body weight, waist circumference, body mass index, lipids and blood pressure during six months of therapy (mean value ± SD)

Parameter	Baseline	3 months	6 months	p (baseline vs. 3 months)	p (baseline vs. 6 months)
BW (kg)	105.30 ± 19.29	102.57 ± 17.84	102.61 ± 17.41	<0.001	<0.001
WC (cm)	115.04 ± 15.47	107.65 ± 12.78	107.91 ± 11.84	<0.001	<0.001
BMI (kg/m^2)	35.06 ± 5.15	34.19 ± 4.93	34.16 ± 4.90	<0.001	<0.001
sBP (mmHg)	125.22 ± 9.94	128.04 ± 9.97	125.00 ± 9.29	0.170	0.954
dBP (mmHg)	78.04 ± 6.51	78.70 ± 4.05	75.65 ± 4.60	1.000	0.400
CHOL (mmol/l)	5.44 ± 1.52	5.50 ± 1.30	5.52 ± 1.16	1.000	1.000
TGL (mmol/l)	3.44 ± 2.58	3.11 ± 2.23	3.27 ± 2.33	0.308	1.000
HDL-c (mmol/l)	1.11 ± 0.22	1.07 ± 0.28	1.08 ± 0.23	0.470	1.000
LDL-c (mmol/l)	2.97 ± 1.14	3.16 ± 1.04	3.05 ± 1.09	0.200	1.000

SD – standard deviation; BW – body weight; WC – waist circumference; BMI – body mass index; sBP – systolic blood pressure; dBP – diastolic blood pressure; CHOL – total cholesterol; TGL – triglycerides; HDL-c – high density lipoprotein; LDL-c – low density lipoprotein

Table 2. The effect of sitagliptin added to insulin glargine and metformin on fasting and prandial glucose levels and glycosylated hemoglobin during six months of therapy (mean value ± SD)

Parameter	Baseline	3 months	6 months	p (baseline vs. 3 months)	p (baseline vs. 6 months)
Fasting glucose levels (mmol/l)	9.92 ± 2.58	8.75 ± 3.02	8.62 ± 2.28	0.192	0.032
Prandial glucose levels (mmol/l)	11.32 ± 3.50	10.14 ± 3.09	9.66 ± 2.71	0.184	0.012
HbA1c %	9.06 ± 1.16	7.91 ± 1.10	8.20 ± 1.14	<0.001	<0.001

HbA1c – glycosylated hemoglobin A1c

Table 3. The effect of sitagliptin added to insulin glargine and metformin on circulating insulin, c-peptide and glucagon levels during six months of therapy (mean value ± SD)

Parameter	Baseline	6 months	p (baseline vs. 6 months)
Insulin (μIU/ml)	16.55 ± 10.10	19.56 ± 13.98	0.492
C-peptide (ng/ml)	1.05 ± 0.99	1.50 ± 1.52	0.163
Glucagon (ng/l)	48.43 ± 16.81	43.61 ± 16.41	0.086

Table 4. The effect of sitagliptin added to insulin glargine and metformin on HOMA-B and HOMA-IR indices during six months of therapy

Parameter	Baseline	6 months	p (baseline vs. 6 months)
HOMA-B	18.33 ± 15.59	35.05 ± 22.71	0.076
HOMA-IR	3.79 ± 1.91	4.09 ± 1.54	0.092

glargine titration was 9.92 ± 2.58 mmol/l. Postprandial blood glucose level, before the addition of sitagliptin, was 11.32 ± 3.50 mmol/l.

After three months of treatment with 100 mg of sitagliptin added to insulin glargine and metformin, the average fasting blood glucose levels were reduced to 8.75 ± 3.02 mmol/l, while after six months it was 8.62 ± 2.28 mmol/l. The average postprandial blood glucose value was 10.14 ± 3.09 mmol/l after three months and 9.66 ± 2.71 mmol/l after six months of treatment. Glycosylated hemoglobin A1c (HbA1c) was 9.06 ± 1.16% before initiating sitagliptin add-on therapy. It significantly decreased after three (8.01 ± 1.11%) and six (8.29 ± 1.13%) months of introducing sitagliptin in therapy. It was only after six months of triple combination therapy that the average fasting and postprandial blood glucose values achieved a significant reduction compared to values at start (Table 2).

Insulin, c-peptide and glucagon values were evaluated at start and after six months of therapy. The average plasma insulin value was 16.55 ± 10.10 μIU/ml before, and 19.56 ± 13.98 μIU/ml six months after addition of 100 mg of sitagliptin to insulin glargine and metformin.

At the same time, plasma c-peptide values significantly increased from 1.05 ± 0.99 ng/ml to 1.50 ± 1.52 ng/ml. Glucagon levels decreased from 48.43 ± 16.81 ng/L to 43.61 ± 16.41 ng/L after six months of sitagliptin add-on therapy (Table 3).

Sitagliptin added on to insulin glargine and metformin did not significantly change insulin secretion and insulin resistance, evaluated through HOMA-B and HOMA-IR models. Non-significant increase in basal insulin secretion was the only observed change (Table 4).

DISCUSSION

Our study has shown that there is a place for the addition of sitagliptin to obese subjects with type 2 diabetes inadequately controlled by insulin glargine and metformin. Sitagliptin add-on therapy significantly lowered weight, BMI and waist circumference. It increased basal insulin secretion and lowered glucagon levels. However, target fasting and prandial glucose levels were not achieved, although levels of HbA1c were significantly reduced after three and six months of therapy. This indicated that optimal titration of insulin glargine to target fasting glucose levels had to be performed before the addition of sitagliptin. This was not the case in our group of participants.

The safety and tolerability of sitagliptin, the first dipeptidyl peptidase-4 (DPP-4) inhibitor used in clinical practice, was previously tested in many trials. It has been used as monotherapy, or in combination with metformin, sulfonylurea, pioglitazone, or insulin [11]. It reduces HbA1c for 1%, fasting plasma glucose for 1 mmol/l, and postprandial glucose levels for 2.6 mmol/l, during 24 weeks of treatment, and does not induce hypoglycemia and weight gain [12]. Nausea is tolerable and no other adverse events

have been reported. Sitagliptin therapy in combination with metformin has been shown to increase circulating insulin levels, c-peptide and HOMA B. It had no influence on HOMA IR, when compared to placebo. Sitagliptin has been shown to reduce postprandial lipemia [13]. In subjects with renal impairment, sitagliptin can be safely used in a reduced dose of 50 mg when creatinine clearance is 30–50 ml/min., and in dose of 25 mg when creatinine clearance is less than 30 ml/min. [14]. Urinary albumin levels are reduced on sitagliptin therapy in subjects with diabetes and albuminuria [15]. The results of the recently completed TECOS trial (Trial to Evaluate Cardiovascular Outcomes after Treatment with Sitagliptin) has shown that sitagliptin does not increase major adverse cardiovascular events, hospitalization for heart failure, or other adverse events in 14.671 high cardiovascular risk subjects with diabetes [16].

Two clinical problems with DPP-4 inhibitors use have not been resolved so far. The question that bothers many physicians is whether it is justified to use sitagliptin in long-term diabetes on insulin therapy. Will this kind of combination therapy lower the dose of insulin, decrease HbA1c, induce hypos and will it be weight neutral? The second dilemma is the use of DPP-4 inhibitors in obese subjects. Glucagon-like peptide-1 analogues are preferable in obese subjects, as they successfully reduce weight. However, they are injectables, and some patients do not like this form of therapy. Hence, our study addressed both questions.

Very few clinical studies have been published on combined use of sitagliptin with insulin analogues. An extension of the EASIE study, randomized controlled study performed on 515 subjects that tested the combination of sitagliptin or insulin glargine with metformin, as initial therapy for type 2 diabetes [17], was similar to ours. In the extension of the EASIE study, sitagliptin was added to therapy of 37 subjects not attaining goal of HbA1c less than 7%, after 24 weeks of treatment of insulin glargine and metformin [18]. Half of the subjects attained HbA1c of less than 7% on 12 weeks of triple therapy of sitagliptin, metformin and insulin glargine.

A study with aims similar to ours assessed the effect of sitagliptin or exenatide added to insulin glargine and metformin, compared to glargine and metformin alone. In enrolled 48 subjects with diabetes duration of 6 ± 1 years and with a BMI of 31.7 ± 3.4 kg/m^2 [19]. During the four weeks of the study, HbA1c was reduced significantly in all groups, with better prandial control on incretin therapy. Subjects on exenatide lost weight, while sitagliptin was weight neutral. Subjects on insulin glargine and metformin gained in weight. As titration of insulin was allowed, the dose of insulin glargine remained the same on incretin therapy, while it increased for five units in the glargine and metformin group. When compared to our study, the subjects in our group were more obese. Sitagliptin did induce significant reduction in fasting and prandial blood glucose levels, HbA1c, weight and waist circumference. It is probable that this could have also happened in the study by Arnolds et al. [19] with its longer duration. The dose of insulin glargine remained stable for SIX months in our study, while it was titrated in the study by Arnolds et al. [19], which represents a difference in study design. Both studies have shown that it is justified to add sitagliptin to insulin glargine and metformin in obese patients with longer duration of diabetes, thus resolving the above mentioned clinicians' dilemmas.

Sitagliptin therapy decreases appetite and glucagon levels, even in subjects with low residual insulin secretion [20]. It improves quality of life [21]. The question of biomarkers that could adequately specify the positive effects of incretin therapy in obese subjects with diabetes remains open [22, 23]. HOMA indices, used in our study, are not standardized. In clinical studies, they have been used with caution, indicating only whether the effect of the drug is in favor of increased insulin secretion or decreased insulin resistance [24]. Although our study was done on a small group of subjects with great variations in HOMA indices, it did show that the main effect of sitagliptin therapy, added to insulin glargine and metformin in obese subjects, is to increase insulin secretion. It did not induce hypoglycemia, nor did it provoke an increase in blood pressure and lipids, proving its safety profile.

CONCLUSION

The results of our study have shown that it is justified to add sitagliptin to the treatment of obese subjects with type 2 diabetes inadequately controlled by insulin glargine and metformin. This kind of add-on therapy significantly decreased fasting and prandial glucose levels and HbA1c. Increase in residual insulin secretion, measured through c-peptide levels and HOMA-B index, and decrease in glucagon levels, may explain its favorable metabolic effects. It seems that the success of this treatment combination depends on the early initiation of insulin therapy, which spares residual insulin secretion. Another prerequisite for successful treatment is previous titration of insulin glargine to target fasting glucose levels.

Sitagliptin is safe in obese subjects with diabetes. It significantly reduced body weight and waist circumference. The subjects did not experience severe hypoglycemia. Blood pressure and lipids remained unchanged. Until GLP-1 analogues and bariatric surgery become a widely available method of treatment for obese subjects with diabetes, a safe and effective combination of sitagliptin, insulin glargine, and metformin may be considered.

doi: 10.2298/SARH1610497Z

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Утицај комбинације инсулина гларгин, метформина и ситаглиптина на инсулинску секрецију, инсулинску резистенцију и метаболичке параметре код гојазних особа са дијабетесом мелитусом типа 2

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КРАТАК САДРЖАЈ

Увод За лечење гојазних са дијабетесом типа 2 неопходна је комбинација лекова, због многоструких патогенетских механизама умешаних у настанку гојазности и дијабетеса.

Циљ рада Циљ овог рада је био испитивање ефекта додавања ситаглиптина комбинацији метформина и инсулина код гојазних особа са дијабетесом типа 2 .

Методе рада У испитивање су биле укључене 23 гојазне особе са дијабетесом мелитусом типа 2, неадекватно лечене са метформином и инсулином гларгин. Титрација инсулина гларгин током месец дана претходила је увођењу ситаглиптина у дози од 100 *mg*. Индекс телесне масе (ИТМ), обим струка (ОС), гликемије наште и два сата постпрандијално мерени су једном месечно. Липиди и гликозилирани хемоглобин (*HbA1c*) евалуирани су једном у три месеца, а *c*-пептид и глукагон на почетку и након шест месеци терапије. Израчунати су хомеостатски индекси инсулинске секреције (*HOMA B*) и резистенције (*HOMA IR*).

Резултати Испитаници су били просечне старости 58,65 ± 7,62 година, ИТМ 35,06 ± 5,15 *kg/cm²*, ОС 115,04 ± 15,47 *cm* и просечне дужине трајања *T2DM* 4,11 ± 2,57 година. Титрацијом инсулина гларгин, током месец дана, нису постигнуте циљне вредности јутарње гликемије. Укључивање 100 *mg* ситаглиптина довело је до значајног смањења ОС и ИТМ током три месеца, уз одржавање ефекта до шест месеци терапије. Вредности *HbA1c* значајно су смањене након три и шест месеци терапије. *C*-пептид се значајно повећао, док се ниво глукагона смањио. Инсулин и *HOMA* индекси су остали непромењени.

Закључак Ситаглиптин може побољшати контролу дијабетеса и довести до мањег смањења у телесној маси гојазних особа на метформину и инсулину гларгин. Титрација инсулина гларгин до оптималне јутарње гликемије је предуслов успеха ове комбинације.

Кључне речи: ситаглиптин; гларгин; гојазност; дијабетес

Примљен • Received: 19/08/2015 **Ревизија • Revision:** 10/12/2015 **Прихваћен • Accepted:**14/12/2015

doi: 10.2298/SARH1610497Z

Treatment of stump complications after above-knee amputation using negative-pressure wound therapy

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SUMMARY

Introduction The stump wound complications after above-knee amputation lead to other problems, such as prolonged rehabilitation, delayed prosthetic restoration, the increase in total treatment cost and high mortality rates.

Objective To evaluate the safety and outcomes of negative-pressure wound therapy (NPWT) using Vacuum-Assisted Closure (VAC®) therapy in patients with stump complication after above-knee amputation (AKA).

Methods From January 2011 to July 2014, AKA was performed in 137 patients at the University Cardiovascular Clinic. Nineteen (12.4%) of these patients (mean age 69.3 ± 9.2 years) were treated with NPWT. The following variables were recorded: wound healing and hospitalization time, rate of NPWT treatment failure, and mortality.

Results AKA was performed in 17 (89.5%) patients after the vascular or endovascular procedures had been exhausted, while urgent AKA was performed in two (10.5%) patients due to uncontrolled infection. The time before NPWT application was 3.1 ± 1.9 days and the duration of the NPWT use ranged from 15 to 54 days (mean 27.95 ± 12.1 days). During NPWT treatment, operative debridement was performed in 12 patients. All the patients were kept on culture-directed intravenous antibiotics. The average hospital length of stay was 34.7 days (range 21–77 days). There were four (20.9%) failures during the treatment which required secondary amputation. During the treatment, one (5.3%) patient died due to multi-organ failure after 27 days.

Conclusions The use of NPWT therapy in the treatment of AKA stump complication is a safe and effective procedure associated with low risk and positive outcome in terms of wound healing time and further complications.

Keywords: above-knee amputation; wound complication; negative-pressure wound therapy (NPWT)

INTRODUCTION

Lower extremity amputation definitely remains the therapeutic option for the management of ischemic disease that fails revascularization, extensive traumatic tissue loss, infection and ischemic disease of the leg where attempts of revascularization failed [1]. Major lower extremity amputations, especially above-knee amputation (AKA), are associated with high morbidity and mortality rates [2, 3]. Previous study reports 11% rate of stump complications [2]. Also, patients after AKA have the worst functional outcomes and survival rates of all other levels of amputation. In addition, the stump wound complications lead to other problems, such as the increase in total treatment cost, prolonged rehabilitation, delayed prosthetic restoration, and finally the reduction in the quality of life of patients [4–7].

Majority of the published papers are focused on the treatment of diabetic foot ulcers, venous leg ulcers and postoperative groin wound infection [8–11]. However, reports of the use of negative-pressure wound therapy (NPWT) after AKA and stump complications are limited to a few patients [12, 13].

To further elucidate this issue, we report of 19 patients with stump complications treated with NPWT after AKA.

OBJECTIVE

The purpose of this study was to evaluate the safety and outcomes of NPWT using Vacuum-Assisted Closure (VAC®) therapy in patients with stump complication after AKA.

METHODS

Patients involved in the study

From January 2011 to July 2014, AKA was performed on 137 patients at our University Cardiovascular Clinic. Nineteen (12.4%) of these patients with a mean age of 69.3 ± 9.2 years were included in this study based on the following criteria: (1) AK amputation; (2) wound infection or secondary skin necrosis resulting in failure to heal, further requiring additional intervention (debridement); (3) patients treated with NPWT using VAC® system. Exclusion

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criterion was massive tissue necrosis, which required early re-amputation.

Preoperative evaluation consisted of the following: clinical examination, ankle-brachial index, multi-slice computed tomography angiography (Lightspeed VCT, GE Healthcare, Milwaukee, WI, USA), and appropriate laboratory tests. The indication for AKA was made by attending vascular surgeon, only if all other options for vascular and endovascular procedures were either exhausted or contraindicated. All stump complications in the series were recorded. Initially, ceftriaxone (2 g / 24 hours) and/or amikacin (1.5 g / 24 hours) were administered to all the patients, who signed the informed consent form for use of their data for the analysis. The study was approved by our hospital's Ethical Committee. Standard descriptive statistics methods were used (number and means), median, minimum, and maximum values. Cox univariate and multivariate analyses were carried out to assess predictors of NPWT treatment failure. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Negative-pressure wound therapy

If any signs of stump (primary closed) complication appeared (infection, secretion, or skin necrosis), the wound would be opened and assessed daily for at least three days. If there was no sign of massive tissue necrosis, the indication for NPWT would be considered set. In case of an uncontrolled infection (e.g. necrotizing fasciitis or gas gangrene), guillotine amputation was performed to sustain the infection. The wound was assessed daily by the attending surgeon. In all the patients wound swabs were taken routinely before the application of the negative pressure wound device.

The NPWT system used in this study was Vacuum-Assisted Closure (VAC®, KCI Medical, San Antonio, TX, USA) and it consisted of three components: 1) a negative pressure-generating unit with a disposable canister, 2) a pad with evacuation tube, and 3) a reticulated, open cell sterile polyurethane or a dense open-pore polyvinyl alcohol foam dressing, cut to fit the open amputation stump wound. Non-occlusive wound dressings were used as protection for exposed vessels. VAC® was applied to the wound as specified by the manufacturer's guidelines [14].

Negative pressure used to treat these patients ranged from 75 mmHg to 125 mmHg. The treatment was continued until several goals were achieved: 1) the wound swabs were negative; 2) sufficient granulation tissue formation for possible secondary suture; 3) absence of necrotic tissue, and 4) absence of secretion from the wound three days after VAC® system removal.

However, the treatment was terminated if NPWT caused progressive worsening of the local ischemic process and necrosis. The polyurethane foam was changed regularly in three-day intervals. When necessary, surgical debridement was performed to healthy muscle and fat. Antibiotics were administered according to the results of antibiogram, generated from intra- or post-operatively obtained wound swab.

RESULTS

During the observation period, 19 (12.4%) of 137 patients met study criteria and were treated with NPWT. Baseline demographic characteristics of enrolled patients, indication for treatment, and type of anesthesia administered are shown in Table 1.

Table 1. Demographic characteristics of enrolled patients

Variable	n = 19	%
Median age (years)	69.3 ± 9.2	
Male sex	15	78.9
Smoking	13	68.4
HTA	14	73.7
HLP	16	84.2
DM	14	73.7
Family	8	42.1
CAD	11	57.9
Prior stroke	9	47.4
Limb side (left)	12	63.2
General anesthesia	5	26.3
Spinal anesthesia	14	73.7
Urgent case	2	10.5
Staged procedure	17	89.5

HTA – hypertension; HLP – hyperlipoproteinemia; DM – diabetes mellitus; Family – family history of atherosclerotic disease; CAD – coronary artery disease

The mean patient population age was 69.2 ± 9.2 years (range 51–82 years) and the patients were predominantly male (78.9%). Staged procedure was performed in 17 (89.5%) patients, while urgent AKA was performed in two (10.5%) patients due to uncontrolled infection. In 17 patients (staged procedure), 49 open vascular (2.9 per patient) and 24 endovascular (1.4 per patient) procedures were performed before AKA. Remaining two patients with uncontrolled infection were admitted to our (tertiary) institution; however, after our assessment and patient status, the revascularization procedures were contraindicated. The time before NPWT application was 3.1 ± 1.9 days and the duration of therapy ranged from 15 to 54 days (mean 27.95 ± 12.1 days). During the therapy, operative debridement was performed in 12 patients, after a mean of 19.2 days. All the patients were kept on culture-directed intravenous antibiotics. The swab culture results are shown in Table 2. The majority of the infections were polymicrobial and two patients had negative swab cultures.

When the conditions were met (sterile swab culture, sufficient granulation tissue formation and absence of necrotic debris), the secondary suturing was done. All the patients were followed in our vascular surgery wound clinic until the

Table 2. Culture results from wounds

Culture result	N
Polymicrobial	7
Klebsiella	2
Methicillin-resistant *Staphylococcus aureus*	2
Serratia	2
Enterococcus	2
Proteus vulgaris	1
Escherichia coli	1
Negative	2

doi: 10.2298/SARH1610503B

wounds completely healed. Our practice is to use NPWT only in the institutional setting because of the increased risk of morbidity and hemorrhaging in these patients. The average hospital length of stay was 34.7 days (range 21–77 days).

There were four (20.9%) failures during the treatment. Re-amputation was required in three of these four patients, and coxofemoral disarticulation in the remaining one, all caused by massive stump tissue necrosis. During the treatment, one patient died due to multi-organ failure 27 days after the amputation.

Univariate analysis evaluating the factors such as age, sex, risk factors for vascular disease, presence of cardiac artery diseases, previous vascular/endovascular intervention, unilateral iliac or deep femoral artery occlusion and limb side, failed to identify any variable predictive of NPWT failure.

DISCUSSION

The main result of this study is that NPWT can have a significant role in the treatment of AKA stump complications. Our study shows that the NPWT reduces morbidity, total treatment cost, the rate of secondary amputations, and the mortality rate. Treatment with NPWT has been used for over two decades. The first paper was published by Fleischman et al. [15] in 1993, reporting the treatment of soft tissue damage caused by open fracture.

In recent years, the advance in technology has extended the use of NPTW to more extensive and complicated wounds, such as diabetic foot wounds, post-surgery diabetic foot wounds, inflammatory ulcers, dehisced sternal wounds, open abdominal wounds, and traumatic wounds [16].

Additional indications for NPWT in vascular surgery are *ulcus cruris* treatment, MESH-grafting, secondary healing, and infected wounds after several vascular operations/intervention, amputations, lymphatic fistulas, soft tissue and skin infections, and infections of vascular grafts [12].

However, majority of these papers reported their results using NPWT in the treatment of diabetic foot ulcers, venous leg ulcer, and vascular groin infection [8–11].

On the other hand, reports on NPWT use in patients with stump complication are limited to a few patients [12, 13].

Unfortunately, major lower extremity amputation is still a commonly performed operation that is indicated in patients with failed attempts of revascularization, extensive traumatic tissue loss, and infection [1]. Further problem after major lower extremity amputation is a significant risk of perioperative morbidity and mortality. Aulivola et al. [2] found significantly higher 30-day mortality rate for AKA patients (16.5%) than for below-knee amputation (BKA) patients (5.7%). A consistent result of 13.3% 30-day mortality rate for AKA was shown in series published by Feinglass et al. [3]. During the study period in our series, 30-day mortality in all of the patients (137 AKA) was 5.1%, and only one (out of 19 patients) died, which occurred 27 days after the index intervention. Also, it has been shown that stump complications increase mortality risk from cardiac complications, pneumonia, renal failure, and stroke [1, 2, 3, 17, 18]. Regarding lower mortality rate than that in previous series [2, 3], we could hypothesize that the use of NPWT in these patients reduces mortality risk.

Regarding the morbidity after AKA, Aulivola et al. [2] reported an 11% rate of the stump complication, which is similar to our series. Patients requiring guillotine amputation as a consequence of sepsis are a special problem. Reported mortality rate in these patients is 14.3% (including AKA and BKA) [2]. In our study, both patients that underwent guillotine amputation had extended postoperative periods (47 and 36 days), and after secondary suture the wounds were completely healed.

We had four (20.9%) treatment failures. Re-amputation was required in three of these four patients, and coxofemoral disarticulation in the remaining one, all caused by massive stump tissue necrosis. However, even Cox univariate analysis failed to identify any variable predictive of VAC® failure. These four patients had unilateral iliac or deep femoral artery occlusion verified with preoperative multi-slice computed tomography (MSCT) angiography. We can conclude that patients like these should not be candidates for NPWT and that local findings (absence of the necrotic mass during the first several days after the amputation) can be misleading.

There are several advantages of NPWT which could be applied in the treatment of AKA wound complications. NPWT decreases the healing time of the wound, reduces the bacterial counts in the wound, possibly decreases the need for future amputations, and significantly improves the results in the subjective pain scores at the follow-up [9, 11, 17–20].

In a recently published case report by Richter and Knudson [13], NPWT was used in a AKA wound with extremely large tissue deficit. After multiple surgical procedures and three months of NPWT, the residual limb improved to a prosthetic-ready state.

In case of wound complications after AKA, general practice in our hospital is to use NPWT until the following goals are achieved: two or more negative swab cultures, presence of sufficient granulation tissue formation, absence of secretion from the wound for three days after NPWT system removal, and absence of necrotic tissue. When these criteria are met, we perform secondary suturing for several reasons: AKA wound defect is too large for second intention healing, and it reduces the length of hospitalization and hospital related complication rate. Also, we avoid the use of skin grafts in this scenario. In addition, this method allows early rehabilitation and prepares the patient to begin prosthetic restoration.

There are some limitations of this study – a relatively small number of patients included (n = 19), and the lack of control group (i.e. patients treated without NPWT or any other therapeutic option).

CONCLUSION

The use of NPWT in treatment of an AKA stump complication is safe and effective. It is also associated with low risk and good outcome. NPWT is not recommended in cases of unilateral iliac or deep femoral artery occlusion verified by preoperative MSCT angiography.

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Терапија негативним притиском у лечењу компликација ампутационог патрљка након натколене ампутације

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КРАТАК САДРЖАЈ

Увод Компликације ампутационог патрљка након натколене ампутације представљају комплексан проблем који води ка продуженом лечењу пацијената, порасту трошкова лечења и повећаној стопи смртности.

Циљ рада Циљ овог истраживања је процена безбедности и ефикасности терапије негативним притиском код пацијената са компликацијама ампутационог патрљка након натколене ампутације.

Методе рада У периоду од јануара 2011. до јула 2014. године натколена ампутација је урађена код 137 пацијената. Код 19 (12,4%) пацијената (просечне старости 69,3 ± 9,2 година) због компликација ампутационе ране примењена је терапија негативним притиском. Праћене су следеће варијабле: зарастање ране и време хоспитализације, неуспех терапије негативним притиском и смртност.

Резултати Натколена ампутација је урађена код 17 (89,5%) пацијената након што су васкуларне или ендоваскуларне могућности реваскуларизације исцрпљене, а у два (10,5%) пацијента због прогресивне и неконтролисане инфекције. Просечно време трајања терапије негативним притиском било је 27,95 ± 12,1 дана (распон 15 до 54 дана). Током терапије хируршка обрада ране урађена је код 12 пацијената. Просечно време хоспитализације је било 34,7 дана (распон 21–77 дана). Неуспех терапије је забележен код четири пацијента (20,9%), што је резултирало реампутацијом. Током третмана, код једног (5,3%) пацијента дошло је до смртног исхода након 27 дана као последица мултиорганског попуштања.

Закључак Терапија негативним притиском у лечењу компликација ране након натколене ампутације је безбедан и ефикасан терапијски избор, праћен ниским ризиком и добрим исходом у погледу зарастања ране.

Кључне речи: натколена ампутација; компликације ране; терапија негативним притиском

Примљен • Received: 08/07/2015 **Ревизија • Revision:** 11/02/2016 **Прихваћен • Accepted:**18/02/2016

doi: 10.2298/SARH1610503B

Factors that predict walking ability with a prosthesis in lower limb amputees

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SUMMARY

Introduction Identification of predictive factors for walking ability with a prosthesis, after lower limb amputation, is very important in order to define patient's potentials and realistic rehabilitation goals, however challenging they are.

Objective The objective of this study was to investigate whether variables determined at the beginning of rehabilitation process are able to predict walking ability at the end of the treatment using support vector machines (SVMs).

Methods This research was designed as a retrospective clinical case series. The outcome was defined as three-leveled ambulation ability. SVMs were used for predicting model forming.

Results The study included 263 patients, average age 60.82 ± 9.27 years. In creating SVM models, eleven variables were included: age, gender, cause of amputation, amputation level, period from amputation to prosthetic rehabilitation, Functional Comorbidity Index (FCI), presence of diabetes, presence of a partner, restriction concerning hip or knee extension, residual limb hip extensor strength, and mobility at admission. Six SVM models were created with four, five, six, eight, 10, and 11 variables, respectively. Genetic algorithm was used as an optimization procedure in order to select the best variables for predicting the level of walking ability. The accuracy of these models ranged from 72.5% to 82.5%.

Conclusion By using SVM model with four variables (age, FCI, level of amputation, and mobility at admission) we are able to predict the level of ambulation with a prosthesis in lower limb amputees with high accuracy.

Keywords: amputation; rehabilitation; recovery of function; support vector machines

INTRODUCTION

Lower limb amputation represents one of the classical rehabilitation problems amenable to intervention by a physiatrist. Because of the aging of the population and the increased incidence of diabetes, the number of amputations is expected to increase in the future [1].

One of the crucial moments in the period after lower limb amputation is the decision whether the patient will be a proper candidate for a prosthesis. This decision is not always easy, and factors that could predict rehabilitation outcome in these patients are only partially understood [2]. The physiatrist, the therapist and especially the health insurance are all interested in the costs of prosthetics and rehabilitation treatment on the one hand, while, on the other hand, patients and their families are interested in the highest possible functional outcome after major limb amputation. In Serbia, after lower limb amputation, patients are examined by a physiatrist, who is responsible for prescribing a prosthesis. This examination often takes place several months after the amputation due to medical or administrative reasons. The number of patients that will be able to walk with a prosthesis after lower limb amputation vary among authors in the range of 50–90% [3, 4, 5].

Many factors potentially influence walking ability with a prosthesis. Patients with dysvascular amputations often have diabetes mellitus, and both conditions are associated with the reduction of physical and cognitive capacities. These reductions can affect the prosthetic use [6]. The influence of comorbidities on functional outcome in patients with lower limb amputation is questionable. There is often a lack of clear connection between comorbidities and walking ability [7–11]. Lower limb amputation constitutes more than 95% of all amputation, while the most common level is transtibial [12, 13]. It is generally accepted that the functioning of these patients is worse in higher amputation level and in older age [6, 8]. Information on influence of gender on functionality, on the other hand, is relatively scarce [14]. It is generally accepted that men are more often affected by lower limb amputation [15]. Whether gender has any influence on functional outcome in these patients remains an open question. While some researchers have found lower functionality in women, others did not find significant difference [9, 14]. Residual limb hip extensor muscle strength was shown to be a strong predictor of the walking distance of these patients [7], while the presence of residual limb contracture was negatively linked

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to prosthetic ambulation in the literature [7, 9]. We must not underestimate the influence of social support on the functional outcome of lower limb amputees [8]. Patients with good social support appear to show greater mobility after amputation [16].

Identification of predictive factors for walking ability in these patients is very important in order to define a patient's potential and realistic rehabilitation goals. Most of the articles used linear or logistic regression models, which were formed and analyzed on one sample of patients, i.e. regression models were not tested on unknown patients [6, 7, 8, 10, 14]. On the other hand, some more advanced regression models could be useful in the analysis of this kind of data which are very "noisy", uncertain or incomplete.

In the last few decades, the applications of machine learning and optimization methods in the procedures of medical diagnoses and prognosis have become very common because of their efficiency in cases where only small amount of data are available or they are incomplete or noisy.

In this paper, we used a relatively new method of machine learning for making the mathematical model for predicting the level of walking ability with a prosthesis after lower limb amputation. We have proposed a prediction model based on support vector machines (SVMs) [17], where a set of input variables was optimized using global optimization algorithm named genetic algorithm (GA) [18]. SVM is a soft computing method which refers to learning from experimental data and human knowledge by transferring them into analytical models. SVM is a learning method, which overlaps with statistics in many ways, but it's not a statistical method. SVM acts as one of the best approaches of data modeling, based on the principle of structural risk minimization, avoiding local minima and handling large amounts of data very well. In recent years, SVM has found application in many wide medical applications including digital imaging [19], medical signal processing [20], and various prediction problems [21, 22].

OBJECTIVE

The objective of this study was to investigate whether variables determined at the beginning of rehabilitation process are able to predict the ability of lower limb amputees to walk with a prosthesis after treatment using SVM models.

METHODS

This study was designed as a retrospective clinical case series. It encompassed patients with lower limb amputation who underwent prosthetic rehabilitation treatment at the Medical Rehabilitation Clinic of the Clinical Centre of Vojvodina in the city of Novi Sad, in the period from 2000 to 2009. We searched the archive in the given period with the key diagnosis of "amputation." All medical records found underwent a thorough review. Inclusion criteria were as follows: unilateral transtibial or transfemoral amputation, patients who received their first prosthesis. We excluded patients who did not get prosthesis, patients who underwent prosthetic fitting and gait training earlier, patients who underwent prosthetic fitting and gait training in outpatient manner, patients with bilateral amputation (toe amputation excluded), patients who due to the complications could not finish prosthetic fitting and gait training, and patients with incomplete medical documentation.

We collected data about the level of amputation (transfemoral/transtibial), age, gender, the period from amputation to prosthetic rehabilitation, the cause of amputation (dysvascular, trauma, other), the number of comorbid conditions was assessed by Functional Comorbidity Index (FCI) [23]. Two physicians scored the presence of 18 comorbidities independently, while in case of a disagreement, each diagnosis was achieved through consensus. We noted the presence of phantom pain, the presence of hip or knee extension restriction in residual limb which was defined as a hip extension less than 0° or a knee extension less than -10° measured with classical goniometer. Residual limb hip extensors strength was graded according to manual muscle testing. Furthermore, we identified whether patients had a partner or not. We also recorded the mobility level of these patients at admission and grouped our findings into three different levels: the first consisted of those patients who were ambulatory with crutches or a walker even outdoors; the second consisted of the patients who were able to walk with crutches or a walker only indoors and needed a wheelchair for outdoors mobility, and the third were the patients who were not able to ambulate with crutches or a walker and needed a wheelchair all the time.

At the end of the rehabilitation treatment, the patients were divided into three functional levels: the patients who were unable to walk independently with a prosthesis, the patients who were able to walk independently with a prosthesis only indoors, and finally, the patients who were able to walk with a prosthesis outdoors.

The data were summarized descriptively with frequency and percentage tables, Student's t-test was used to compare age, FCI, and the time from amputation until prosthetic rehabilitation, while χ^2 test was used for comparisons of functional outcome categories and the level of amputation for females and males. $P \leq 0.05$ was considered statistically significant.

SVMs were used for creating mathematical models for predicting the level of walking ability with a prosthesis.

SVM learning algorithm (classifier) attempts to learn the input–output relation by using a training data set $X = \{[x_i, y_i]\}, i = 1, ..., m$, where the inputs x are n-dimensional vectors and the labels y are discrete. There are two phases when applying supervised learning algorithms for problem-solving. The first phase is the so-called learning phase where the learning algorithms design a mathematical model of a dependency, classifiers (in a classification i.e., pattern recognition) based on the training data given. The second phase is the test and/or application phase. In this phase, the models developed by the learning algorithms are used to predict the outputs y of the data which are unseen by the learning algorithms in the learning phase. Before an actual application, the test phase is always carried out for checking the accuracy of the models developed in the first phase.

doi: 10.2298/SARH1610507K

GA represents a robust optimization method based on elementary mechanisms of evolution. GA imitates processes of nature selection and reproduction in order to solve a certain optimization problem. The first point of such mathematical evolution is the population of *n* individuals where every individual represents a potential solution. Every individual has its measure of adjustment, i.e. in mathematical sense, the value of optimization criteria. Using operators like selection, crossover and mutation GA attempt, through iterations (generations of population), we achieved the best value of optimization criteria function.

RESULTS

We identified 373 patients with 'amputation' as the primary diagnosis, 110 of which were excluded from this study for the following reasons: 30 patients were bilateral lower limb amputees, 32 patients underwent a prosthetic fitting and training earlier, 27 patients did not receive a prosthesis, nine patients were excluded due to an incomplete medical documentation (there were no data about the rehabilitation outcome), 10 patients were excluded due to complications which prevented further rehabilitation, and two were excluded because the amputation diagnosis referred to upper extremities. A total of 263 patients were included in the study. The characteristics of these patients are given in Table 1.

There were no significant differences between men and women in terms of the average age (60.56 vs. 62.09; t = -1.01; p = 0.313), FCI scores (2.16 vs. 2.33; t = -0.149; p = 0.881), and the period from the amputation to prosthetic rehabilitation (185.57 vs. 189.36; t = -1.048; p = 0.295). Although women suffered from transfemoral

Table 1. Patients' characteristics and the prosthetic rehabilitation outcome

Characteristics		Value
Age (years)	Mean ± SD	60.82 ± 9.27
	Range	24–82
Gender	Male	218 (83%)
	Female	45 (17%)
Cause of amputation	PAD (with or without DM)	237 (90.1%)
	Trauma	16 (6.1%)
	Other	10 (3.8%)
Amputation level	Transtibial	93 (35%)
	Transfemoral	170 (65%)
Period from amputation to prosthetic fitting and training (days)	Mean	186.22
	Range	28–973
Functional comorbidity index (FCI)	Mean ± SD	2.19 ± 1.03
	Range	0–5
The most common comorbidities from FCI	PAD	236 (89.7%)
	DM	172 (65.4%)
	Myocardial infarct	37 (14.1%)
	Upper gastrointestinal disease (ulcer, hernia, reflux)	30 (11.4%)
	Visual impairment (cataract, glaucoma)	23 (8.8%)
	Stroke or TIA	22 (8.4%)
Phantom pain	Yes	52 (27.8%)
	No	135 (72.2%)
	Missing data	76
Partner	Yes	192 (75.6%)
	No	62 (24.4%)
	Missing data	9
Restriction of hip or knee extension	Yes	38 (15.5%)
	No	207 (84.5%)
	Missing data	18
Residual limb hip extensor strength according to MMT	Grade 2	74 (29.2%)
	Grade 3	167 (66.0%)
	Grade 4	12 (4.7%)
	Missing data	10
Mobility at admission	Ambulatory with crutches / a walker outdoors	132 (50.2%)
	Ambulatory with crutches / a walker indoors only, a wheelchair outdoors	56 (21.3%)
	Mobile only with a wheelchair	75 (28.5%)
Functional outcome after rehabilitation	Unable to walk	18 (6.8%)
	Walk indoors only	71 (27%)
	Walk outdoors	174 (66.2%)

Values are presented as mean value ± standard deviation and as the number of patients with percentage (%).
PAD – peripheral arterial disease; DM – diabetes mellitus; TIA – transient ischemic attack; MMT – manual muscle testing

level of amputation more often, this difference was not significant [136 (62.4%) vs. 34 (75.6%), $\chi^2 = 2.831$; p = 0.092]. On the other hand, men reached significantly higher functional level at the end of rehabilitation treatment ($\chi^2 = 6.672$; p = 0.036) (Table 2).

As expected, the walking ability of the patients with transtibial level of amputation with a prosthesis was much better than in the case of the patients with transfemoral level ($\chi^2 = 14.047$; p = 0.001) (Table 3).

In order to create optimal SVM model for predicting the level of walking ability after lower limb amputation, we optimized a set of input variables using GA. Every individual of GA population represents a "bit mask" which determines which variable will be used in the SVM model.

Table 2. Functional outcome levels at the end of the rehabilitation treatment in men and women

Gender		Unable to walk with a prosthesis	Walk with a prosthesis indoors only	Walk with a prosthesis outdoors	Total	χ^2	p
Men	N	12	55	151	218	6.672	0.036
	%	5.5	25.2	69.3	100.0		
	Σ%	66.7	77.5	86.8	82.9		
Women	N	6	16	23	45		
	%	13.3	35.6	51.1	100.0		
	Σ%	33.3	25.5	13.2	17.1		
Total	N	18	71	174	263		
	%	6.8	27.0	66.2	100.0		
	Σ%	100.0	100.0	100.0	100.0		

N – number of patients

Table 3. Level of amputation and functional outcome at the end of the rehabilitation treatment

Amputation level		Unable to walk with a prosthesis	Walk with a prosthesis indoors only	Walk with a prosthesis outdoors	Total	χ^2	p
Transtibial	N	5	13	75	93	14.047	0.001
	%	5.4	14.0	80.6	100.0		
	Σ%	27.8	18.3	43.1	35.4		
Transfemoral	N	13	58	99	170		
	%	7.6	34.1	58.2	100.0		
	Σ%	72.2	81.7	56.9	64.6		
Total	N	18	71	174	263		
	%	6.8	27.0	66.2	100.0		
	Σ%	100.0	100.0	100.0	100.0		

Table 4. Selected variables and accuracy of SVM classifiers on test data

Variables		Number of selected variables					
		4	5	6	8	10	11
Age		×			×	×	×
Gender			×	×	×	×	×
Period from amputation to prosthetic fitting and training							×
Partner			×		×	×	×
Cause of amputation				×	×	×	×
Amputation level		×		×		×	×
FCI		×	×	×	×	×	×
Diabetes mellitus					×	×	×
Residual limb hip extensor strength				×	×	×	×
Restriction of hip or knee extension			×	×	×	×	×
Mobility at admission		×	×			×	×
Accuracy	%	82.5	77.5	77.5	77.5	77.5	72.5
	Predicted/Actual	33/40	31/40	31/40	31/40	31/40	29/40

FCI – Functional Comorbidity Index

Table 5. Predicted versus actual functional outcome levels

ACTUAL	PREDICTED			
	Unable to walk	Able to walk with a prosthesis indoors only	Able to walk with a prosthesis outdoors	Total
Unable to walk	0	1	0	1
Able to walk with a prosthesis indoors only	0	9	3	12
Able to walk with a prosthesis outdoors	0	3	24	27
Total	0	13	27	40

doi: 10.2298/SARH1610507K

Optimization criteria was the accuracy of classification on set test data. In order to compare the influence of different variables and a different number of variables with the accuracy of prediction models, we created several SVM models (Table 4).

Interestingly, our first model with four variables was the most accurate one. That model included age, amputation level, FCI, and mobility at admission to the Clinic, and successfully predicted 33 of 40 functional outcome levels (Table 5). Gender, FCI, and residual limb contracture were the most frequent variables in our models, while the period from amputation to prosthetic fitting and training was present in only one model (the one which included all variables).

Our model failed to predict the functional level for a patient who was unable to walk with a prosthesis, while the accuracy for those able to walk with a prosthesis indoors and outdoors was 69.2% and 88.9%, respectively.

DISCUSSION

The prediction of walking ability after lower limb amputation is crucial in the rehabilitation process of these patients. It represents the base for the prosthesis prescription. The purpose of this study was to explore the possibilities of functional level prediction in these patients. Although it is clear that walking ability with a prosthesis depends on more than one factor, the predominant predictors are still vague.

In our analysis, we recognized two groups of significant variables. The first group consisted of those variables that were used in the SVM model which was the most accurate (with four variables), and the second consisted of variables that were selected for the majority of the models. We assumed that these variables were the most important for our prediction and we took a closer look at them.

During the 10-year period of our research, the majority of patients with lower limb amputation were men. This was similar to other researches [24, 25]. In the literature, however, data about gender influence on prosthetic rehabilitation outcome is scarce [14]. Lefebvre and Chevan [26] found transfemoral level of amputation more often in women, which could explain lower functional levels in female patients. In our study, women suffered from transfemoral level of amputation more often, although this difference did not reach any significance. On the other hand, men had a significantly higher walking ability with a prosthesis. It is well known that women reach lower walking speed and lower step length in comparison to men [27, 28, 29]; therefore, we can presume that the difference in gender will be more profound after lower limb amputation. Our models included gender in five of six models, which indicates that gender could be considered as a predictor for ambulation with a prosthesis.

People who lived with their partners showed increased social support, which could improve the outcome after lower limb amputation [16]. The presence of a partner was an important variable in our study as well. It was present in four SVM models.

Age was an important predictor for walking ability after lower limb amputation in our study, since it was selected in the first model with the highest accuracy. This is consistent with other researchers [7, 30].

We found that transfemoral level of amputation was the most common level in our study, which was inconsistent with other researchers, who argued that the transtibial level of amputation was the most frequent one [24, 31]. The reason for this distinction probably lies in the fact that patients reach vascular surgeons too late in our country; in other words, their primary disease reaches the advanced stage and the level of amputation has to be higher. It is of a great importance to preserve knee joint, if possible, keeping in mind functional outcome of these patients [32]. Our study agrees with these findings, highlighting that the level of amputation was an important factor in the prediction, given it was part of the most accurate model.

Walking with a prosthesis requires higher energy consumption than walking with both intact lower extremities [33]. Comorbidities such as peripheral artery disease, myocardial infarcts, angina pectoris, heart failure or diabetes reduce cardiovascular capacity in these patients, and also the walking ability with a prosthesis. In our study, each of the six models included FCI, which was interpreted as having a great significance for this variable, although we were not sure whether it was independent of the other variables or not. It would be possible that FCI emerging among other variables consisted of unbalanced data if it was collected with the lowest degree of error. However, De Laat et al. [34] in their study found that FCI was connected to the independence of lower limb amputees in climbing stairs. FCI was not used too often in the literature, probably because it is a relatively new index. Its orientation to function is crucial, and we are expecting a larger number of studies using this index in the future.

Some authors argue that the presence of contractures could be a negative prognostic factor for successful prosthetic ambulation [9]. Our findings were similar, and the presence of the residual limb restriction of hip or knee extension was included in five of six models. Raya et al. [7] found that residual limb hip extensors strength is a very important predictor of walking with a prosthesis, which is in line with our findings.

The functional mobility after lower limb amputation is an important predictor of rehabilitation outcomes [6]. We divided mobility at admission in three groups according to the patients' ability to use crutches. This variable was included in four of six models, including the most accurate one.

Our models predicted walking ability after prosthetic rehabilitation with high accuracy. It should be emphasized that these results were achieved on samples unknown to our models. Every model was tested on an unknown data set – in other words, we randomly chose 40 patients whose data was excluded from the training set when the models were formed. Then we tested given SVM models on 40 previously unseen patients' data. As far as we know, this was the first study where SVMs were used in order to predict functional outcome after lower limb amputation.

In addition, quality of data could be analyzed in a mathematically more formal way. This means that the best

prediction results were obtained with only four input arguments (82.5%), while the accuracy of prediction decreases with more arguments in input vector (Table 4). In our case, a relatively small number of input arguments (maximum 11) could not lead to curse of dimensionality problem, i.e. the model complexity. We assume that the quality of the collected data directly leads to unbalanced data sets, which presents a challenge when training a classifier and SVMs. Therefore, we propose GA optimization approach to select features (input parameters), which gets the highest classification accuracy.

Despite meeting our goals, this study had limitations which are characteristic of a retrospective analysis. The quality of data relied on the reliability of medical documentation. Therefore, certain limitations in our study warrant further consideration. Firstly, manual muscle testing and measurement of joint range of motion was performed by many therapists (more than 10), which could put the reliability of these measurements in question. Secondly, similarly to all retrospective studies, we also had a problem with the missing data and we did not include the factors such as phantom pain in further analysis due to the fact that a substantial number of patients' histories were missing this information. Similarly, we could not identify enough data about cognitive and social factors which could be important predictors of the functional outcome in these patients. Despite all aforementioned facts, our models predict functional outcome with high accuracy.

CONCLUSION

By using an SVM model with four variables (age, FCI, level of amputation, and mobility at admission) we can predict the level of ambulation with a prosthesis in lower limb amputees with high accuracy.

For future researches we suggest using new variables, such as cognitive, social variables, and phantom pain in order to estimate walking ability with a prosthesis more precisely.

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Фактори који утичу на способност ходања уз помоћ протезе код болесника после ампутације ноге

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КРАТАК САДРЖАЈ

Увод Идентификовање фактора који могу да предвиде способност за ход уз помоћ протезе након ампутације доњих екстремитета веома је важно како би се дефинисали потенцијали пацијената и поставили реални рехабилитациони циљеви.

Циљ рада Циљ ове студије био је да се испита, уз помоћ алгоритама базираних на потпорним векторима (*SVM*), да ли фактори утврђени на почетку рехабилитационог процеса могу да предвиде исход рехабилитације.

Методе рада Ово истраживање дизајнирано је као ретроспективна клиничка серија случајева током које су анализиране историје болести пацијената. Исход је дефинисан као тростепена способност хода уз помоћ протезе. У формирању модела за предвиђање коришћени су *SVM*.

Резултати У студију је укључено 263 пацијента просечне старости 60,82 ± 9,27 година. У формирање *SVM* модела укључено је једанаест варијабли: старост, пол, узрок ампутације, ниво ампутације, период од ампутације до протетичке рехабилитације, функционални коморбидитетни индекс (*FCI*), присуство шећерне болести, присуство партнера, ограничена екстензија кука или колена резидуалног екстремитета, мишићна снага екстензора резидуалног екстремитета, мобилност при пријему. Формирано је шест модела са 4, 5, 6, 8, 10 и 11 варијабли. Генетски алгоритми (ГА) коришћени су да би се одабрале најбоље варијабле за предвиђање нивоа оспособљености за ход са протезом. Прецизност модела кретала се од 72,5% до 82,5%.

Закључак Користећи *SVM* модел са четири варијабле (старост, *FCI*, ниво ампутације и способност кретања при пријему), можемо у високом проценту предвидети ниво способности за ход уз помоћ протезе особа са ампутацијом доњих екстремитета.

Кључне речи: ампутација; рехабилитација; опоравак функције; потпорни вектори

Примљен • Received: 21/08/2015 **Ревизија • Revision:** 18/01/2016 **Прихваћен • Accepted:** 20/01/2016

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):514-520
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1610514V
UDC: 616.35-072.1; 616.3-089.5

Manual versus target-controlled infusion of balanced propofol during diagnostic colonoscopy – A prospective randomized controlled trial

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SUMMARY

Introduction There is an increasing interest in balanced propofol sedation (BPS) for colonoscopy in outpatient settings. Propofol is a potent anesthetic agent for this purpose and has a narrow therapeutic range, which increases a risk of cardiovascular and respiratory complications in case of improper administration.

Objective The aim of this study was to compare patients' safety and comfort of endoscopists in two methods of BPS targeting deep sedation – propofol target-controlled infusion (TCI) and manual intravenous titration technique (MT) – during colonoscopy.

Methods This prospective randomized controlled trial included 90 patients (class I or II of the American Society of Anesthesiologists) deeply sedated with propofol, coadministered with small doses of midazolam and fentanyl. Propofol was given by MT technique (45 patients) or by TCI (45 patients). The following adverse effects were recorded: hypotension, hypertension, bradycardia, tachycardia, hypoxemia, bradypnea, apnea, hiccupping, and coughing, as well as endoscopist's comfort during colonoscopy by means of a questionnaire.

Results The MT group compared to the TCI group had a lower mean arterial pressure in the 10th minute after the beginning ($p = 0.017$), and at the end of colonoscopy ($p = 0.006$), higher oxygen saturation in the fifth minute ($p = 0.033$), and in the 15th minute ($p = 0.008$) after the beginning of colonoscopy, and lower heart rate at the beginning of the procedure ($p = 0.001$). There were no statistically significant differences in adverse events. Endoscopist's comfort during colonoscopy was high 95.6% in the TCI group vs. 88.9% in the MT group ($p = 0.069$).

Conclusion MT is clinically as stable as TCI of propofol for deep sedation during colonoscopy, and endoscopists experienced the same comfort during colonoscopy in both groups. Thus, both combinations are suitable for deep sedation during diagnostic colonoscopy.

Keywords: balanced propofol sedation; deep sedation; colonoscopy; endoscopist's comfort; target-controlled infusion

INTRODUCTION

Colonoscopy is an important invasive procedure, but an uncomfortable, painful, and unpleasant experience for a lot of patients [1]. For colonoscopy in outpatient settings, deep sedation in spontaneously breathing patients is increasing in popularity, despite a relatively high incidence of cardiovascular and respiratory suppression [2].

Propofol is increasingly used for sedation during colonoscopy due to its pharmacological properties – rapid onset of action and short recovery profile, moderate antiemetic, analgesic, and amnestic effect [3, 4, 5]. However, propofol induces respiratory and cardiac depression which is dose-dependent and may put patients at risk. Therefore, there is an increasing interest in balanced propofol sedation (BPS) – with addition of benzodiazepines and/or opioids in small doses, the dose of propofol may be reduced [6, 7].

Propofol has narrow therapeutic range and the current standard administration technique in colonoscopy is intermittent administration of propofol bolus [8], according to desired sedation depth [4]. Among various methods which are now available for administration of propofol, target-controlled infusion (TCI) is one of the most sophisticated ones [9]. The basic principle of TCI is that anesthesiologist sets and then adjusts target concentrations of propofol. The infusion rate is adjusted automatically according to a validated pharmacokinetic model (Marsh's or Schnider's) [10, 11], and TCI maintains present concentration of propofol in the plasma (Cp) or the brain (Ce).

OBJECTIVE

The aim of this clinical study was to compare patients' safety and endoscopists' comfort in two methods of BPS targeting deep sedation – propofol TCI with manual intravenous (i.v.) titration technique (MT) during colonoscopy.

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METHODS

We conducted a prospective randomized controlled clinical trial which included 90 patients, comparing patients' safety and endoscopists' comfort of the two different administration techniques of propofol to patients receiving concomitantly small doses of midazolam and fentanyl. The patients were one-to-one randomized into two groups – MT and TCI group – using a random-numbers table. The study took place at the Endoscopy Department of the Clinic for Gastroenterology and Hepatology, Clinical Centre of Serbia, Belgrade, during a period of six months (from April through October, 2013). Seven expert endoscopists with similar clinical experience performed colonoscopies in the trial. Propofol administration and dose adjustments were carried out by one anesthesiologist, with the help of trained nurses. The study was approved by Institutional Ethics Committee of the Clinical Centre of Serbia (No. 4183/01.08.2012.) and written informed consent was obtained from all the patients.

We included 90 patients, who signed the informed consent, of both sexes, 18–65 years old (body weight from 50 kg to 120 kg), classified into group I or II according to the American Society of Anesthesiologist (ASA), recruited from the practices and scheduled for diagnostic outpatient colonoscopy with deep sedation. Indications for colonoscopy were as follows: screening for colorectal cancer, diarrhea, constipation, and bleeding. The following patients were excluded from the study: those allergic to the study drugs, patients with previous problems with anesthesia or sedation, patients with history of stridor, snoring or sleep apnea, patients with neck abnormalities, and those classified into groups III or IV of Mallampati classification [12], patient with neuropsychiatric, cardiac, respiratory, renal disorders, those in pregnant state, and with history of large-bowel surgery. If, for any reason, the endoscopist could not complete the procedure, the patient was excluded from the final analysis.

All the patients underwent an overnight fast and bowel cleansing by drinking 4 l of polyethylene glycol electrolyte solution (Fortrans, Beaufour Ipsen Industrie, Dreux, France). In the endoscopy room, intravenous access was obtained and each patient received 8 ml/kg/h of isotonic saline solution in the form of infusion. Oxygen was supplemented with a mask (6 l/min.). Pre-induction medication for all the patients was as follows: midazolam (Dormicum, Roche Pharma, Reinach, Switzerland, 5 mg/5 ml) in a bolus of 2 mg for the patients up to 70 kg, and 3 mg for those over 70 kg, and fentanyl (Fentanyl, Janssen-Cilag, Baar, Switzerland, 0.05 mg/ml) in a bolus of 1 ml for the patients between 50 kg and 60 kg, 1.5 ml for the patients between 60 kg and 80 kg, and 2 ml for those over 80 kg. Both drugs were administrated slowly (>60 seconds), two minutes before propofol. The patients in the MT group (n = 45) received propofol intravenously (Diprivan, Astra-Zeneca, Stocholm, Sweden, 10 mg/ml), in a bolus of 0.5 mg/kg, and then 10–20 mg were titrated every one to two minutes. The TCI group (n = 45) received propofol with TCI pump (Alaris PK, Cardinal Health, Dublin, OH, USA), according to the Schnider's pharmacokinetic model, with the initial Ce of 2.5 μg/ml. This concentration was increased or decreased by 0.5–1 μg/ml until the deep level of sedation was achieved. After the deep sedation level had been achieved, colonoscopy was performed. The Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale was used to document the patients' responsiveness scores [13]. The patients who lost response to verbal commands and eyelash reflex (MOAA/S = 2) were considered to be in deep sedation.

Administration of propofol was stopped at the end of the colonoscopy by reaching the base of cecum and seeing the landmark of ileocecal valve.

The patients were monitored at five-minute intervals; heart rate, systolic and diastolic arterial pressure, then mean arterial blood pressure were measured automatically using patient monitor (Mec-1000, Mindray Medical International Limited, Shenzhen, China), as well as blood oxygen saturation (SpO_2) using pulse oximeter (Oxipac, Draeger, Lübeck, Germany); respiration rate per minute was recorded by visual inspection and palpation. Endoscopist used Olympus (Olympus Corporation, Tokyo, Japan) and Pentax (Pentax Corporation, Tokyo, Japan) video-endoscopes. The following observed adverse events in both groups (MT and TCI) were recorded and compared [14]: hypotension [mean arterial pressure (MAP) < 60 mmHg], hypertension (MAP > 105 mmHg), bradycardia [heart rate (HR) < 45 beats/min.], tachycardia (HR > 115 beats/min.), hypoxemia (blood oxygen desaturation, SpO_2 < 92% for longer than 30 seconds), bradypnea (number of respirations < 6/minute), apnea (cessation of breathing) and other adverse events related to colonoscopy sedation (coughing, hiccupping) [15]. Each use of the following maneuvers was also registered: lifting the chin, increase in oxygen flow, placement of an oropharyngeal airway, assisted ventilation with bag-mask, and endotracheal intubation.

After colonoscopies we asked the endoscopists to assess the degree of difficulty of colonoscopy with an 11-point (0–10) rating scale, with 0 being "very easy" and 10 "extremely difficult". The endoscopists also assessed the patients' sedation using verbal scale for the quality of sedation (excellent, good, fair, poor) [16], and patients' comfort based on the observation of defensive reactions during colonoscopy [17]. The movement was rated as none (no movement), mild (face grimacing or small movement), moderate (movement without the need to discontinue the procedure) or severe (movement requiring discontinuation of the procedure). We also asked the endoscopists to assess the overall satisfaction with procedure using a five-point verbal scale for comfort (excellent, very good, good, fair, poor) [18].

Statistical analysis

The calculation of sample size was made for two independent samples, with equal number of patients in each group (1:1). Probability of type 1 statistical error (alpha) was set to 0.05, and power of the study to 80%. The effect size was calculated from the observed difference in time to opening of the eyes after target-controlled versus manually-

controlled infusion of propofol in the study of Passot et al. [19] (4.6 ± 2.0 minutes vs. 6.8 ± 2.5 minutes). The sample size was calculated by G*Power software version 3.0.10 (Heinrich Heine University Düsseldorf, Düsseldorf, Germany) [20], and it turned out to be 45 patients per group.

Numeric variables were described by central tendency measures (the mean) and by measures of statistical dispersion (the standard deviation). Categorical variables were described by percentages. The Student's t-test was used for comparison of the study groups after confirming normal distribution of data within the groups. Pearson's χ^2 test was used for testing differences in categorical variables among the study groups. The differences were considered significant if probability of null hypothesis was less than 0.05. All calculations were performed by IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Ninety patients in total were enrolled in the study, and 45 of them were allocated to each of the two groups. Baseline characteristics of the patients were similar in both groups, and are shown in Table 1. The duration of colonoscopy in the MT group was 10.33 ± 5.16 minutes, and in the TCI group 9.66 ± 5.18 minutes (p = 0.543). Total dose of propofol in the MT and TCI groups were 155.77 ± 52.63 mg and 148.97 ± 58.46 mg, respectively (p = 0.563). Changes in MAP, HR, respiration rate, oxygen saturation and levels of sedation during colonoscopy are presented in Table 2. The MAP values before sedation and during colonoscopy were similar in both groups. However, in the 10th minute during colonoscopy, the MAP in the MT group was significantly lower in comparison to the TCI group (86.50 ± 9.04 vs. 92.39 ± 6.37 mmHg, p = 0.017), and the same was observed at the end of the colonoscopy (86.55 ± 9.28 vs. 91.50 ± 7.05 mmHg, p = 0.006). HR at the beginning of colonoscopy was significantly higher in the MT group in comparison to the TCI group (79.55 ± 11.17 vs. 71.80 ± 10.61 beats/minute, p = 0.001), but during the procedure the difference in HR between the groups disappeared. In both groups oxygen saturation during the procedure was in the range from 97% in the MT to 100% in the TCI group. The saturation was significantly lower in the MT group, compared with the TCI group, in the fifth (98.84 ± 1.67 vs. 99.48 ± 0.82%, p = 0.033) and the 15th minute (97.38 ± 2.26 vs. 99.60 ± 0.51%, p = 0.008) of the procedure. In regard to the respiration rate, it was lower in the TCI group in the fifth minute (12.26 ± 2.75 vs. 15.42 ± 4.0 min^{-1}, p = 0.000) and at the end of the procedure (13.28 ± 2.17 vs. 15.1 ± 3.0 min^{-1}, p = 0.001).

Table 1. Demographic characteristic of the patients

Demographic characteristics	MT group (n = 45)	TCI group (n = 45)	p
Sex (male/female), n	15/30	19/26	0.384[b]
Age (years), mean ± SD	49.02 ± 11.91	50.35 ± 11.21	0.586[a]
Weight (kg), mean ± SD	75.06 ± 14.54	73.60 ± 13.51	0.621[a]
Height (cm), mean ± SD	173.31 ± 9.12	173.02 ± 8.76	0.879[a]
BMI (kg/m^2), mean ± SD	24.88 ± 3.90	24.47 ± 3,24	0.586[a]
Alcohol consumption (yes/no), n	0/45	3/42	0.078[b]
Smoking (yes/no), n	18/27	21/24	0.523[b]
ASA (I/II), n	29/16	25/20	0.389[b]

MT – manual technique; TCI – target-controlled infusion; BMI – body mass index; ASA – American Society of Anesthesiologists; n – number in group; SD – standard deviation; [a]Student's t-test; [b]χ^2 test; statistical significance p < 0.05

Table 2. Monitored parameters before and during colonoscopy

Measuring points		Immediately before sedation (basic value)	Start of colonoscopy	During colonoscopy after 5 min.	During colonoscopy after 10 min.	During colonoscopy after 15 min.	During colonoscopy after 20 min.	The end of colonoscopy
MAP (mmHg)	MT group (n = 45)	110.16 ± 13.61	87.00 ± 9.31	87.55 ± 9.73	86.50 ± 9.04	78.12 ± 11.63	82.50 ± 8.66	86.55 ± 9.28
	TCI group (n = 45)	105.12 ± 10.93	88.77 ± 7.69	88.26 ± 8.17	92.39 ± 6.37	86.00 ± 4.59	85.00 ± 5.00	91.50 ± 7.05
	p	0.066[a]	0.327[a]	0.719[a]	0.048[a]	0.066[a]	0.677[a]	0.001[a]
HR (beats/ min.)	MT group (n = 45)	87.17 ± 17.15	79.55 ± 11.17	71.66 ± 10.83	70.30 ± 7.34	81.50 ± 8.43	69.00 ± 6.92	73.15 ± 11.03
	TCI group (n = 45)	82.28 ± 15.04	71.80 ± 10.61	68.30 ± 10.00	69.33 ± 8.15	74.18 ± 8.86	75.00 ± 0.00	68.77 ± 10.92
	p	0.154[a]	0.000[a]	0.146[a]	0.685[a]	0.088[a]	0.203[a]	0.062[a]
Oxygen saturation (%)	MT group (n = 45)	98.95 ± 1.41	99.22 ± 1.37	98.84 ± 1.67	98.70 ± 1.80	97.38 ± 2.26	98.50 ± 0.70	98.93 ± 1.83
	TCI group (n = 45)	98.93 ± 1.75	99.51 ± 0.69	99.48 ± 0.82	99.47 ± 0.66	99.60 ± 0.516	100.00 ± 0.00	99.35 ± 0.77
	p	0.947[a]	0.213[a]	0.038[a]	0.062[a]	0.01[a]	0.095[a]	0.159[a]
Respiration rate per minute	MT group (n = 45)	17.06 ± 2.91	14.00 ± 3.81	15.42 ± 4.05	14.80 ± 3.07	14.50 ± 2.77	15.00 ± 3.46	15.11 ± 3.03
	TCI group (n = 45)	15.95 ± 3.05	12.66 ± 2.79	12.26 ± 2.75	12.95 ± 3.24	15.20 ± 3.42	14.00 ± 3.46	13.28 ± 2.17
	p	0.081[a]	0.062[a]	0.000[a]	0.064[a]	0.647[a]	0.721[a]	0.000[a]
MOAA/S score	MT group (n = 45)	5*	2.24 ± 0.43	2.15 ± 0.36	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	/
	TCI group (n = 45)	5*	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	/
	p	/	0.000[a]	0.056[a]	/	/	/	

MT – manual technique; TCI – target-controlled infusion; MAP – mean arterial pressure; HR – heart rate; SD – standard deviation; MOAA/S – Modified Observer's Assessment of Alertness/Sedation, scale being as follows: 6 = agitated; 5 = responds to name in normal tone; 4 = lethargic response to name in normal tone; 3 = responds to name called loudly; 2 = responds to mild prodding/shaking; 1 = does not respond to mild prodding/shaking; and 0 = does not respond to deep-stimulus "sternal rub"
* immediately before sedation (base value) = 5; measured values refer to the measured points from the start until the end of the colonoscopy
[a] Student's t-test;
Values are mean ± SD; statistical significance p < 0.05.

Table 3. Endoscopist's comfort and judgment of patient's comfort

Questions		MT group (n = 45)	TCI group (n = 45)	p
How would you rate difficulty of colonoscopy (mean ± SD), rating scale (0–10)		5.20 ± 1.75	5.5 ± 2.37	0.422a
What was patient's sedation for colonoscopy like, n (%)	Excellent	41 (91.1)	43 (95.6)	0.536b
	Good	3 (6.7)	2 (4.4)	
	Fair	1 (2.2)	/	
	Poor	/	/	
What was patient's comfort during colonoscopy like, n (%)	No movements	40 (88.9)	43 (95.6)	0.375b
	Mild movements	2 (4.4)	2 (4.4)	
	Moderate movements	1 (2.2)	/	
	Severe movements	2 (4.4)	/	
What was your comfort during colonoscopy like, n (%)	Excellent	40 (88.9)	43 (95.6)	0.069b
	Very good	/	2 (4.4)	
	Good	3 (6.7)	/	
	Fair	2 (4.4)	/	
	Poor	/	/	

MT – manual technique; TCI – target-controlled infusion; SD – standard deviation;
[a]Student's t-test; [b]χ^2 test; statistical significance $p < 0.05$

Table 4. Procedure-related time and sedative doses

Procedure/sedation times	MT group (n = 45) (mean ± SD)	TCI group (n = 45) (mean ± SD)	p
Induction time (min.)	3.31 ± 0.55	3.28 ± 0.62	0.859[a]
Time of deeper sedation (min.)	11.86 ± 5.21	10.93 ± 5.25	0.400[a]
Total sedation time (min.)	19.55 ± 5.25	18.4 ± 5.67	0.319[a]
Early eye-opening time, (min.)	5.91 ± 1.12	5.44 ± 1.40	0.086[a]
Duration of colonoscopy (min.)	10.33 ± 5.16	9.66 ± 5.18	0.543[a]
Propofol (mg)	155.77 ± 52.63	148.97 ± 58.46	0.563[a]
Midazolam (mg)	2.60 ± 0.49	2.53 ± 0.50	0.529[a]
Fentanyl (mL)	1.59 ± 0.36	1.52 ± 0.40	0.415[a]

MT – manual technique; TCI – target-controlled infusion; SD – standard deviation;
[a]Student's t-test; statistical significance $p < 0.05$

Values of MAP, HR, SpO_2 and respiration rate per minute in both groups were significantly above the minimum acceptable values of 60 mmHg, 45 beats/minute 92% and six breaths per minute, respectively. Great majority of the patients from both groups reached the desired level of sedation (MOAA/S score = 2), from the start until the end of colonoscopy, but the patients in the TCI group in the fifth minute during the colonoscopy had deeper sedation level than the patients in the MT group (2.00 ± 0.00 vs. 2.24 ± 0.43, p = 0.001). Deep sedation level was achieved in both groups without risk of oxygen desaturation. None of the patients required increase in oxygen flow, placement of an oropharyngeal airway, the assisted ventilation with bag-mask, or endotracheal intubation. However, the chin had to be lifted in one patient from the MT group.

Occurrence rate of adverse events related to sedation were similar in both groups, with only one hiccupping in the TCI group and two in the MT group (p = 0.554) and without coughing in both groups.

Endoscopists' comfort during colonoscopy and their judgment of patients' comfort is shown in Table 3. All endoscopists completed the questionnaire. There were no statistical differences between the groups. Difficulty rate of colonoscopy was 5.2 ± 1.7 in the MT group vs. 5.5 ± 2.37 in the TCI group (p = 0.422). Endoscopists registered five patients (11%) with movements in the MT group, whereas in TCI there were two patients with movements (p = 0.375). The overall satisfaction with patients' sedation was high, 91.1% described it as „excellent" in the MT group vs. 95.6% who described it as „excellent" in the TCI group (p = 0.536). Also, endoscopists' assessment of their own comfort was high, 95.6% in the TCI group vs. 88.9% in the MT group (p = 0.069).

Mean values of propofol, midazolam, fentanyl doses, administered to the study groups, and the parameters of sedation and procedural time are shown in Table 4. The observed differences were not significant. In the TCI group, Ce_{min} = 1 μg/ml, Ce_{max} = 4.5 μg/ml, and mod = 2.3 (TCI range of propofol: 1 μg/ml – 4.5 μg/ml, mod = 2.3)

DISCUSSION

As far as we know, this is the first randomized, prospective study which compared administration of propofol by titration with TCI following intravenous administration of midazolam and fentanyl for achieving deep sedation in diagnostic colonoscopy. However, despite numerous publications describing propofol use in colonoscopy, there are limited data studying the incidence of cardiorespiratory complications of BPS targeted to deep sedation. Our study shows that although several cardiopulmonary parameters were more stable numerically in the TCI group, the MT group also maintained the values sufficiently above the lower limit indicated. No patients in both groups required additional oxygen, oropharyngeal airway, endotracheal intubation, or assisted ventilation. Seven endoscopists participated in the study, and their comfort was the same in both groups.

Our results of endoscopists' comfort are similar to those of Chiang et al. [21]: they compared the propofol administration by TCI using Schnider's model with manually

controlled propofol infusion, with both groups premedicated with alfentanil, for the same-day bidirectional endoscopy in deep sedation. They reported high endoscopists' satisfaction score in both groups, but nurse anesthetists had to additionally assist the upper airways throughout the procedure for the group of patients with manually controlled propofol infusion. This result is in correlation with ours: the chin had to be lifted in one patient, and four patients' movements were registered in the MT group. Unlike our research, in this study the TCI with propofol gave less haemodynamic and respiratory adverse events than manually controlled propofol infusion.

The question whether sedation technique influences endoscopist's comfort was investigated by Mazanikov et al. [22] Anesthesiologist-managed propofol sedation with constant propofol infusion in deep sedation had no impact on the degree of gastroenterologist's satisfaction when compared with patient-controlled moderate sedation with propofol/remifentanil in endoscopic retrograde cholangiopancreatography [22]. Also, the study of Stonel et al. [23] showed that endoscopists had similar satisfaction during colonoscopy, compared with patient-controlled sedation using TCI of propofol with anesthesiologist-administered propofol bolus technique targeting moderate sedation.

Advantage of TCI over manual regimen was shown in the study of Chan et al. [17], in which they compared TCI with propofol using Marsh's pharmacokinetic model with intermittent bolus of sedative cocktail (midazolam + alfentanil + small doses of propofol) during deep sedation for colonoscopy; the TCI group produced less hypoxia, hypotension and bradycardia than with intermittent bolus of sedative cocktail. The advantages of the TCI were also shown in the study of Absalom et al. [24] with closed-loop control of moderate sedation for colonoscopy by using bispectral index. None of the patients became apneic, required airway support or became hemodinamically unstable while sedated. On the other hand, Eberl et al. [25] reported less hemodynamic stability with combination of alfentanil and TCI of propofol than when alfentanil alone or combination of midazolam and fentanyl were used for achieving moderate sedation during colonoscopy. The propofol group experienced more hypotensive events, but less oxygen desaturation episodes, similar as with the „opioid-only" group.

Both propofol alone and propofol in combination with opiates and/or benzodiazepines are frequently used during colonoscopy to achieve moderate levels of sedation [3]. Lee et al. [7] found no significant differences between the balanced propofol sedation and conventional groups (midazolam and meperidine) with regard to the rates of cardiopulmonary complications, but BPS provided significantly higher level of endoscopist's satisfaction. Increasing interest in BPS titrated to deep sedation is shown in the study of Ho et al. [26]: comparing alfentanil and fentanyl in BPS during gastroscopy and colonoscopy they reported the same safety profile.

In our hospital, patients and endoscopists favor a high-quality sedative and pain-free colonoscopy. Therefore, the ASA level of deep sedation (MOAA/S = 2) was set in our research [13, 27]. In a study of 17,999 endoscopic procedures performed over 8 years, the authors concluded that deep sedation during endoscopic procedures is safe and adverse events occurred in a small proportion of patients (4.5%) [28]. In the present study, deep sedation level was achieved in both groups without cardiorespiratory risk. Also, deep sedation showed excellent endoscopists' comfort and low incidence of adverse events such as hiccup and cough [29, 30]. El Chafic et al. [15] reported that the rate of coughing was very low – in 757 patients deeply sedated for endoscopy only 13% had at least one cough and 3% a prolonged cough. Although the patients from both groups were without coughing in our study, hiccupping was more frequent in the MT group (6.7% vs. 3.3%) which did not affect excellent comfort of the endoscopists.

The limitations of our study include the patients' selection bias as they were all of ASA I and II status and below 65 years of age. It should be noted that colonoscopy examinations in Serbia are not routinely done in sedation. They are indicated by gastroenterologist for the patients who are afraid of examination, or have a low pain tolerance, or previous unsuccessful examinations. It is also possible that we missed episodes of hypotension larger than the recorded ones, but the shorter intervals of pressure measuring during the procedure, which approximately lasted 15 to 20 minutes, as well as invasive precise blood pressure monitoring, were inconvenient for the patients. In this study, seven specialists were performing all colonoscopies. Ideally, future studies should be done with a single specialist to insure consistency of the procedure.

CONCLUSION

Both techniques of administration of balanced propofol, MT and TCI, provide the same safety and endoscopist's comfort in deep sedation, thus both combinations are suitable during diagnostic colonoscopy. TCI might have some advantages, since several cardiopulmonary indicators were more stable numerically. For a strategy to be introduced to daily practices, a method has to be not only clinically effective but also cost-effective. From that point of view further observation is warranted regarding cost-effectiveness of TCI in comparison to MT.

ACKNOWLEDGEMENT

Trial registration: the study was approved by Institutional Ethics Committee of the Clinical Centre of Serbia (No. 4183/01.08.2012) and in ISRCTN registry with study ID ISRCTN51098340.

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Поређење болусне и циљаном концентрацијом вођене примене пропофола током дијагностичке колоноскопије – проспективна рандомизована контролисана студија

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КРАТАК САДРЖАЈ

Увод Све је веће интересовање за примену балансиране седације пропофолом (*BPS*) током колоноскопије у амбулантним условима. Пропофол је потентан анестетик са малом терапијском ширином, а неправилним интравенским давањем повећава се ризик од кардиоваскуларних и респираторних компликација.

Циљ рада Циљ ове студије је био да се пореди безбедност пацијента и комфор ендоскописте применом две методе *BPS* у дубокој седацији: циљано контролисаном инфузијом (*TCI*) и давањем пропофола у болусима у одређеним временским интервалима (*MT*) током колоноскопије.

Методе рада Ово је проспективна рандомизована контролисана студија са 90 пацијената који су испунили услове за укључивање у студију (класификације I или II Америчког удружења анестезиолога), дубоко седираних пропофолом уз претходно интравенско давање малих доза мидазолама и фентанила. Пропофол је даван *MT* (45 пацијената) или *TCI* (45 пацијената) техником. Бележена су следећа нежељена дејства: хипотензија, хипертензија, брадикардија, тахикардија, хипоксемија, брадипнеа, апнеа, штуцање и кашаљ, а кроз упитник је испитан комфор ендоскописте током колоноскопије.

Резултати *MT* група је у поређењу са *TCI* имала нижи средњи артеријски притисак у десетом минуту после почетка ($p = 0,017$) и на крају колоноскопије ($p = 0,006$), виши степен сатурације у петом минуту ($p = 0,033$) и у петнаестом минуту ($p = 0,008$) после почетка колоноскопије, као и спорији пулс на почетку процедуре ($p = 0,001$). Није било статистички значајне разлике у испољавању нежељених догађаја током примене пропофола на ова два начина. Комфор ендоскописта током колоноскопије је био висок, 95,6% у *TCI* према 88,9% у *MT* групи ($p = 0,069$).

Закључак *MT* је клинички безбедна као и *TCI* пропофола, а комфор ендоскописта током колоноскопије је исти у обе групе, тако да су обе технике адекватне за дубоку седацију током дијагностичке колоноскопије.

Кључне речи: балансирана седација пропофолом; дубока седација; колоноскопија; комфор ендоскописте

Примљен • Received: 12/11/2015 **Ревизија • Revision:** 29/03/2016 **Прихваћен • Accepted:** 01/04/2016

Streptococcus pneumoniae serotype distribution in Vojvodina before the introduction of pneumococcal conjugate vaccines into the National Immunization Program

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SUMMARY

Introduction *Streptococcus pneumoniae* is the most common causative agent of bacterial pneumonia and meningitis. Mandatory childhood immunization against pneumococcal diseases is introduced in the new Law on Protection of Population against Communicable Diseases in Serbia.

Objective The objective of this study was to determine the prevalence of pneumococcal serotype distribution in Vojvodina region before routine use of pneumococcal conjugate vaccine in Serbia.

Methods A total of 105 isolates of *Streptococcus pneumoniae* were collected in the period from January 2009 to April 2016. Based on the results of serotyping in the National Reference Laboratory, we analyzed distribution of circulating serotypes and coverage of conjugate and 23-valent polysaccharide pneumococcal vaccines in different age groups.

Results Among 105 isolates, a total of 21 different serotypes of *Streptococcus pneumoniae* were determined. The most frequent serotypes were 3 (21.9%), 19F (20.0%), and 14 (10.5%). The serotype coverage of pneumococcal conjugate vaccines (PCV7, PCV10, and PCV13) was 48.6%, 54.3%, and 84.8%, respectively, while pneumococcal polysaccharide vaccine (PPV23) covered 89.5% of the total number of isolates in all age groups. Serotypes included in PCV7, PCV10, and PCV13 represented 72.0%, 76.0%, and 88.0% of the total number of isolates in children ≤5 years, respectively. Vaccine serotype coverage of PCV13 and PPV23 ranged from 87.1% to 90.3% in adults 50–64 years of age, and 77.8% to 85.2% in adults ≥65 years old.

Conclusion Serotype distribution of *Streptococcus pneumoniae* in the population fairly overlaps with the serotypes contained in pneumococcal vaccines, so that implementation of childhood immunization is justified. The study was done in the Province of Vojvodina but the findings may be applied to Serbia as a whole.

Keywords: *Streptococcus pneumoniae*; serotypes; vaccine serotypes

INTRODUCTION

Streptococcus pneumoniae (pneumococcus) is the most common causative agent of bacterial pneumonia and meningitis. Prior to introduction of pneumococcal conjugate vaccines, 6–11 serotypes accounted for ≥70% of all invasive pneumococcal disease (IPD) occurring in children worldwide [1]. Incidence rates, as well as case fatality rates of invasive pneumococcal diseases (IPD) are the highest in children under two years old and elderly, even in developed countries. In the European Union (EU)/ European Economic Area (EEA) countries in 2012, average case fatality rate was 11%, ranging 4–29% [2].

Development of pneumococcal vaccines and immunization enabled efficient prevention of infections. Pneumococcal polysaccharide vaccine (PPV23), which contains 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F) was licensed in 1983. PPV23 is poorly immunogenic in the most vulnerable group, children younger than two years of age. Therefore, pneumococcal conjugate vaccines (PCVs) were developed, including pneumococcal polysaccharides conjugated with highly immunogenic protein carrier. First conjugate vaccine, PCV7, was licensed in Europe in 2001 and contains seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F). Ten-valent conjugate vaccine (PCV10) was licensed in 2009 and contains additional three serotypes (1, 5, and 7F). The last, 13-valent vaccine (PCV13), licensed in 2010, contains all previously mentioned and three more serotypes (3, 6A, and 19A). Unlike the PPV23, conjugate vaccines trigger both humoral and cellular immune response and are therefore efficient in all age groups including infants and children under two years of age [1].

Conjugate vaccines are used in 29 EU/EEA countries, while in 23 countries they are included in their respective national immuniza-

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tion programs (NIPs) [2]. In Serbia so far, pneumococcal vaccines were only used for immunization according to clinical indications [3]. Mandatory childhood immunization against *Streptococcus pneumoniae* is introduced in the new Law on Protection of Population against Communicable Diseases, (LPPCD) adopted in March 2016 [4].

World Health Organization considers that it should be a priority to all countries to include conjugate vaccine in national immunization programs for children. Countries are encouraged to conduct appropriate surveillance of IPD and to monitor the impact of vaccination, including the occurrence and magnitude of replacement disease. Surveillance should be introduced at least two years prior to and maintained at least five years after the immunization introduction [1]. It is also recommended to follow up the trends in serotype distribution in order to (i) monitor changes, (ii) guide most adequate strategy of immunization, and (iii) assess the impact of immunization on the population structure of circulating pneumococcal serotypes [1, 2, 5].

Surveillance of IPD in Serbia is based on reporting of clinically diagnosed illness with different clinical presentations (pneumonia, sepsis, bacterial meningitis) with etiological agent being identified in a small number of cases. New LPPCD introduces reporting of IPD as a separate entity that will improve surveillance [4].

OBJECTIVE

The objective of this study was to determine the prevalence of pneumococcal serotype distribution in Vojvodina region before routine use of pneumococcal conjugate vaccine in Serbia.

METHODS

A total of 105 pneumococcal isolates were collected from the same number of patients in the period from January 2009 to April 2016. The youngest patient was a newborn, five days old, while the oldest one was 82 years old. In children under five years of age, adults 50–64 years of age and older than 65, a total of 25, 31, and 27 isolates were obtained, respectively.

Analyses encompassed isolates from middle ear aspirate (14), bronchoalveolar lavage fluid (4), tissue bioptate (1), and wound swab (1). Invasive strains (83) were obtained from blood (48), cerebrospinal fluid (17), pleural fluid (17), and pericardial fluid (1) (Table 1).

A total of 52 *Streptococcus pneumoniae* strains were isolated in the clinical microbiological laboratory of the Institute of Public Health of Vojvodina from the clinical specimens of patients treated in the Clinical Centre of Vojvodina (Clinic for Otorhinolaryngology, Clinic for Internal Medicine and Clinic for Infectious diseases) as well as from the Institute for Child and Youth Health Care of Vojvodina. Forty-three isolates were collected from microbiological laboratory of the Institute for Pulmonary Diseases of Vojvodina, Institute for Cardiovascular Diseases of Vojvodina, Institute for Oncology of Vojvodina, and the remaining 10 strains were obtained from Regional Public Health Institutes in Vojvodina (Sombor, Kikinda, Subotica). All isolates were identified using standard microbiological methods – colony morphology, optochin susceptibility, and bile solubility.

Streptococcus pneumoniae isolates were transported in Amies transport media to the National Reference Laboratory for Streptococci, Institute for Microbiology and Immunology, School of Medicine, Belgrade. Capsular serotyping was done by the Quellung reaction, using the antisera from Statens Serum Institut (Copenhagen, Denmark).

We analyzed whether the isolates are covered by pneumococcal vaccines related to the target groups for the immunization by conjugate vaccines (children ≤5 years old) and PCV13 and PPV23 (adults 50–64 years of age and elderly ≥65 years).

RESULTS

Among 105 isolates, a total of 21 different serotypes of *Streptococcus pneumoniae* were determined, whereas only one isolate was non-typable. The most frequent serotypes were 3 (21.9%), 19F (20%), and 14 (10.5%) (Graph 1).

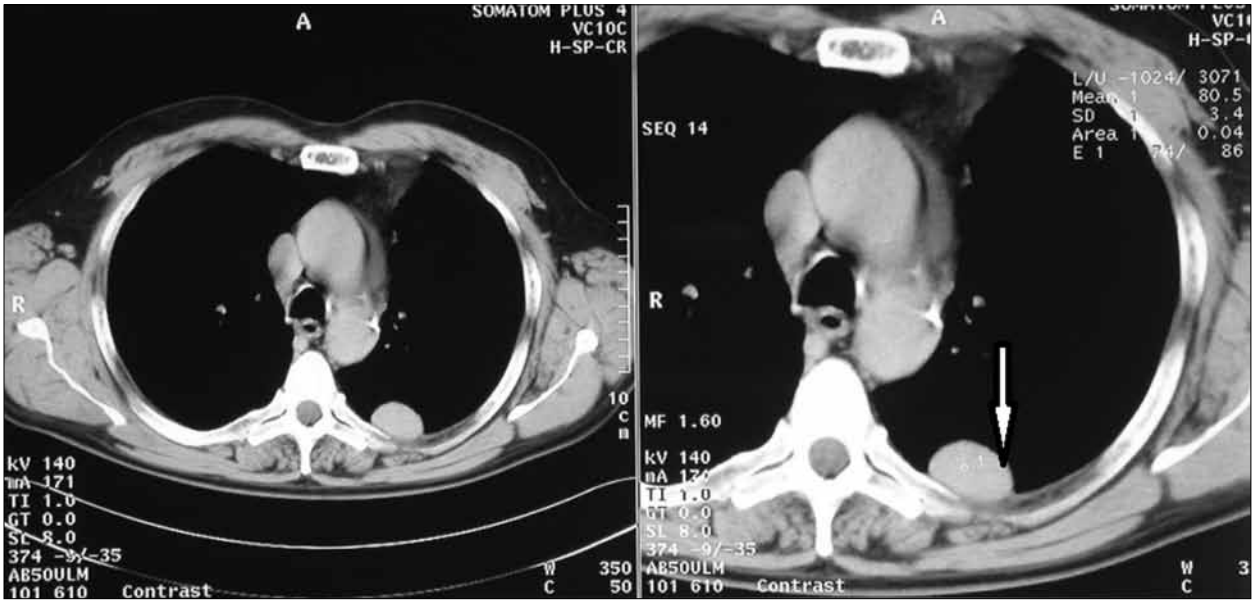
Serotypes included in seven-, 10-, and 13-valent conjugate vaccines and a 23-valent polysaccharide vaccine represented 48.6%, 54.3%, 84.8%, and 89.5% of the total number of isolates, respectively (Table 2).

Among 25 isolates from children ≤5 years of age, a total of 11 different serotypes of *Streptococcus pneumoniae* were detected, out of which three are not included in conjugate vaccines (11A, 23A and 33F). The most frequent serotypes were 19F (44%) and 14 (16%), which are incorporated in all three conjugate vaccines. The serotype coverage by PCV7, PCV10, and PCV13 in this age group were 72%, 76%, and 88%, respectively. In a subset of children ≤2 years of age, 20 strains were isolated and 19F was the most common one (10 out of 20 strains). Among children younger than two years of age, coverage with PCV7, PCV10, and PCV13 was 56%, 60%, and 72%, respectively. Three out of five strains isolated from children aged two to five years belonged to type 14, one was 19F, and the remaining 33F is present in PPV23 only.

Table 1. Type and number of examined specimens

Type of specimens	Number (%)
Blood	48 (45.7)
Cerebrospinal fluid	17 (16.2)
Blood and cerebrospinal fluid	2 (1.9)
Pleural fluid	17 (16.2)
Pericardijal punctate	1 (1.0)
Middle ear aspirate	14 (13.3)
Bronchial aspirate or bronchial lavage	4 (3.8)
Tissue bioptate	1 (1.0)
Wound swab	1 (1.0)
Total	105 (100)

doi: 10.2298/SARH1610521P



Graph 1. Circulating serotypes of Streptococcus pneumoniae in Vojvodina in the period from January 2009 to April 2016

Serotypes included in PPV23 and PCV13 represented 90.3 % and 87.1% of the total number of isolates in adults 50–64 years of age. The most frequent was serotype 3 (32.3%), which is present in both vaccines. Serotypes 8 and 9N presented only on PPV23 were detected in two isolates, which is 6.4% of the total number of isolates in this age group.

Vaccine coverage among adults aged ≥65 years were 85.2% and 77.8% for PPV23 and PCV 13, respectively. In this age group, the most frequent serotypes were 3 (25.9%), 19F (18.05%) and 6A (11.1%), incorporated in both vaccines. Serotypes present only in PPV23 (8, 9N, 11A, 17F) accounted for 18.5% of all isolates in the oldest age group.

DISCUSSION

There are over 90 serotypes of *Streptococcus pneumoniae* with only 20 of them responsible for >80% of IPD cases in all age groups, and 13 leading serotypes caused 70–75% of IPD cases in children worldwide, before the introduction of immunizations [6]. Distribution of serotypes still varies geographically, according to age, clinical form and severity of illness, and is also subject to change over time [1].

In countries that introduced conjugate vaccine in NIP for children, direct and indirect (herd) protection rapidly reduced IPD incidence across all age groups. [7]. However, PCV immunization led to changes in the circulating serotypes and serotype replacement was detected. Significant reduction of the vaccinal serotypes and an increased frequency of serotypes not included in vaccine were noticed [7]. Before the introduction of PCV7, the most frequent types were those included in the vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F). At the end of the 20th century these serotypes caused 59% of IPD cases in adults and 87% in children [7]. After the introduction of PCV7 in the United States in 2000, most European countries have gradually introduced this vaccine in their vaccination schedules between 2006 and 2009 [8]. They reported a drastic reduction of vaccine preventable serotypes in IPD cases, but the emergence of serotype 19A was noticed. After the introduction of PCV13 in the United States in 2010, the occurrence of serotype 19A decreased by 58% [9]. However, even after introduction of PCV10, a significant reduction in frequencies of both PCV10-related IPD and 6A and 19A (not included in PCV10) was observed, suggesting that PCV10 may also provide cross-protection against vaccine-related serotypes [10].

In this study we provide data on serotype distribution in Vojvodina region in Serbia in the period before the introduction of mandatory vaccination against IPD in children, which is implemented in the new National Immunization Schedule for 2016. Among 105 isolates, a total of 21 different serotypes of *Streptococcus pneumoniae* were determined and the most frequent in all age groups were serotype 3 (21.9%), which is included only in PCV13, and PPV23, followed by serotypes 19F (20.0%) and 14 (10.5%), which are included in all the vaccines. Unlike the serotype 3, which is susceptible to antibiotics, 19F and 14 are most often antibiotic-resistant types [2]. Our results indicate that one third of all pneumococcal isolates and 60% of isolates from children ≤5 years old belong to 19F and 14 serotypes, which is worrisome. Clinical impact of pneumococcal antibiotic resistance is a worldwide problem. High level of pneumococcal resistance has already been documented in our country, especially in 19F and 14 serotypes. Besides the effect on IPD, it was demonstrated that the introduction of PCV has been associated with a reduction in antimicrobial-resistant pneumococcal disease. This is particularly important in countries such as ours, with relatively high incidence rate of pneumococcal resistance to penicillin and macrolides. In the period from 2009 to 2011, 34% and 36% of pneumococcal invasive isolates in Serbia were nonsusceptible to penicillin and erythromycin,

Table 2. Circulating serotypes of *Streptococcus pneumoniae* by age groups for immunization in Vojvodina in the period from January 2009 until April 2016 and coverage by conjugate vaccines and PPV23

Vaccine	Serotype	Isolates in children 2 years of age		Isolates in children 2–5 years of age		Isolates in children ≤5 years of age		Isolates in adults 50–64 years of age		Isolates in elderly ≥65 years of age		All Isolates	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
PPV23 serotypes, PCV13 serotypes, PCV10 serotypes, PCV7 serotypes	4	0	0.0	0	0.0	0	0.0	4	12.9	1	3.7	6	5.7
	6B	2	8.0	0	0.0	2	8.0	1	3.2	0	0.0	4	3.8
	9V	0	0.0	0	0.0	0	0.0	1	3.2	1	3.7	3	2.9
	14	1	4.0	3	12.0	4	16.0	3	9.7	1	3.7	11	10.5
	18C	0	0.0	0	0.0	0	0.0	1	3.2	0	0.0	2	1.9
	19F	10	40.0	1	4.0	11	44.0	2	6.5	5	18.5	21	20.0
	23F	1	4.0	0	0.0	1	4.0	2	6.5	1	3.7	4	3.8
	Subtotal (PCV7 serotypes)	14	56.0	4	16.0	18	72.0	14	45.2	9	33.3	51	48.6
	1	1	4.0	0	0.0	1	4.0	0	0.0	0	0.0	1	1.0
	5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	7F	0	0.0	0	0.0	0	0.0	2	6.5	1	3.7	5	4.8
	Subtotal (PCV10 serotypes)	**15**	60.0	**4**	16.0	19	76.0	16	51.6	10	37.0	57	54.3
	3	1	4.0	0	0.0	1	4.0	10	32.3	7	25.9	23	21.9
	6A	1	4.0	0	0.0	1	4.0	1	3.2	3	11.1	6	5.7
	19A	1	4.0	0	0.0	1	4.0	0	0.0	1	3.7	3	2.9
	Subtotal (PCV13 serotypes)	18	72.0	4	16.0	22	88.0	27	87.1	21	77.8	89	84.8
PPV23 serotypes	2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	8	0	0.0	0	0.0	0	0.0	1	3.2	2	7.4	3	2.9
	9N	0	0.0	0	0.0	0	0.0	1	3.2	1	3.7	2	1.9
	10A	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	11A	1	4.0	0	0.0	1	4.0	0	0.0	1	3.7	2	1.9
	12F	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	15B	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	17F	0	0.0	0	0.0	0	0.0	0	0.0	1	3.7	2	1.9
	20	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	22F	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
	33F	0	0.0	1	4.0	1	4.0	0	0.0	0	0.0	1	1.0
Subtotal (PPV23 serotypes) (6A excluded)		18	72.0	5	20.0	23	92.0	28	90.3	23	85.2	94	89.5
Non vaccinal serotypes	23A	1	4.0	0	0.0	1	4.0	0	0.0	0	0.0	2	1.9
	15A	0	0.0	0	0.0	0	0.0	1	3.2	0	0.0	1	1.0
	15C	0	0.0	0	0.0	0	0.0	0	0.0	1	3.7	1	1.0
	Non typable	0	0.0	0	0.0	0	0.0	1	3.2	0	0.0	1	1.0
Subtotal		1	4.0	0	0.0	1	4.0	2	6.5	1	3.7	5	4.8
Total		20	80.0	5	20.0	25	100.0	31	100.0	27	100.0	105	100.0

respectively [11]. More than one third (36.4%) of pneumococcal isolates in Serbia in the 2009–2012 period expressed co-resistance to macrolides and penicillin. Multiresistant isolates were significantly more prevalent among children than adults [12].

Serotypes included in pneumococcal seven-valent, 10-valent, and 13-valent vaccines represented 48.6%, 54.3%, and 84.8% of the total number of our isolates, respectively. In children ≤5 years of age, who are the target group for the immunization with conjugate vaccines, coverage of circulating isolates was higher – 72% for PCV7, 76% for PCV10, and 88% for PCV13, because majority of serotypes in this age group belonged to serotypes 14 and 19F, which are in all vaccines. The serotypes presented only in PCV13-3 and 19A, were found in two (one from each) of 25 strains isolated in this age group. Average coverage of circulating isolates by PCV7 in European countries in the period before 2008 was 71%, and rose to 78% for PCV10 and to 87% for PCV13 [13]. In Europe and North America, the serotypes included in PCV10 and PCV13 are estimated to cover approximately 80–85% and 85–90% of IPD, respectively, in children younger than five years of age [14]. Nevertheless, the majority of isolates of children in Vojvodina were obtained from younger than two years, who are the target group for conjugate vaccines only. Vaccine-preventable serotypes covered by PCV7,

PCV10, and PCV13 were represented in somewhat lower percentages – 56.0%, 60.0%, and 72.0% of the total number of isolates in the youngest, respectively – but it is still high enough to justify implementation of immunization.

A polyvalent polysaccharide vaccine, PPV23, comprises only T-cell-independent antigens and therefore induces only B-cells that are short-lived and produce low-affinity antibodies. This vaccine is recommended for adults ≥65 years old and for those two years or older at high risk for IPD [15]. PPV23 is not effective in infants and the main advantage of this vaccine is that it protects against 23 types of pneumococci. There were only five isolates obtained from children aged two to five years in Vojvodina, and all of them are covered by PPV23. Still, four out of five strains are also covered by PCVs. PPV23, unlike conjugate vaccines, has no impact on nasopharyngeal carriage and does not alter circulating serotypes. Although the efficacy for prevention of non-bacteriaemic pneumonia is questionable, epidemiological studies showed that the immunization with PPV23 reduced the risk of IPD by 60% [16]. Additionally, recent meta-analysis provided evidence supporting the recommendation for PPV23 to prevent IPD in adults. However, the evidence from randomized clinical trials was less clear with respect to adults with chronic illness. Same meta-analyses did not provide compelling evidence to support the routine use of PPV to prevent all-cause pneumonia or mortality [17]. Out of total number of identified isolates, PPV23 covered 85.2% and 90.3% in elderly and adults 50–64 years of age, respectively. Approximately one third of the isolates in both age groups belonged to type 3 (25.9% in ≥65 and 32.3% in 50–64 age group) which is related to higher risk of death compared to other serotypes [2, 16]. Having in mind higher immunogenicity and effectiveness of PCV13 compared to PPV23, we should consider introduction of this vaccine for adults older than 50 years, especially those at high risk (immunocompromised, persons with functional or anatomic asplenia, those with chronic illness, etc.) similar to practice adopted in some European countries and the United States [18].

CONCLUSION

Although the number of isolates included in this analysis is relatively low, our results contribute to overall comprehension of circulating serotypes of *Streptococcus pneumoniae* in Vojvodina region in the pre-vaccinal period. Serotype distribution of *Streptococcus pneumoniae* in the population fairly overlaps with the serotypes contained in pneumococcal vaccines, so that implementation of childhood immunization is justified. The study was done in the Province of Vojvodina but the findings may be applied to Serbia as a whole. The introduction of PCV13 into the National Immunization Programme should be considered for adults older than 50 years, with risk factors, such as chronic illness.

Further follow-up of circulating serotypes of *Streptococcus pneumoniae* in our population will enable assessment of impact of immunization on distribution and possible changes in the circulation of serotypes.

ACKNOWLEDGMENT

The authors wish to thank all physicians who have participated in the collection of samples for their support and the participating microbiological laboratories for providing the isolates. The study was partially funded by Special Public Health Programme of the Autonomous Province of Vojvodina in the 2012–2015 period. The research was partially funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. ON 175039). Anti-pneumococcal antisera were kindly donated by GlaxoSmithKline plc (London, England, UK) and Pfizer Inc. (New York City, NY, USA).

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Дистрибуција серотипова *Streptococcus pneumoniae* у Војводини у периоду пре увођења конјуговане вакцине у Национални програм имунизације

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КРАТАК САДРЖАЈ

Увод *Streptococcus pneumoniae* је најчешћи изазивач бактеријске пнеумоније и менингитиса. Нови Закон о заштити становништва од заразних болести у Србији предвиђа увођење обавезне имунизације деце против обољења изазваних пнеумококом.

Циљ Циљ ове студије је био да се одреди заступљеност циркулишућих серотипова пнеумокока у Војводини у периоду пре увођења обавезне конјуговане пнеумококне вакцине у Србији.

Метод У периоду од јануара 2009. до априла 2016. године сакупљено је 105 изолата *Streptococcusa pneumoniae*. Анализирали смо резултате серотипизације пнеумокока, која је урађена у Националној референтној лабораторији и утврдили каква је дистрибуција циркулишућих серотипова и њихова покривеност конјугованим вакцинама и 23-валентном полисахаридном вакцином у различитим узрасним групама.

Резултати У колекцији од 105 изолата *Streptococcusa pneumoniae* нађен је 21 серотип. Најзаступљенији су били серотипови 3 (21,9%), 19*F* (20,0%) и 14 (10,5). Подударност изолата са серотиповима садржаним у вакцини била је 48,6% за *PCV*7, 54,3% за *PCV*10 и 84,8% за *PCV*13, док је пнеумококна полисахаридна вакцина (ППВ23) покривала 89,5% од укупног броја изолата. Серотипови садржани у *PCV*7, *PCV*10 и *PCV*13 били су заступљени код 72,0%, 76,0% и 88,0% изолата деце млађе од пет година. Подударност изолата са серотиповима садржаним у *PCV*13 и ППВ23 износила је 87,1% и 90,3% код одраслих старости 50–64 године и 77,8% и 85,2% код старијих од 65 година.

Закључак Заступљеност циркулишућих серотипова *S. pneumoniae* у популацији се у великој мери преклапа са серотиповима садржаним у пнеумококним вакцинама, што оправдава увођење имунизације у дечјем узрасту. Иако је истраживање спроведено у Војводини, налази се могу применити на целу Србију.

Кључне речи: *Streptococcus pneumoniae*; серотипови; вакцинални серотипови

Примљен • Received: 30/05/2016 **Ревизија • Revision:** 23/08/2016 **Прихваћен • Accepted:** 26/08/2016

Relentless placoid chorioretinitis – A case report

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SUMMARY

Introduction Relentless placoid chorioretinitis is an entity which belongs to the group of an atypical intermediate form of primary inflammatory choriocapillaropathies, resembling both acute posterior multifocal placoid pigment epitheliopathy and serpiginous choroiditis, but the retinal distribution and clinical course are not the same. Because of this similarity this entity was termed "AMPPiginous". This entity was first described by Jones et al. in 2000. The aim of our case report is to present a very specific case where the clinical course was progressive, with loss of vision in the affected eye.

Case Outline A 31-year-old man, with no previous ophthalmic diseases, was hospitalized at the Clinic of Ophthalmology, Clinical Center Kragujevac, because of a reduction of vision in the right eye, and scotoma and metamorphopsia in the left eye. The clinical course of retinal lesions in the left eye resembled the changes observed in acute posterior multifocal placoid pigment epitheliopathy, and the right eye changes were between acute posterior multifocal placoid pigment epitheliopathy and serpiginous choroiditis. The diagnosis of relentless placoid chorioretinitis was confirmed after clinical, laboratory, immunological, virological, and angiography examinations.

Conclusion The progressive clinical course of the disease, complemented by multimodal imaging and extensive laboratory diagnostics, has led us to the diagnosis of relentless placoid chorioretinitis. The combined anti-inflammatory and immunomodulatory therapy led to the stabilization of visual acuity of the left eye as opposed to the right, where there has been no recovery.

Keywords: anti-inflammatory therapy; immunomodulatory therapy; multimodal imaging; primary inflammatory choriocapillaropathies

INTRODUCTION

Relentless placoid chorioretinitis (RPC) is a relatively new and rare entity, which belongs to the atypical intermediate form of primary inflammatory choriocapillaropathies (PICCP), which was first described in 2000 by Jones et al. [1]. RPC is an entity in which multiple inflammatory, deep white-creamy lesions resembling those seen in acute posterior multifocal placoid pigment epitheliopathy (APMPPE) and serpiginous choroiditis (SC) develop. Unlike APMPPE, the lesions in RPC continue to expand in size and number with a relentless course over many months. Unlike SC, the lesions of RPC are multifocal and eventually involve all areas of the retina, including the anterior periphery prior to involvement of the posterior pole and macula. Therefore, RPC belongs to the group of an atypical intermediate form of PICCP [2]. PICCP is a heterogeneous group of diseases with a common pathogenetic mechanism – choriocapillaris non-perfusion with subsequent retinal ischemia above, which depends on oxygen and nutrients from choriocapillaris. Clinical differences in PICCP are explained by the level and severity of inflammatory insults at the level of choriocapillaris circulation [2]. Etiology is undefined; some suggest an autoimmune/inflammatory cause triggered by an exogenous agent [3]. Biochemical and serological examinations complemented by multimodal imaging and functional parameters facilitate diagnosis and management [4]. Multimodal imaging includes fluorescein angiography (FA), indocyanine green angiography (ICGA), fundus autofluorescence (FAF), and optical coherence tomography (OCT). FA provides information regarding the localization and extent of posterior segment inflammatory diseases [4]. ICGA is dominant to diagnose diseases of choriocapillaris, as well as for monitoring course of disease and effect of therapy [5]. OCT could help in the differential diagnosis of RPC, because RPC may rarely lead to severe macular atrophy [6]. Therapy should be adjusted to the course of inflammatory diseases because we know the structures that are primarily affected. The treatment includes hospitalization, patient monitoring, with anti-inflammatory [7], immunomodulatory [8–12], and anti-angiogenic therapy [13]. Most patients treated with prolonged systemic corticosteroid therapy recover their prior vision. However, maintenance of remission is possible if immunosuppressive therapy is involved in the early stages of the disease [2]. Also, vision can decrease significantly when fovea is involved [14]. Complications of RPC could be choroidal neovascularization, subretinal fibrosis, and epiretinal membrane formation [15].

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CASE REPORT

A 31-year-old male was hospitalized complaining of decreased vision in his right eye for the previous two days. The patient denied any previous ophthalmic diseases, febrile or flu-like episodes. On initial examination, visual acuity was 0.05 on the right eye and 1.0 (Snellen charts) on the left. Color vision (Ishihara plates) on the left was preserved. Intraocular pressure in both eyes was 16 mmHg. The anterior segments were quiet, and there were no vitreous cells. Fundus examination (Visucam, Carl Zeiss AG, Oberkochen, Germany) showed lesions characterized as multiple creamy-white at the level of choriocapillaris, with an inflamed retina above in the mid periphery, posterior pole and praeequatorialis, but in the right eye the inflammation was a damaged macula. On the right eye, the perimetry (central 30-2 and macular threshold, Humphrey Systems Instruments, Carl Zeiss AG) showed an absolute absence of sensitivity and, on the left, objective, multifocal scotoma decreased sensitivity. A fluorescein angiogram (Visucam FA, Carl Zeiss AG) showed an area of subretinal placoid lesions characterized in the early stages by visible hypofluoroscence, which indicates choriocapillaris nonperfusion, and in the late stage of progress show hyperfluoroscence, depending on the severity of the ischemic process (Figure 1) (unlike APMPPE, active lesions do not show blocked fluorescence). The previously described changes were observed in both eyes in the region of the equatorial zone; in the right, the changes had much more extensive involvement of the macula. Visual evoked potentials showed the left eye had a proper cortical response, while cortical response was not generated in the right eye. Optical coherence tomography (Cirrus OCT, Carl Zeiss Meditec AG, Jena, Germany) of the right eye presented macular atrophy [central macular thickness (CMT) was 142 μm], and left eye thickness of the retinal pigment epithelium and mild retinal pigment epithelium detachment in the macula (CMT was 282 μm). Biochemical, antinuclear factor, rheumatoid factor, angiotensin-converting enzyme, serum lysozyme levels, and lupus anticoagulant were all normal. Serological tests on syphilis, human immunodeficiency virus, varicella zoster virus, herpes simplex virus, mycoplasma pneumoniae, cytomegalovirus, toxoplasmosis, rubella, *Borrelia burgdorferi* were within reference ranges. Chest X-rays were normal and tuberculin skin test (Mantoux test) was negative. After the previous examination, the patient was diagnosed with RPC in the right eye, and with APMPPE in the left. The patient was prescribed parenteral corticosteroid therapy (1 mg/kg/day). Only three days after the hospitalization, the disease progression was as follows: visual acuity amaurosis on the right, and best corrected visual acuity on the left eye was 0.8. Hence, we administered cyclosporine (5 mg/kg) as well, after which best corrected visual acuity on the left eye was 1.0, but on the opposite eye the amaurosis remained because the macula and mid-periphery of the retina was already damaged. Characteristic local findings as well as progressive, almost aggressive course of the disease, suggest a rare clinical syndrome – a relentless placoid chorioretinitis. We monitored the patient monthly during the first six months, and since there was no progression of the disease on the opposite eye, we reduced cyclosporine to a dose of 2 mg/kg after six months. Regular two-month control check-ups were performed for the next six months. Local funduscopic findings and findings obtained by the fundus camera remained unchanged. The patient had no side effects to the drug, but because of the stable fundus, we discontinued cyclosporine administration. After 18 months from the disease onset, fundoscopic findings of both eyes remained unchanged.

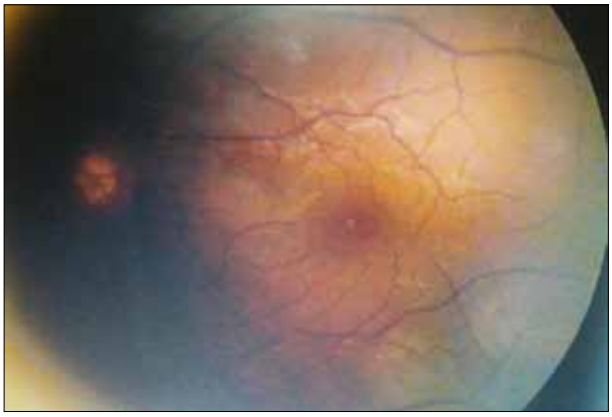

Figure 1. Image shows multiple areas of hyperfluoroscence in the mid-periphery and posterior pole, capturing the macula, only three days after hospitalization

DISCUSSION

PICCP are caused by an inflammation producing choriocapillaris non-perfusion and show the characteristic local retinal and subretinal lesions on fundus examination. The differential diagnosis includes some of the forms of PICCP and some systemic diseases. Unlike APMPPE, the lesions in RPC continue to expand in size and number, while lesions in APMPPE are reversible and without scars **sans** immunosuppressive therapy. In contrast to SC, the lesions of RPC are multifocal and involve all areas of the retina, including the anterior periphery prior to involvement of the posterior pole and macula [2]. The progressive outer retinal necrosis syndrome in the early stages of the disease is characterized by multifocal deep retinal lesions. However, lesions rapidly progress to total retinal necrosis with retinal detachment, and laboratory findings on herpes zoster virus was negative [16, 17]. Ocular ischemic syndrome (choroidal ischemia) is a rare condition associated with severe artery occlusive disease leading to ocular hypoperfusion. Choroidal hypoperfusion results with lesions resembling those in RPC in the posterior pole or the mid-periphery. However, prolonged ischemia results in retinal detachment and retinal atrophy [18]. Infectious ocular diseases like acute retinal necrosis [17], systemic diseases like systemic lupus erythematosus [19], AIDS [20], and non-Hodgkin's lymphoma [21] may have similar fundoscopic lesions, but all of them have vitritis and/or mild or severe anterior uveitis, as well as laboratory analyses results beyond reference values, which differentiates them

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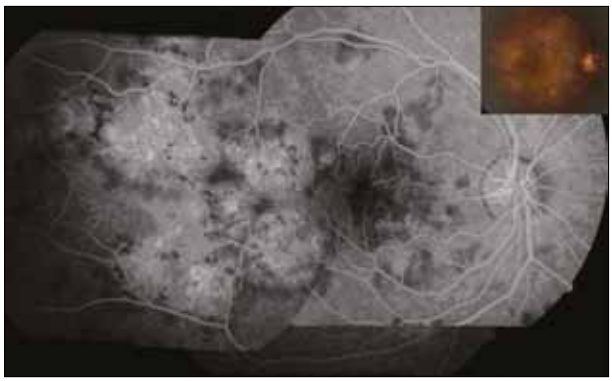

Figure 2. Fundus examination of the left eye presents multiple creamy-white lesions at the level of choriocapillaris

from RPC. Characteristic local findings which were confirmed with additional diagnostic tests, as well as a progressive, almost aggressive course of the disease indicate a clinical syndrome – a relentless placoid chorioretinitis. Application of corticosteroid therapy alone has not proved as effective as expected. Among patients where the disease progresses rapidly, as was the case with our patient, with some complications present, or if vision is threatened, the addition of immunosuppressive therapy may lead to the stabilization of the disease. Combined, anti-inflammatory and immunosuppressive therapy should be administered for a sufficiently long period of time, as early discontinuation of such therapy can lead to the reactivation of the disease. But in the described case even the combined and long-term therapy of 12 months did not lead to an improvement because the macula was damaged, which led to permanent loss of vision on the right eye; however, we managed to stop the process in the left eye (Figure 2).

NOTE

The paper has been presented at the 1st Congress of Ophthalmologists of Republic of Srpska with International Participation, Bijeljina, 29th to 31st of May 2015, where it has been published in the form of an abstract (Jovanović S, Obradović Lj, Jovanović M, Jovanović Z. A case of ampiginous choriocapillaritis syndromes – Case report. Proceedings and Abstract Book of the 1st Congress of Ophthalmologists of the Republic of Srpska with International Participation. Bijeljina; p. 156–7.

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Рефракторни плакоидни хориоретинитис – приказ болесника

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КРАТАК САДРЖАЈ

Увод Рефракторни плакоидни хориоретинитис је ентитет који припада групи атипичних интермедијарних форми примарних инфламаторних хориокапиларопатија, подсећајући и на акутни задњи мултифокални плакоидни пигментни хориоретинитис и на серпигинозни хориоретинитис, али ретинална дистрибуција и клинички ток нису исти. Због такве сличности овај ентитет се може наћи у литератури и под термином „ампигинозни". Описан је први пут од Џона и осталих. Циљ приказа случаја је веома специфичан случај јер је клинички ток био прогресиван са губитком видне функције.

Приказ болесника 31-годишњи мушкарац, без ранијих офталмолошких обољења, хоспитализован је на Клинику за офталмологију Клиничког центра Крагујевац због пада вида на десном оку, и са скотомима и метаморфопсијом на левом оку. Клинички ток ретиналних лезија на левом оку личио је на промене описане код акутне постериорне мултифокалне пигментне епителопатије, а на десном оку промене су биле између акутне постериорне мултифокалне пигментне плаоидне епителопатије и серпигинозног хороидитиса. Дијагноза рефракторног плакоидног хориоретинитиса је потврђена након клиничких, лабораторијских, вирусолошких и ангиографских испитивања.

Закључак Прогресиван клинички ток болести, употпуњен мултимодијалним имиџингом и опсежном лабораторијском дијагностиком, довели су нас до дијагнозе рефракторног плакоидног хориоретинитиса. Комбинована антиинфламаторна и имуномодулаторна терапија су довеле до стабилизације видне функције левог ока за разлику од десног, где није дошло до опоравка.

Кључне речи: антиинфламаторна терапија; имуномодулаторна терапија; мултимодијални имиџинг; примарне инфламаторне хориокапиларопатије

Примљен • Received: 22/12/2015 **Ревизија • Revision:** 27/04/2016 **Прихваћен • Accepted:** 15/06/2016

doi: 10.2298/SARH1610527O

Central mucoepidermoid carcinoma of the mandible – A case report

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SUMMARY

Introduction Mucoepidermoid carcinoma, compared to other tumors of salivary glands, occurs in 5–10% of cases. Histopathologically, it is divided into a well differentiated tumor that is of low-grade of malignancy, and a medium and poorly differentiated tumor of high grade of malignancy. Central mucoepidermoid carcinoma (CMEC) of the mandible was firstly described by Lepp in 1936, on a 66-year-old female patient. CMEC is characterized by atypical clinical image and radiological manifestation.

Case Outline A 55-year-old female patient was examined at the Clinic of Dentistry in Niš, Serbia, with anamnestic data regarding the presence of painless swelling in the right side of the mandible. Considering the histopathological results and presence of enlarged lymph nodes, right hemimandibulectomy and tumour excision from pterygomandibular space followed by supraomohyoid neck dissection was done. In due course, postoperative radiotherapy was applied (60 Gy).

Conclusion CMEC represents a rare tumor, characterized by local tissue destruction and ability to metastasize. Initial biopsy represented the key in preoperative planing. Radical excision with neck lymph node dissection followed by postoperative radiotherapy in our case represent a successful method of treating CMEC of the mandible.

Keywords: central carcinoma; mandibular swelling; biopsy; surgical therapy

INTRODUCTION

Mucoepidermoid carcinoma (MEC), compared to other types of tumor of salivary glands, occurs in 5–10% of the cases. It may be present in large salivary glands (86% in parotid gland, 8% in submandibular, and 6% in sublingual gland) [1]. Most frequent intraoral localization of MEC are small salivary glands of the hard palate with 41.1% [2]. Histopathologically, there are three modes of MEC – well differentiated tumor with low-grade of malignancy, medium, and poorly differentiated tumors with high grade of malignancy. By observing intraoral localization, a well differentiated tumor is encountered in 58.4% of the cases, medium differentiated in 38.3%, while poorly differentiated tumor is present in 3.2% of the cases [3].

Central mucoepidermoid carcinoma (CMEC) of the mandible was described in literature for the first time by Lepp [4] in 1936, in a 66-year-old female patient. However, this is a very rare localization of the tumor, which belongs to primary intraosseous carcinomas. Assumptions are that CMEC can originate from ectopic glandular tissue, transformed mucosa cells, existing untreated odontogenic cist or intra-bony propagation of epithelial cells from maxillary sinus and submucous salivary glands [5]. CMEC is characterized by atypical clinical image and radiological manifestation [6].

CASE REPORT

A 55-year-old female patient was referred to the Clinic of Dentistry in Niš, Serbia, with a painless swelling in the right region of the mandible, present for the previous 30 days. The patient said that four years prior to this she underwent an extraction of lower lateral teeth for prosthetic rehabilitation of the jaw, which included the extraction of the lower right impacted wisdom tooth. Extraoral palpation revealed an extremely hard swelling in the region of the body, angle and ramus of the mandible (Figure 1). Enlarged lymph nodes were also palpated in levels Ib and II of the neck, smaller than 1 cm in diameter, movable and painless. Intraoral examination revealed a complete lack of teeth in the upper and lower jaw and expanding

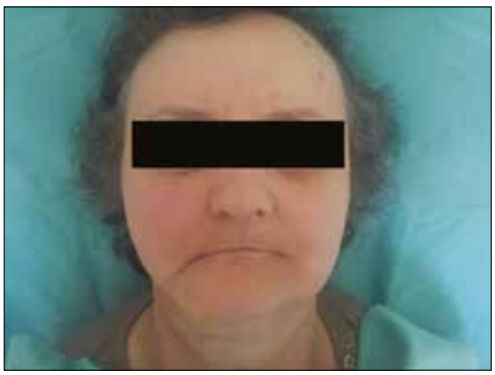

Figure 1. Swelling in the right region of the mandible

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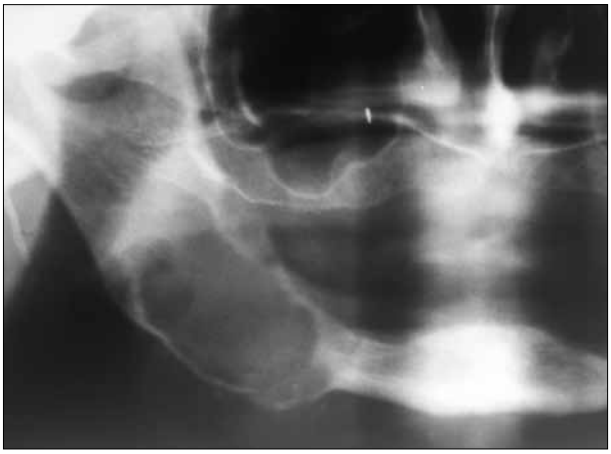

Figure 2. Presence of multi-locular irregular osteolytic lesion in the region of the body, corner, and branch of the mandible

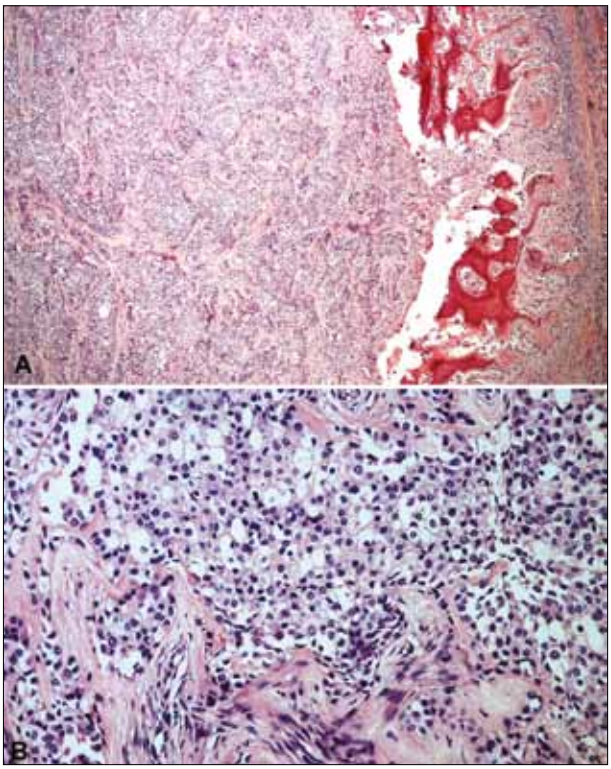

Figure 3. Mucoepidermoid carcinoma composed of basal, intermediate, and mucous cells (HE; A: ×4; B: ×20)

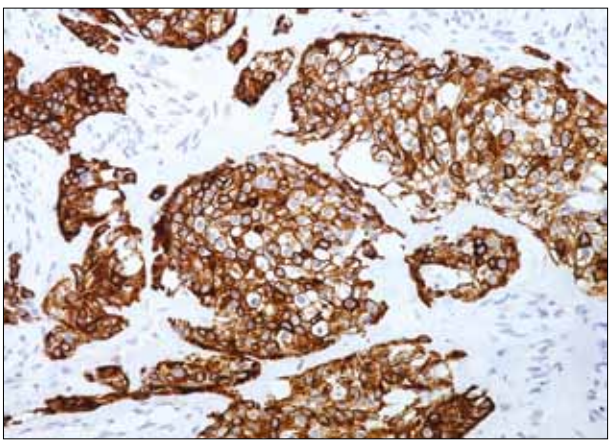

Figure 4. Tumor cells randomly positive on CK7 (cytoplasmic staining; ×20)

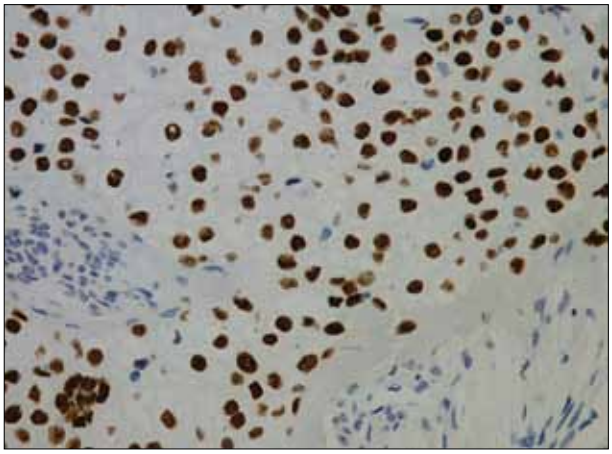

Figure 5. Tumor cells randomly positive on P63 (nuclear positive staining; ×40)

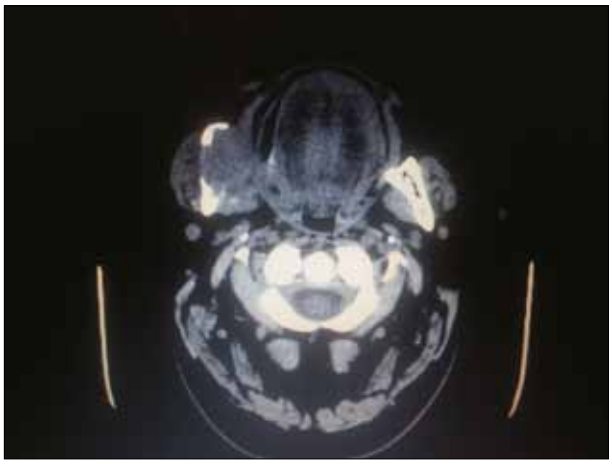

Figure 6. Multi-slice computer tomography (MSCT) revealed that lingual and buccal cortices are destroyed by tumour

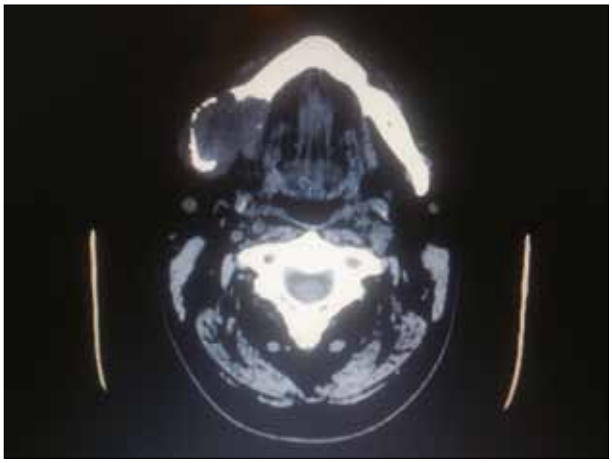

Figure 7. MSCT revealed an extension of tumor mass in the pterygomandibular space

tumor mass in the region of the body and angle of the mandible. The abovementioned region was covered with intact mucosa. Panoramic radiograph revealed a multilocular irregular osteolytic lesion in the region of the body, angle, and ramus of the mandible (Figure 2).

An incisional biopsy was performed due to large spectrum of pathological entities that could be diagnosed, and the result was a low-grade MEC (Figures 3–5). The tumor

doi: 10.2298/SARH1610531K

was composed of basal intermediary and mucosal cells. Presence of mucin was proven by alcian-blue PAS (AB-PAS) staining. Immunohistochemically, tumor cells were positive to CK7 and P63, and negative to S100. Magnetic resonance imaging revealed an expansive tumor formation with signs of bone destruction of the body, angle, and ramus of mandible, an extension of tumor mass to pterygomandibular space, as well as enlargement of lymph nodes at neck levels IIb, Ib, and IIa (Figures 6 and 7).

Considering the histopathological results and presence of enlarged lymph nodes, right hemimandibulectomy and tumour excision from pterygomandibular space, followed by supraomohyoid neck dissection, were performed. Operative histopathological result confirmed the presence of low-grade mucoepidermoid carcinoma, while the neck result was negative. Postoperative radiation therapy was applied after a successful postoperative period. The patient was followed up for the next two years through regular medical checkups every three months and then every six months. Clinical examination and insight into ultrasonography of the neck did not reveal signs of recurrence of the tumor or appearance of secondary deposits in the neck. A reconstruction of the postoperative defect of the mandible was proposed, which the patient refused.

DISCUSSION

CMEC is a very rare tumor, illustrated by only 200 described cases of this lesion in literature [7]. In average, it represents 2–3% of all MEC described in literature [8]. It is usually seen in women in the fourth and fifth decade of life, often in the region of the mandibular body [9]. Criteria that clearly confirm the diagnosis of CMEC include the following: 1. intact cortical bone; 2. radiological proof of bone destruction; 3. absence of primary tumor that would metastasize to the mandible; 4. absence of odontogenic tumor; 5. histopathological verification; 6. presence of intracellular mucin [10].

Degree of development of the disease is defined by the state of the surrounding bone [11]. Stage I represents an intact cortical bone, stage II intact cortical bone with minimal signs of expansion, and stage III represents a cortical destruction with encompassing surrounding periosteum. Our patient could be classified as stage III due to presence of buccal and lingual cortex of the mandible. Our patient had no metastases, although literature data show 9% of metastases in regional lymph nodes. Distant metastases are described in the region of the lungs and the brain [12].

True origin of the CMEC is still not explained in detail. Bouquot et al. [13] present data that intrabony presence of small salivary glands was spotted in 0.3% of the upper and lower jaw, which could be one of the causes. Ellis et al. [14] also presents data that confirm ectopic glandular tissue to be a rare pathological entity even in cases of histologically proven CMEC. Brookstone and Huvos [15] show evidence that CMEC is related to the presence of odontogenic cists in 32% of cases, while 1–2% of jaw tumors are related to the presence of impacted wisdom teeth.

Considering that our patient said that four years previously she had an extraction of the lower impacted wisdom tooth, it is possible that malignant transformation of the remaining cyst epithelial cells around the impacted tooth led to the creation of the CMEC.

The treatment included surgical removal of the tumor and post-operative radiation therapy. More conservative surgical methods like curettage, enucleation, and marginal resection of the jaw, with or without post-operative radiation, lead to relapse of primary disease in 40% of cases, while in the case of segmented resection of the mandible the relapse is in 4% of the cases [5]. Gradation of the tumor is one of the most important predicting factors for the survival of patients suffering from CMEC. High grade is characterized by larger bone destruction, enlarged frequency of relapses, and presence of regional and distant metastases [16].

CMEC represents a rare tumor, with causes still not fully defined in detail, which is characterized by signs of local destruction of tissue and ability to metastasize. Long-lasting asymptomatic clinical period, atypical radiological manifestation and wide spectrum of pathological entities can present a problem for the differential diagnosis during planning of the treatment. Initial biopsy represents the key to creating an operative plan. Radical excision with neck lymph node dissection followed by postoperative radiotherapy in our case represents successful method of treating CMEC of the mandible.

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Централни мукоепидермоидни карцином доње вилице – приказ болесника

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КРАТАК САДРЖАЈ

Увод Мукоепидермоидни карцином (МЕК) у односу на остале туморе пљувачних жлезда јавља се у 5 до 10% случајева. Хистопатолошки је подељен на добро диферентоване туморе који су ниског степена малигнитета, средње и лоше диферентоване туморе високог степена малигнитета. Централни мукоепидермоидни карцином (ЦМЕК) доње вилице је први описао Леп 1936. године, код 66-годишње пацијенткиње. ЦМЕК се одликује атипичном клиничком сликом и радиолошком презентацијом.

Приказ болесника Пацијенткиња старости 55 година јавила се на Клинику за Стоматологију у Нишу са анамнестичким подацима о присуству безболног отока у пределу доње вилице са десне стране. У складу са хистопатолошким резултатима и присуством увећаних лимфних нодуса дренажне зоне врата, урађена је супраомохиоидна лимфаденектомија праћена хемимандибулектомијом и екстирпацијом тумора у птеригомандибуларној области. Након уредно протеклог постоперативног тока спроведена је радиотерапија.

Закључак ЦМЕК представља редак тумор који карактеришу знаци локалне деструкције ткива и способност метастазирања. Иницијална биопсија представљала је кључ стварања оперативног плана. Хируршка ресекција инфилтрисаног подручја, лимфаденектомија врата и постоперативна радиотерапија били су успешни у лечењу ЦМЕК.

Кључне речи: централни карцином; оток доње вилице; биопсија; хируршка терапија

Примљен • Received: 23/10/2015 **Ревизија • Revision:** 13/01/2016 **Прихваћен • Accepted:** 26/01/2016

Epithelioid hemangioma in the oral mucosa – A case report

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SUMMARY

Introduction Epithelioid hemangioma is an uncommon benign vasoproliferative neoplasm that usually manifests as multiple red nodules in middle-aged adults.

Case Outline A 52-year-old male patient presented with a one-year history of a nodular lesion in the left buccal mucosa measuring 3 cm. The clinical hypothesis was lipoma. An excisional biopsy revealed a circumscribed lesion composed of lobules of vessels with perceptible or poor lumina, associated with a prominent inflammatory infiltrate consisting of eosinophils, histiocytes and chronic inflammatory cells. The endothelial cells composing the lesion had an epithelioid morphology and contained abundant eosinophilic cytoplasm. Immunohistochemistry for CD34, factor VIII, collagen IV, alpha-smooth muscle actin, and mast cells, as well as histochemical staining with Weigert's orcein were performed.

Conclusion Vascular proliferations of soft tissues are a diverse and morphologically complex group of lesions that are difficult to diagnose. This report presents a case of oral epithelioid hemangioma, highlighting relevant morphological and immunohistochemical features that could help distinguish this condition from other neoplasms.

Keywords: angiolymphoid hyperplasia with eosinophilia; epithelioid hemangioma; oral mucosa

INTRODUCTION

First described by Wells and Whimser in 1969 as angiolymphoid hyperplasia with eosinophilia, epithelioid hemangioma (EH) is an uncommon benign vasoproliferative neoplasm whose etiology and pathogenesis continue to be a matter of debate [1, 2, 3]. The term epithelioid hemangioma was proposed by Enzinger and Weiss in 1983 [1]. Despite the acceptance of this term since 1983, other terms have been used to describe this lesion, including angioblastic hyperplasia with eosinophilia, nodular angioblastic lymphoid hyperplasia with eosinophilia, lymphofolliculosis, pseudopyogenic granuloma, atypical pyogenic granuloma, and inflammatory angiomatous nodule [4].

EH commonly affects young adults and manifests as red or brown multiple nodules or small papules in the intradermal or subcutaneous region of the head and neck [1, 2, 4, 5, 6]. Extracutaneous lesions located in muscles, bone, and salivary glands are uncommon, and lesions involving oral mucosa are rarely observed [1, 4, 6, 7].

Histologically, EH is composed of well-developed, but frequently immature, vascular structures lined by aggregates of epithelioid- or histiocytoid-like endothelial cells that contain abundant eosinophilic cytoplasm. The endothelial cells are usually associated with a prominent inflammatory infiltrate consisting of dispersed lymphocytes, eosinophils, histiocytes and mast cells [1, 3, 6]. Most lesions are well circumscribed and have a lobular architecture. Lymphoid follicles with a germinal center may be present [1, 4, 5]. Although recurrence is observed in one third of cases of EH, metastasis is extremely rare and surgical excision generally leads to cure [5].

This case report presents an oral EH, highlighting morphological and immunohistochemical features that are important for the differential diagnosis of this lesion.

CASE REPORT

A 52-year-old black male patient was admitted to the Surgery and Oral-Maxillofacial Traumatology Service with main complaint of having a lump in the cheek that started to appear one year previously. The patient was a non-smoker and non-drinker, had no history of systemic alterations, and laboratory parameters were within the normal range. Intraoral clinical examination revealed a sessile swelling in the left buccal mucosa with a smooth erythematous

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Figure 1. View of oral mucosa lesion; note the swelling in the left buccal mucosa with a smooth erythematous surface, which measured 3 cm in its major diameter

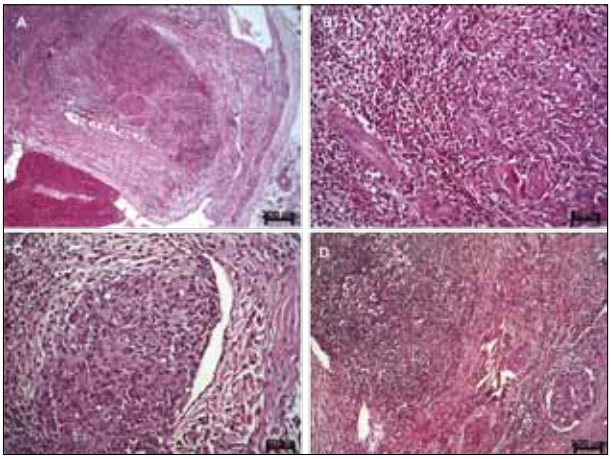

Figure 2. Histological sections of EH stained with hematoxylin-eosin: (A) well-delimited lesion exhibiting proliferation of endothelial cells in a lobular arrangement; (B) note the lobule composed of epithelioid endothelial cells near vascular spaces and associated with numerous eosinophils; (C) detail of the blood vessel wall surrounded by epithelioid cells; (D) left, lymphoid follicles; right, blood vessels with a thickened wall surrounded by epithelioid cells

surface, which was tender to palpation and measured 3 cm in its major diameter (Figure 1). The patient reported no pain to manipulation and there were no signs of an association with dental trauma or infection. Under the clinical hypothesis of lipoma, the patient was submitted to an excisional biopsy. The lesion was removed with safety margins and exhibited defined limits, no adherence to deeper planes, and no signs of excessive bleeding.

The specimen was sent for histopathological analysis, which showed a well-delimited lesion characterized by lobular proliferations. The lesion was composed of epithelioid endothelial cells with eosinophilic cytoplasm located near large vascular spaces or immature vessels. Vascular damage characterized by thrombosis and fibrointimal proliferation was seen in some wider vessels. Another finding was the abundant presence of eosinophils, sometimes near

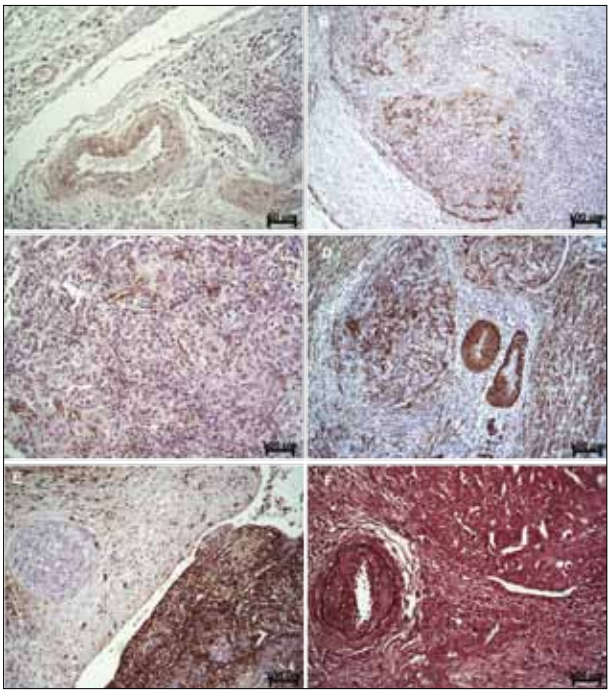

Figure 3. Histological sections of EH submitted to immunohistochemistry (A–E) and histochemistry (F): (A) note the immunostaining for collagen IV around blood vessels; (B) epithelioid endothelial cells and small-caliber vessels immunostained for factor VIII in a lobular arrangement; (C) CD34-positive vessels in an area rich in epithelioid endothelial cells and eosinophils; (D) strong immunostaining for α-SMA in vessels of different calibers; (E) right, note the presence of mast cells amidst numerous epithelioid endothelial cells; (F) Weigert's orcein staining around intact blood vessels, demonstrating the presence of elastic fibers; note the absence of elastic fibers in an area rich in endothelial cells.

lymphocytes, as well as formation of lymphoid follicles (Figures 2A–D). Mitotic figures and cellular atypia were absent. Immunohistochemistry for collagen IV, factor VIII, CD34, alpha-smooth muscle actin (α-SMA) and mast cells was performed, as well as histochemical staining with Weigert's orcein (Figures 3A–F). The antibodies, sources, dilutions and antigen retrieval methods used are shown in Table 1.

Immunohistochemical staining was positive for collagen IV and factor VIII around blood vessels (Figures 3A and 3B). The epithelioid endothelial cells were positive for factor VIII (Figure 3B), and CD34-positive vessels were observed in areas rich in epithelioid endothelial cells and eosinophils (Figure 3C). Strong α-SMA immunostaining was detected in small- and medium-caliber vessels (Figure 3D). Numerous mast cells were also found, especially in areas rich in epithelioid cells (Figure 3E). Staining with Weigert's orcein revealed the absence of elastic fibers in areas rich in epithelioid endothelial cells (Figure 3F).

The diagnosis of EH was based on the clinical and histopathological features. The patient has been followed up for approximately 18 months and shows good healing and no signs of recurrence.

DISCUSSION

This case report presents a 52-year-old patient with EH involving the cheek mucosa. Although uncommon, a search

Table 1. Specifications of the antibodies used

Antibody	Manufacturer	Clone	Antigen retrieval	Dilution	Incubation
Collagen IV	Dako	CIV22	Citrate pH6	1:25	1 hour / room temperature
Factor VIII	Dako	F8/86	Citrate pH6	1:100	1 hour / room temperature
CD34	Dako	QBEND10	Citrate pH6	1:200	1 hour / room temperature
α-SMA	Dako	1A4	Citrate pH6	1:150	1 hour / room temperature
Tryptase	Dako	AA1	Trypsin 1%	1:50	1 hour / room temperature

of the English-language literature for cases of EH involving the oral cavity identified 40 case reports, including the present case. The mean age of the patients was 37.7 years (range: 3–82 years). There was a slight predilection for young adult men (24 of 40 cases) (Table 2). In contrast, cutaneous EH more commonly affects women in the third and fourth decades of life [6]. There was a predominance of cases involving the lips (18 of 40 cases), while the cheek mucosa was affected in nine of the cases reported (Table 2). Involvement of the cheek mucosa seems to be less common and usually manifests as a nodule, features observed in the present case.

Clinical presentation of EH is nonspecific and is therefore often confused with epidermal cyst, angioma, pyogenic granuloma, Kaposi's sarcoma, salivary gland tumor, lymphoma and squamous cell carcinoma, among other less frequently cited conditions [1, 6]. In the present case, the nodular feature mimicked lipoma, an entity not yet suggested by other authors (Table 2). EH can also manifest as pruritus, macula and ulcer; however, the nodular form is the most common [1, 7, 9, 13, 16, 19, 21, 22, 23, 27].

In view of clinical similarities that EH shares with other diseases, histopathological examination is essential for establishment of the diagnosis. In the present case, the diagnosis was based on the typical histopathological features described in other studies [1, 2, 3, 6]. The morphological characteristics consisted of well-developed vessels and some immature vessels lined by aggregates of epithelioid endothelial cells associated with an exuberant component of inflammatory cells, particularly eosinophils and lymphocytes. According to Aggarwal and Keluskar [6], eosinophils typically account for 5–15% of the infiltrate, but can reach up to 50% in rare cases. Occasionally, the infiltrate is devoid of eosinophils. An interesting finding reported by Sun et al. [1] and Aggarwal and Keluskar [6] was the presence of cytoplasmic vacuoles in endothelial cells, a feature not observed in the present case. At low magnification, most of the lesions described by Sun et al. [1] also exhibited a characteristic lobular arrangement similar to the case reported here. Other features shared with the present case were the presence of lymphoid follicles, which are typical of EH, and the absence of cellular atypia [1, 2, 3]. In contrast to the seven cases reported by Sun et al. [1] which were non-encapsulated lesions, a well-defined capsule was observed in the present case. Furthermore, similar to the case reported recently by Kumar et al. [3], evidence of thrombi inside dilated vessels was found in the present patient, suggesting the occurrence of vascular damage.

Although these morphological features are well established, it is important to differentiate oral EH from other vascular tumors that also consist of epithelioid-like endothelial cells since some of these tumors exhibit a different biological behavior, prognosis and treatment. The differential diagnosis of EH includes epithelioid angiosarcoma, epithelioid hemangioendothelioma, hobnail hemangioma, and epithelioid angiomatous nodule [1, 4, 5]. Epithelioid angiosarcoma is characterized by an infiltrative and destructive growth pattern and is composed of highly pleomorphic cells, in addition to the presence of atypical mitotic figures and areas of necrosis [5]. Epithelioid hemangioendothelioma also exhibits an infiltrative growth pattern in the form of cords and nests of spindle-shaped, discretely pleomorphic endothelial cells amidst a hyaline and myxoid stroma [6]. However, well-defined vascular canals are rarely found [6]. In contrast to EH, epithelioid hemangioendothelioma is associated with high rates of morbidity and mortality due to recurrence and regional metastasis [5]. Hobnail hemangioma is characterized by a biphasic growth pattern consisting of well-defined dilated vessels lined by prominent "hobnailed" endothelial cells and neoplastic vessels in the deep portion of the lesion. This tumor is extremely rare in the oral mucosa [5]. Finally, epithelioid angiomatous nodule shares many clinical and histopathological features with EH; however, this lesion seems to be confined to the dermis [5].

EH should also be differentiated from Kimura's disease and bacillary angiomatosis. Kimura's disease also exhibits a prominent inflammatory infiltrates associated with blood vessels and lymphoid follicles with germinal centers, but aggregates of epithelioid endothelial cells with abundant eosinophilic cytoplasm are absent [5, 27]. Moreover, Kimura's disease manifests as nodules or deep tumors accompanied by lymphadenopathy and marked peripheral eosinophilia, representing a chronic inflammatory process and not originating from proliferation of endothelial cells [5, 6]. There has been extensive debate whether EH is a variant of Kimura's disease. However, today it is accepted that the two diseases are independent and represent separate entities with distinct clinical and histopathological features [6]. Bacillary angiomatosis usually develops in immunodeficient patients and is mainly caused by infection with *Bartonella henselae*. Microscopic analysis shows abundant neutrophils associated with clusters of bacteria [5].

Although some peculiar morphological features help recognize EH and exclude other vascular lesions, immunohistochemical markers can be used to confirm the vascular nature of epithelioid-like cells or even to better characterize the lesion. These epithelioid endothelial cells are positive for endothelial cell markers such as factor VIII and CD34, a fact observed in the present study. Similarly,

Sun et al. [1] detected less prominent staining for CD34 compared to factor VIII. Immunostaining for α-SMA was useful in demonstrating the myopericytic layer around immature blood vessels. A similar immunoreactivity pattern for these markers has been observed in other studies [1, 2]. In the present case, collagen IV staining permitted identification of small immature vessels with indistinguishable or inconspicuous lumina. Positive immunostaining for mast cells confirmed the presence of these cells, which have also been detected in EH by other authors [1, 3].

The pathogenesis of EH continues uncertain and two strong hypothesis have been proposed, which consider it to be a reactive lesion or true neoplasm. The features that support the reactive condition would be the preference of EH for the surface of soft tissues, the presence of central areas containing epithelioid endothelial cells in an

Table 2. Summary of clinical data of previously reported EH in oral mucosa

Age (years)	Sex	Lesion	Location	Preoperative diagnosis	Reference
37	Female	Nodule	Lower lip	Kaposi's sarcoma	Rosai and Akerman [8]
28	Female	Macule	Palate	Lymphoma	Saxe and Kahn [9]
22	Male	Nodules	Upper and lower lip	-	Dickens [10]
28	Male	Nodule	Upper lip	Salivary gland adenoma	Buckerffeld and Edwards [11]
46	Male	Nodule	Upper lip	-	Eveson and Lucas [12]
13	Male	Pruritus	Lower lip	-	Buchner et al. [13]
28	Male	Nodules	Oral mucosa	Pyogenic granuloma	Massa et al. [14]
32	Male	Nodule	Upper lip	-	Peters et al. [15]
31	Male	Macule	Togue	-	Iguchi et al. [16]
25	Female	Nodule	Palate	-	Moran et al. [17]
42	Female	Nodule	Buccal mucosa	-	Kabani et al. [18]
82	Male	Ulcer	Togue	Squamous cell carcinoma	Razquin et al. [19]
48	Male	Nodule	Togue	-	Artazkoz et al. [20]
55	Male	Ulcer	Lower lip	Squamous cell carcinoma	Lopez and Battaglino [21]
17	Male	Nodule	Buccal mucosa	Pyogenic granuloma	Toeg et al. [22]
12	Male	Ulcer	Buccal mucosa	-	Toeg et al. [22]
65	Male	Nodule	Upper lip	EH	Renshaw and Rosai [23]
43	Female	Nodule	Upper lip	EH	Renshaw and Rosai [23]
3	Male	Nodule	Upper lip	EH	Renshaw and Rosai [23]
34	Female	Pruritus	Lower lip	EH	Renshaw and Rosai [23]
59	Male	Nodule	Buccal mucosa	-	Misselevich et al. [24]
30	Female	Nodule	Upper lip	-	Bartralot et al. [25]
27	Female	Nodule	Buccal mucosa	-	Martin-Granizor et al. [26]
54	Male	Nodule	Lower lip	-	Martin-Granizor et al. [26]
43	Female	Nodule	Togue	-	Martin-Granizor et al. [26]
30	Female	Nodule	Upper lip	-	Mariatos et al. [4]
23	Male	Ulcer	Togue	Malignant tumor	Shimoyama et al. [27]
60	Male	Nodule	Buccal mucosa	-	Tsuboi et al. [28]
56	Male	Ulcer	Togue	-	Park et al. [7]
42	Male	Nodule	Palate	Salivary gland adenoma	Sun et al. [1]
48	Male	Nodule	Togue	Vascular tumor	Sun et al. [1]
52	Male	Nodules	Togue	Vascular tumor	Sun et al. [1]
65	Female	Nodule	Togue	Pyogenic granuloma	Sun et al. [1]
31	Female	Nodule	Upper lip	Vascular tumor	Sun et al. [1]
8	Male	Nodule	Lower lip	Pyogenic granuloma	Sun et al. [1]
32	Female	Ulcer	Togue	Squamous cell carcinoma	Sun et al. [1]
65	Female	Nodule	Lower lip	-	Miteva et al. [2]
25	Female	Nodule	Buccal mucosa, upper and lower lip	Atypical granuloma	Aggarwal and Keluskar [6]
30	Female	Nodule	Gingiva	-	Kumar et al. [3]
52	Male	Nodule	Buccal mucosa	Lipoma	Present

doi: 10.2298/SARH1610535H

indeterminate pattern, peripheral vessels surrounded by the proliferation of epithelioid cells, and vascular damage characterized by fragmentation of the elastic lamina, fibrointimal proliferation and rupture of the muscle wall [29]. From this perspective, some authors believe that local trauma may trigger cell proliferation of the reactive condition [3, 26]. On the other hand, the invasive growth of EH observed in some cases and possible recurrence suggest a true neoplastic process [1, 3, 4].

Sun et al. [29] suggested local hypoxia mediated by congenital vascular malformation or trauma to play an important role in the pathogenesis of EH. Hypoxia would be responsible for proliferation of endothelial cells, especially by promoting synthesis of vascular endothelial growth factor (VEGF) that results in formation of a vascular tumor. Additionally, inflammatory cells, such as eosinophils and mast cells, contribute to proliferation of endothelial cells in EH. Hypoxia has been shown to stimulate degranulation of mast cells and eosinophils, increasing the expression of hypoxia-inducible factor 1 (HIF-1) and VEGF and thus inducing the proliferation of endothelial cells [30].

Some features observed in the present case, such as evidence of vascular damage including thrombosis and fibrointimal proliferation, seem to support the reactive condition. However, the positive staining of epithelioid endothelial cells, which lined the vessels or formed aggregates, for the vascular markers cited above favors the neoplastic nature of this lesion. Another finding that complements and supports the neoplastic nature of the present case is the absence of elastic fibers, demonstrated by staining with Weigert's orcein amidst epithelioid endothelial cell proliferations, which rules out the hypothesis that the proliferation of these cells would have been triggered by vascular damage. In the present case, elastic fibers were only found surrounding the wall of intact blood vessels.

Considering the possible neoplastic nature of the lesion, according to Miteva et al. [2] it remains unclear whether EH arises from the exclusive proliferation of endothelial cells derived from blood vessels or, in fact, exhibits a biphasic pattern, also involving proliferation of endothelial cells derived from lymphatic vessels. The authors therefore performed an immunohistochemical analysis using D2-40 and observed an increase in the number of lymphatic vessels inside the tumor. On the basis of these results, the authors suggested that the lymphoid component of EH may result from lymphangiogenesis.

Treatment of EH is often a challenge. Intralesional corticosteroids, cryotherapy, laser cauterization and irradiation have been used, but are not very effective. Surgical excision and follow-up continue to be the most recommended treatment [3, 4, 6]. Most patients with oral EH (Table 2) were submitted to complete surgical resection (37 of 40 cases) without additional therapy, and recurrence was relatively rare, reported in only five studies published in the literature [3, 8, 9, 14, 20]. Local recurrence is common in one third of patients with cutaneous EH, but is rare in the oral mucosa [1, 3]. The present case is consistent with the literature since the lesion was treated by complete surgical excision, showing no signs of recurrence so far. As expected, none of the cases of oral EH was associated with metastases.

EH in the oral mucosa is uncommon and the clinical and histopathological differential diagnosis with other lesions is necessary, including non-neoplastic proliferative processes or neoplasms of vascular, lymphoid, glandular and epithelial origin. For this purpose, careful morphological analysis should be performed based on the peculiarities of each entity. Immunohistochemical analysis may be used in some cases.

NOTE

The abstract of the paper has been published in Oral Surgery, Oral Medicine, Oral Pathology, and Oral Radiology (Vidal MTA, Gurgel CA, Leitão ÁCGH, Ramos EAG, Xavier FCA, Ramalho LMP, Santos JN. Epithelioid Hemangioma Located in The Buccal Mucosa: Case Report. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014; 117(2):e155).

Written informed consent for publication of this case report and any accompanying images was obtained from the patient.

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Епителиоидни хемангиом слузокоже уста – приказ болесника

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КРАТАК САДРЖАЈ

Увод Епителиоидни хемангиом је ретка бенигна вазопролиферативна неоплазма која се обично испољава код одраслих особа средњег животног доба у виду црвених израслина.

Приказ болесника Болесник стар 52 године јавио се са квржастом лезијом пречника 3 *cm* с леве стране слузокоже уста, насталом годину дана раније. Клиничка претпоставка била је да се ради о липому. Ексцизиона бипсија показала је омеђену лезију сачињену од режњева судова са слабо приметним или малим луменом, који се доводе у везу са израженим инфламаторним инфилтратом сачињеним од еозинофила, хистиоцита и хроничних инфламаторних ћелија. Ендотелијске ћелије које су чиниле лезију имале су епителиоидну морфологију и садржале су веће количине еозинофилне цитоплазме. Извршена је имунохистхемијска анализа за *CD*34, фактор VIII, колаген VI, *α*-актин глатких мишића и мастоците, као и хистохемијско бојење орцеином Вајгертовом методом.

Закључак Разнородна и морфолошки сложена група лезија тешка за дијагностиковање сачињава васкуларне пролиферације меког ткива. Овај приказ представља случај оралног епителиоидног хемангиома, при чему је акценат на релевантним морфолошким и имунохистохемијским особинама које могу помоћи да се ово стање диференцира од других неоплазми.

Кључне речи: ангиолимфоидна хиперплазија са еозинофилијом; епителиоидни хемангиом; слузокожа уста

Примљен • Received: 29/10/2015 **Ревизија • Revision:**15/01/2016 **Прихваћен • Accepted:** 20/01/2016

Symptomatic isolated thoracic splenosis 11 years after abdominal trauma – Case report

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SUMMARY
Introduction Thoracic splenosis is defined as the autotransplantation of splenic tissue into thorax. It occurs due to splenic rupture in association with a diaphragmatic tear on the left side after a traumatic event. It is a rare disease that most commonly remains undiscovered as it is usually asymptomatic.
Case Outline We present a symptomatic case of thoracic splenosis in a 53-year-old smoker male patient with a medical history of abdominal surgery and splenectomy for a thoracoabdominal gunshot. Three years before the medical examination he was suffering from dyspnea, frequent coughing, left pleuritic chest pain and complained about faster fatigue. A chest radiograph obtained during a medical checkup showed a multinodular left pleura-based mass in the upper lobe. Established histopathological diagnosis after surgical removal of the nodule was splenosis. No evidence of malignancy was observed.
Conclusion Splenosis should be considered as a differential diagnosis by the undertaken workup of left pulmonary nodules or masses in patients with a history of trauma.
Keywords: symptomatic thoracic splenosis; splenectomy; thoracoabdominal gunshot

INTRODUCTION

Splenosis, a condition also known as ectopic spleen, is an extremely rare occurrence that is defined as autotransplantation of splenic tissue usually after splenic rupture due to trauma and in association with a subsequent splenectomy [1]. With splenosis, splenic tissue is most commonly seeded into the abdominal cavity or pelvis. Thoracic splenosis occurs less frequently than abdominal splenosis and may be found in 18% of patients after splenic rupture. The diagnosis of thoracic splenosis can be established noninvasively with several diagnostic modalities [2, 3]. Computed tomography (CT) or ultrasonographic imaging should be used to identify areas of possible ectopic tissue, although diagnosis is confirmed postoperatively by means of pathologic analysis. A minimally invasive approach for excision of such masses could be completed with minimal morbidity. Video-assisted thoracoscopic surgery is an option that can serve both diagnostic and therapeutic purposes [4]. Pathologic analysis can rule out such causes as pulmonary metastases, non-Hodgkin lymphoma, or mesothelioma. Some authors reported 57 cases of thoracic splenosis and four symptomatic cases in the English-language literature [5, 6]. Reviewing the MEDLINE database this is the first described case of intrathoracic splenosis in Bosnia and Herzegovina and the second symptomatic case in Europe.

CASE REPORT

A 53-year-old man was referred to our clinic because of dyspnea, frequent coughing, faster fatigue and left chest pain. He used to smoke four cigarette packs per day for 20 years and quit smoking three months before presentation. His surgical history included thoracoabdominal gunshot wound suffered 11 years previously which required an emergency splenectomy, without surgical opening of the chest. His medical history included tachycardia in the past five years. On admission, physical examination was unremarkable, and laboratory tests were normal. Chest radiographs showed oval homogeneous soft tissue opacity, measuring 4.5 × 4 cm in the left lung lobe, between first and second ribs (Figure 1). The chest CT scan revealed two well-circumscribed lobulated, nodular masses, measuring up to 2 cm in diameter in the back and the front of the left upper lobe. These nodules were not calcified. Also, a CT scan of the apical region showed subpleural fibrosis (Figure 2). In our case, technetium-99m (^{99m}Tc) scintigraphy was unavailable, so we achieved diagnosis with fine-needle aspiration, and pathohistological examination. After analysis of a sample of the biggest nodule, obtained by percutaneous aspiration biopsy and by immunochemistry analysis (vimentin+++, LCA+++, CD34-, S-100-, CK-), it was supposed that disease was connected to mesenchymal, cellular, predominantly lymphocytic well-vascularized, probably benign tumor, but no accurate pathologic diagnosis was made.

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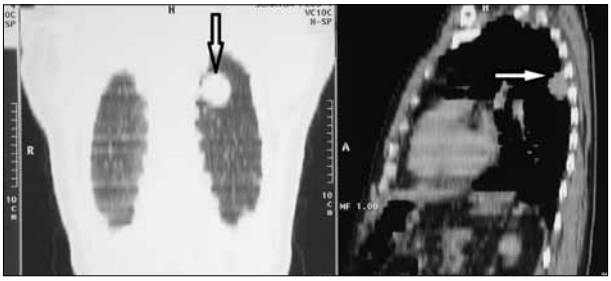

Figure 1. Chest radiograph (anterior and lateral views)

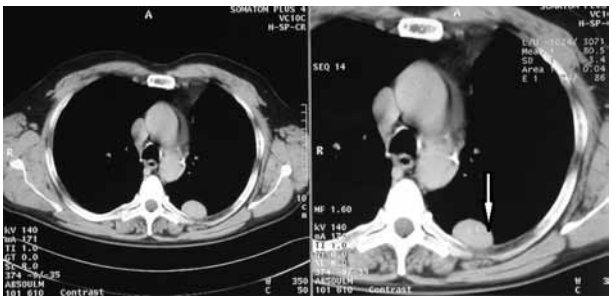

Figure 2. Thoracic computed tomography – lobulated pleural-based mass in the posterior segment of the upper lobe

An oval smooth surface nodule of 4 × 3.5 × 1.5 cm in size was removed from the apical part of the left lung lobe. In cross-section, tissue appeared soft, purple, homogenous, with adjacent several smaller oval solid nodules, also with smooth surface, size ranging from 0.3 cm to 0.7 cm, localized in the parietal pleura (Figure 3a). Histological examination of the relevant sites revealed splenic tissue with white and red pulp, lymphoid follicles, secondary germinal centers and granulocytes, surrounded by a fibrotic ring (Figure 3b). These findings were consistent with splenosis in all the relevant biopsies. No evidence of malignancy was observed.

DISCUSSION

Thoracic splenosis, first reported by Shaw and Shafi [7] in 1937, describes the autotransplantation of splenic tissue into the pleural cavity after splenectomy for traumatic or iatrogenic injury, resulting in multiple nodular implants on the left pleura. As imaging technology improved, splenosis has been noted in up to two thirds of patients after splenectomy for trauma. However, its frequency is likely underestimated because most splenic implants are asymptomatic and are only incidentally discovered during chest X-ray or CT. Abdominal splenosis is the most common type of splenosis, with most common sites of autotransplantation of the spleen being the mesentery, peritoneum, and omentum. Abdominal and pelvic splenosis are often mistaken for abdominal lymphoma, metastatic disease, carcinomatosis, primary renal or hepatic malignancy, adenomas, endometriosis or simple adenopathy [8]. Subcutaneous splenosis is quite rare, with less than 14 reported cases. The differential diagnosis of cutaneous splenosis includes differentiated cutaneous lymphoma, nodular Kaposi sarcoma, and subcutaneous vascular malformations. There is only one case report of intracerebral splenosis in the medical literature. Similarly, due to the spleen's anatomic location, thoracic splenosis arises almost exclusively in the left hemithorax. The most common location of thoracic splenosis occurs in the pleural cavity, and most commonly involves a diaphragmatic tear, or rarely a diaphragmatic hiatus, and small pieces of splenic tissue are displaced into the left hemithorax through the diaphragmatic opening. A second mechanism is the hematogenous spread of splenic pulp, as suggested by case reports of intrahepatic, as well as intracranial splenosis [9, 10, 11]. In fact, all cases of reported thoracic splenosis had diaphragmatic rupture as well as splenic rupture [9, 12]. In our case, spleen nodules were localized in the apex of the left lung with no evidence of them near the diaphragm. Hematogenous dissemination of splenic tissues probably occurred in this patient rather than passing through a diaphragmatic tear. Maximum amount of time that can pass between the initial trauma and the appearance of pulmonary splenosis is 40 years, and minimum is 13 years. The average interval between the initial trauma and diagnosis of thoracic

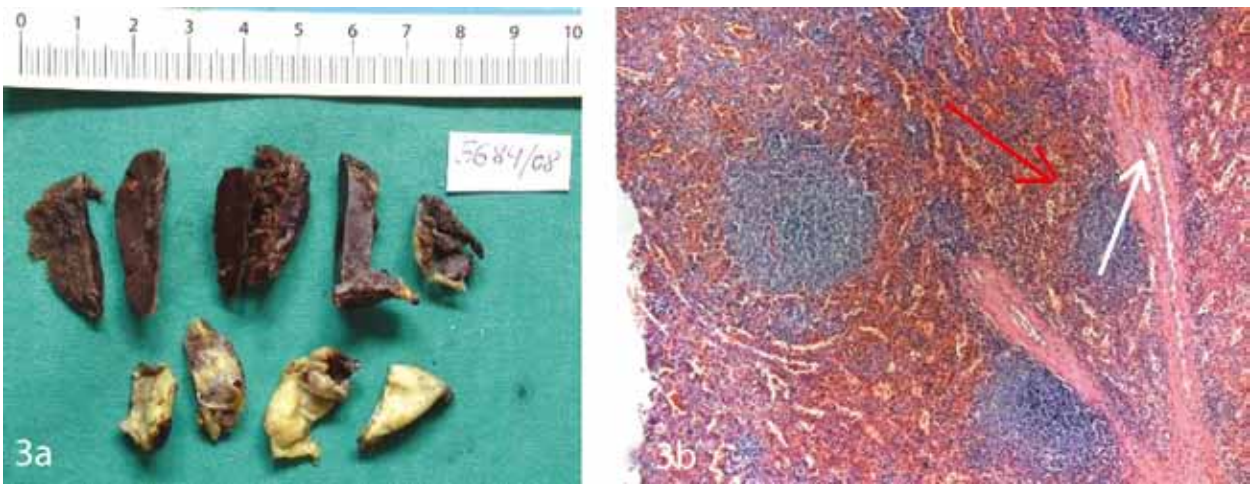

Figure 3. a) Macroscopic appearance of intrathoracic splenosis; b) microscopic appearance (haematoxylin and eosin staining; 100×): red pulp with reticular tissue and wide sinus filled with blood (red arrow); white pulp built of lymphoreticular tissue in the form of lymph nodules (Malpighi bodies) with a central artery (white arrow)

doi: 10.2298/SARH1610541P

splenosis is 21 years [13]. This is the first case of splenosis in which the symptoms of the disease appeared 11 years after the injury. Autotransplanted spleens differ from accessory spleens by blood supply, local perforator arteries versus splenic artery, respectively. Accessory spleens which are few in number occur near the splenopancreatic ligament and gastrosplenic ligament and have been found in up to 40% of patients undergoing autopsies. In contrast, splenosis nodules are numerous (up to 100) appearing in any location, particularly on serosal surfaces, and, though encapsulated, have no well-defined hilum. The splenic implants are sessile or pedunculated reddish blue nodules. These deposits may vary in number, they can be of any shape, and their diameter can range from a few millimeters to 12 cm. Splenosis can occur with either single or multiple nodules – upwards of 400 in some cases [14]. The diagnosis of thoracic splenosis can be established noninvasively with several diagnostic modalities: radionuclide scintigraphy or magnetic resonance imaging. Technetium-99m-labeled heat-damaged erythrocyte scanning is the preferred method because it is significantly more specific than sulfur colloid, indium-111-labeled platelet, or ^{99m}Tc-labeled white blood cell scanning due to diminished liver uptake. Pleural lesion often requires a fine-needle aspiration if a lesion is accessible. Pathologic presentation can be misleading, with lymphocytic infiltrate misdiagnosed as lymphoma [15, 16]. If the diagnosis can be confirmed preoperatively, surgery is not indicated unless the patient is symptomatic. It is usually not necessary to remove the pulmonary nodules because the splenic tissue is slow growing, noninvasive and nonmalignant [17–20]. Four symptomatic cases of thoracic splenosis have been reported; two patients reported having hemoptysis, one patient complained of a productive cough, and a patient reported having pleuritic chest pain [21, 22]. Previously reported case of symptomatic thoracic splenosis has significant interval growth of the splenic tissue and the large size of the mass described. Our patient was indicated for operative treatment because of chest pain. We postulate that the pain in our patient was exhibited itself due to an irritation of the parietal pleura.

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Симптоматска изолована грудна спленоза 11 година после повреде трбуха – приказ болесника

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КРАТАК САДРЖАЈ

Увод Грудна спленоза представља аутотрансплантацију ткива слезине у грудни кош. Настаје због трауматског цепања слезине и истовремене повреде леве дијафрагме. Ово ретко обољење најчешће остаје неоткривено јер није праћено симптомима болести.

Приказ болесника Представљамо случај симптоматске торакалне спленозе код мушкарца старог 53 године, дугогодишњег пушача цигарета који је имао оперативно уклањање слезине због повреде трбуха и грудног коша рањавањем из ватреног оружја. Од симптома болести имао је отежано дисање, кашаљ, брзо замарање и бол у левој страни грудног коша три године пре медицинског прегледа. Симптоми болести су упућивали на карцином плућа. На радиографији грудног коша уочава се туморска маса саграђена од неколико мањих чворова, а локализована уз плеуру у горњем режњу левог плућног крила. После хируршког уклањања туморске масе патохистолошка дијагноза је била торакална спленоза уз одсуство малигне болести.

Закључак Код пацијената са чворовима или туморским масама локализованим у левом плућном крилу и медицинском историјом трауме спленоза мора бити укључена у диференцијалну дијагнозу.

Кључне речи: симптоматска грудна спленоза; спленектомија; рањавање стомака и грудног коша ватреним оружјем

Примљен • Received: 26/11/2015 **Ревизија • Revision:** 10/05/2016 **Прихваћен • Accepted:** 16/05/2016

Single stage surgical treatment of amniotic band syndrome – Case report

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SUMMARY

Introduction Amniotic band syndrome is a rare congenital disorder with clinical presentation of constricting bands in different parts of extremities or whole extremities. Conservative or surgical treatment is provided depending on the type and severity of the anomaly.

Case Outline The paper presents the case of a neonate patient with constriction bands localized on the left leg. During the second week of life, a surgery was indicated, and a single-stage multiple Z-plasty was performed to correct the anomalies on the left lower leg. Postoperative edema in the distal part of the lower leg was easily managed by incisions and drainage. Two months later, the correction of the stricture of the left thigh was managed using the same procedure. The postoperative course was uneventful and the outcome was satisfactory after a two-year follow-up.

Conclusion Evaluation of a patient with amniotic band syndrome, as well as diagnosis, monitoring, treatment and postoperative care, should always be multidisciplinary. A single-stage correction approach provided satisfactory both functional and aesthetic results. Given many morphological variations of the syndrome, a decision on the strategy of treatment should be made individually for each patient.

Keywords: amniotic band syndrome; congenital anomaly; pediatric surgery; treatment; outcome

INTRODUCTION

Amniotic band syndrome (ABS), also known as Streeter's dysplasia, amniotic deformity, adhesion and mutilations, ADAM complex, represents a heterogeneous group of congenital anomalies with the incidence of 1:1,200 to 1:15,000 live births with equal frequency in both sexes [1, 2]. As many as 34 different names corresponding to this entity can be found in the literature [3].

The syndrome refers to a broad spectrum of anomalies including simple or multiple strictures, affecting only a portion or the whole circumference of limbs, oligodactyly, acrosyndactyly, pes equinovarus, cleft lip and palate, hemangioma, anencephaly or intrauterine fetal death due to umbilical cord strangulation [4]. Deformities may involve any portion of the fetus depending on the intrauterine localization. The extremities are involved most commonly, especially distal parts, while neck, chest and abdomen are rarely affected [5, 6].

Being formed inside the uterus, bands exert variable pressure on the tissue. As a result, changes that occur may vary from shallow wrinkles to complete amputation. In most cases, constriction bands extend to the first layer of fascia [6].

Peterson classifies clinical ABS into the following four types: type 1 – the presence of simple constriction ring; type 2 – the emergence of the ring combined with changes of distal parts, with or without lymphedema; type 3 – the presence of the strictures in combination with the fusion of the soft tissues in the distal parts; and type 4 – intrauterine amputation at any level of limb or finger. Deformities are usually caused by merger of soft tissue or lymphedema, but there are also angulations, flexion contractures, and stiff joints [7].

A routine prenatal ultrasound examination could reveal the presence of bands in utero, restricted fetal movements, oligohydramnios or characteristic deformities of the fetus. Besides ultrasound, in prenatal diagnosis, magnetic resonance imaging could give more detailed information [8]. Unfortunately, since prenatal diagnosis of ABS is not always possible, clinical examination after the birth is critical for diagnosis [9, 10].

Surgical treatment is not necessary in cases where vascularization is not compromised and there is no occurrence of lymphedema. However, it is often carried out for aesthetic reasons. If there are signs of lymphedema and functional disorder, urgent surgical treatment is necessary [1, 4].

CASE REPORT

A twin female newborn was sent from the regional hospital to our hospital after detecting anomalies corresponding to the ABS, during the first day of life. Pregnancy began as in vitro fertilization. It was uncomplicated and terminated with cesarean section at term (BM 3,045 g, 48 cm BL, AS 10/10).

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Figure 1. Preoperative clinical findings, constriction rings in upper and lower left leg

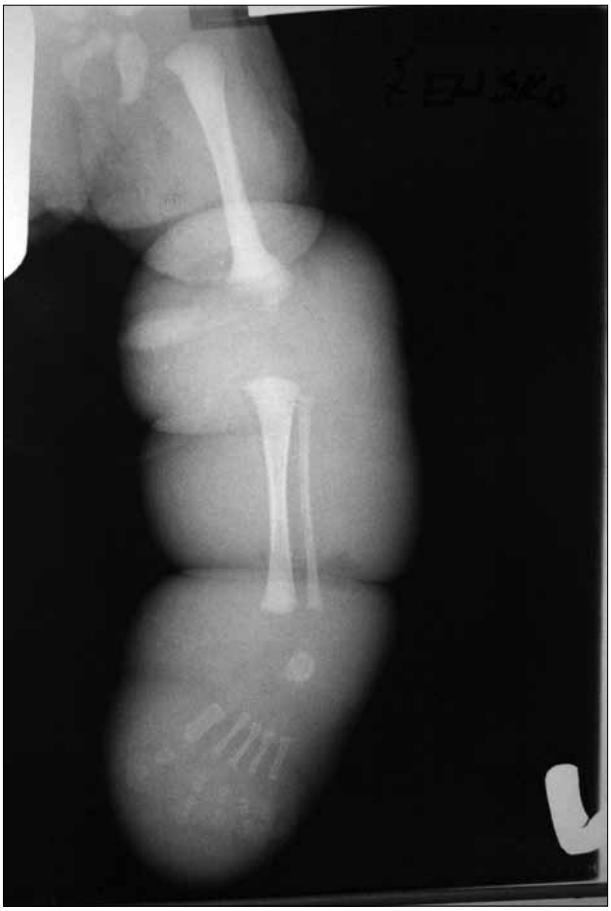

Figure 2. X-ray image of the left leg

A physical examination revealed the annular strictures in the left leg, and lymphedema with interruption of continuity of the skin, subcutaneous tissue and fascia in the both upper and lower leg (Figure 1). Ultrasound of head and abdomen, ECG and X-ray (Figure 2) were performed to detect if there were any other anomalies. Pediatrician and geneticist were consulted as well.

Since no other disorders were detected, an ultrasound and CT scan of lower extremity were conducted. Lymphedema of the entire circumference from the middle third of the left thigh to the lower leg and foot with hypoplasia of bone structures were observed, while muscular structures of the middle third of the lower leg and thigh were not

Figure 3. Excision of the fibrous, subcutaneous tissue and skin of the entire circumference around the stricture

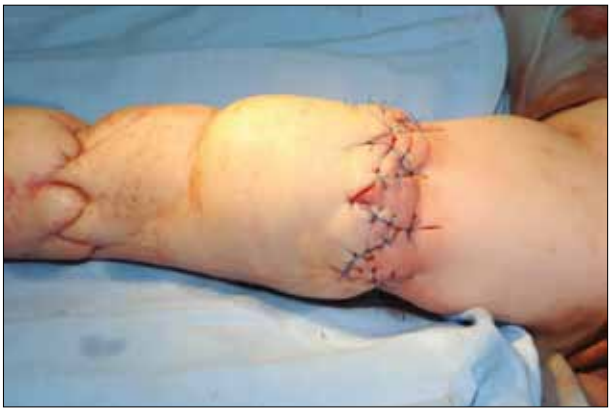

Figure 4. Reconstruction of the defect using multiple Z-plasty (second surgery)

well visualized. Deep veins were elastic, compressible, with satisfactory flow. They could be traced up to the second groove of edematous segment of the left leg. Numerous superficial venous blood vessels along with hyperechogenous muscles were present in edematous segment, while preserved architectonics of a muscle layer was visualized in the proximal non-edematous part of the leg.

The newborn was admitted to the intensive care unit where preoperative preparations were conducted. The surgical treatment of the anomalies was performed on the thirteenth day. The surgical procedure started with excision of the fibrous tissue and skin of the entire circumference of the distal third of the left lower leg about 3cm around the stricture, followed with the reconstruction of the defect using multiple "Z" plasty, forming local triangular lobes. Multiple "Z" plasty was used instead of direct closure to prevent future contracture of the scar or functional impairment which can be expected during the growth due to uneven formation of "normal" scar. Postoperatively, an edema was observed distally from the incision line. Edema resolved after incision and drainage of the area, and on the fourth postoperative day, the child was transferred to the Neonatal surgical ward where compressive bandaging was conducted on daily basis.

Two months later, the correction of the constriction band in the left thigh was performed applying the same technique (Figures 3 and 4). Postoperatively, there was no formation of lymphedema, and the child was discharged.

doi: 10.2298/SARH1610545K

Figure 5. Postoperative result after a year

The child was referred to physical therapy and had periodical check-ups during the recovery. Two years after the treatment, the child had no functional impairment, walking scheme was well preserved, and postoperative scars had satisfactory characteristics (Figures 5 and 6). The plan was to continue regular check-ups in order to monitor the possible changes during the growth and development of the child, as well as assessing the need for any corrective interventions.

DISCUSSION

The cause of the stricture development is not fully understood yet. There are two theories of the ABS onset described in the literature. In 1930, Streeter [11] claimed that before the formation of the embryo primary defect arises from the subcutaneous tissue in the early embryonic period (intrinsic theory). In 1965, Torpin [12], however, suggested that the formation of mesenchymal, amniotic bands is a consequence of premature rupture of membranes, oligohydramnios, protrusion of fetal parts into the chorionic cavity, and vascular compression of parts or entire limbs (extrinsic theory).

This theory could not explain the simultaneous occurrence of conditions such as anal atresia, polydactyly, cleft lip with or without cleft palate. A number of papers indicate that vascular compromise is obvious in the development of craniofacial and abdominal wall defects [13]. Some studies, which have proven the existence of blood vessels anomalies (bifurcation or trifurcation of artery, absence or segmental atresia of large blood vessels),

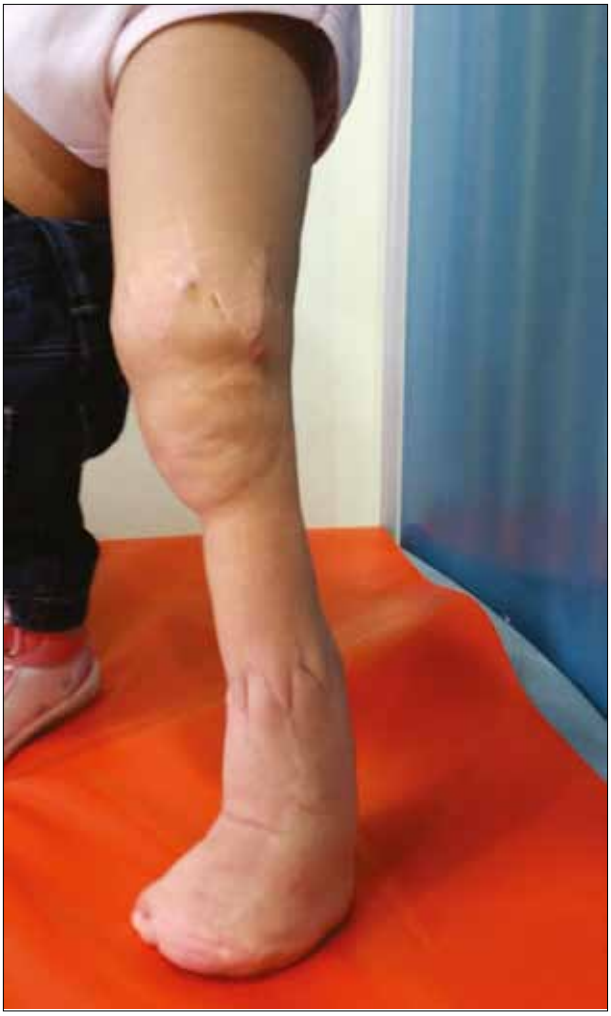

Figure 6. Postoperative result after two years

compared to the contralateral unaffected limb, point out that the same pathogenic mechanism is also involved in developing ABS [14, 15].

The association of malformations of the limbs with malformations of other organs in ABS supports the "intrinsic" theory of vascular accident during the early stages of embryogenesis. Greater incidence of the syndrome in closest relatives supports the idea of the existence of predisposing genetic factors for ABS.

On the other hand, additional factors such as smoking, use of drugs, alcohol, and psychoactive substances, some diseases that affect the vascular system (diabetes), and iatrogenic lesions (injuries to the amniotic membrane during amniocentesis), seem to be equally significant, and all in favor of "extrinsic" theory [16].

Both theories give a good explanation for the onset of ABS, which is most likely caused by the combination of genetic and environmental factors.

Numerous cases of ABS are described in the literature. However, the same two cases of this syndrome have never been described. This emphasizes the importance of an individual approach to the treatment of each patient [16, 17].

Today, several approaches are used in correction of the strictures. The main aim of all surgical techniques is preservation of the function of the affected region, followed

by an improvement of the aesthetic appearance. The most commonly used techniques in solving the strictures are Z-plasties, W-plasties, Mutaf technique, the sine plasty, and direct closure of the defect after the excision of the band in one or two stages [4].

Stevensons, and many other authors indicate that the treatment in two acts reduces the likelihood of vascular complications in distal parts and lymphedema, as well as that closure of the defect without mobilization of subcutaneous adipose tissue could cause the formation of the scar tissue that will have a constrictive effect after surgery (the hourglass phenomenon) [1, 18–22]. In the literature, there are several papers that favor resolving congenital constrictive bands in the single stage, with very satisfactory results [23, 24].

In our case, the strictures were removed in the single-stage procedure, and reconstruction of the defect was performed using Z-plasty. As already mentioned, the goal of multiple Z-plasty was to prevent expected contracture of the scar or functional impairment. Although there was development of lymphedema after removal of the first stricture in the lower leg, after incision and bandaging, lymphedema withdrew on the fourth postoperative day. After eliminating other stricture in the thigh, there were no other complications in the further course of the treatment.

The operating method of removing an amniotic band in one stage have given quite satisfactory functional and aesthetic results. Also, by using the single-stage procedure, multiple surgeries and general anesthesia were avoided. Given the many morphological variations of the syndrome, a decision on the treatment strategy should be made specifically for each individual patient.

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Хируршко лечење синдрома амнионских брида у једном акту – приказ болесника

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КРАТАК САДРЖАЈ

Увод Синдром амнионских брида (*ABS*) ретка је конгенитална аномалија коју карактерише појава стриктура најчешће на деловима или целим екстремитетима. У зависности од степена захваћености и типа аномалије лечење је конзервативно или оперативно.

Приказ болесника У раду је приказан случај рочног новорођенчета са *ABS* локализованим на левој нози. У другој недељи живота се оперативно коригује аномалија на левој потколеници. Постоперативно долази до појаве едема у дисталном делу оперисане потколенице, што се лако решава инцизијом и дренажом. Два месеца касније се оперативно коригује стриктура леве натколенице у једном акту, методом мултипле „*Z*" пластике. Постоперативни ток протиче уредно, стање на контролном прегледу након две године је задовољавајуће.

Закључак Приступ пацијентима са овим синдромом требало би да буде мултидисциплинаран, посебно у погледу дијагностике, праћења и лечења. Оперативни метод уклањања брида у једном акту је дао сасвим задовољавајући функционални и естетски резултат. С обзиром на бројне морфолошке варијације синдрома, одлука о плану лечења треба да буде донета индивидуално за сваког пацијента.

Кључне речи: синдром амнионских брида; конгениталне аномалије; дечја хирургија; лечење; исход

Примљен • Received: 30/09/2015 **Ревизија • Revision:** 25/04/2016 **Прихваћен • Accepted:** 30/06/2016

Srp Arh Celok Lek. 2016 Sep-Oct;144(9-10):550-552
ПРИКАЗ БОЛЕСНИКА / CASE REPORT
DOI: 10.2298/SARH1610550V
UDC: 616.345-006.03

Benign cystic teratoma of the mesosigmoid – Report of a case

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SUMMARY

Introduction Extragonadal intraperitoneal teratomas are very rare, especially those arising from mesentery and mesocolon. In the contemporary literature only 22 cases of such tumors have been published and described.

Case Outline We report a case of a 52-year-old woman with a benign cystic teratoma of the mesosigmoid. The patient presented with mild clinical signs of intestinal obstruction. Computerized tomography of the pelvis and abdomen showed a large 9.7 × 8.9 × 9.4 cm calcified tumor in the lower part of the left hemiabdomen. Extraluminal obstruction was verified by colonoscopy at 35 cm from the anal verge. Intraoperatively, a cystic calcified tumor of the mesosigmoid was found causing extraluminal obstruction of the left colon. The tumor was extirpated and a partial resection of the adherent great omentum was performed. The histopathological examination revealed a benign cystic teratoma.

Conclusion Considering the fact that mesenteric teratomas are extremely rare tumors, it is difficult to designate a general conclusion for an adequate treatment of patients suffering from them. Complete surgical excision is indicated in order to establish a correct histopathological diagnosis and to relieve the patients of symptoms.

Keywords: mesosigmoid benign cystic teratoma; intestinal obstruction; colonic surgery

INTRODUCTION

Teratomas are tumors composed of a mixture of tissues derived from the three embryonic germinal layers and are, consequently, considered to be neoplastic counterparts of embryonic tissues. As in gonadal and extragonadal examples, there are two variants identified – mature and immature teratomas. The histologic appearance and malignant potential of teratomas is determined by the degree of immaturity of the tissue components [1]. Mature teratomas are generally multicystic and composed of a mixture of recognizable mature elements including keratin balls, hair, cartilage and bone. Most benign teratomas are composed of mature cells; however, 20–25% of these also contain immature elements, mostly the neuroepithelium [1]. The most common site of extragonadal teratoma is the sacrococcygeal region followed by neck, mediastinum, central nervous system, paranasal sinuses, liver, uterine cervix, stomach, abdominal wall, omentum and peritoneum [2]. Extragonadal intraperitoneal teratomas, especially those arising from mesentery and mesocolon, are very rare – only 22 cases of such tumors have been published and described in the contemporary literature [3].

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CASE REPORT

A 52-year-old woman presented to the emergency room with clinical signs of mild intestinal obstruction, which were associated with irregular stool, abdominal discomfort and distension. On the manual abdominal examination, she was found to have a slightly distended abdomen and a palpable nontender mass in the left hemiabdomen, with no signs of peritonism. She had no previous abdominal operations. Rectal examination was unremarkable. Common laboratory tests were in normal ranges, including tumor markers: CEA = 1.7 ng/ml, CA19-9 = 4.0 U/ml, CA125 = 41 U/ml and *Echinococcus* IgG At = 11.4 U/ml. Computerized tomography of the pelvis and abdomen showed a large calcified tumor in the lower part of the left hemiabdomen with smooth walls and 9.7 × 8.9 × 9.4 cm in size (Figures 1 and 2). Extraluminal obstruction with intact mucosa was verified at 35 cm from the anal verge by colonoscopy.

Considering the clinical symptoms, computerized tomography scan, and endoscopic findings, elective laparotomy was performed. Intraoperative findings revealed a cystic tumor of the mesosigmoid, causing extraluminal obstruction of the left colon, with no interference with blood vessels of the mesosigmoid. The tumor was enucleated and a partial resection of the adherent mesosigmoid and the great omentum was performed (Figures 3 and 4).

Macroscopic examination of the resected specimen showed a solid, calcified tumor mass with hair and cartilage inside its capsule. The histopathological examination revealed benign cystic teratoma. The postoperative course was uneventful and the patient was discharged on postoperative day seven. The patient was free of symptoms during a 12-month follow-up period.

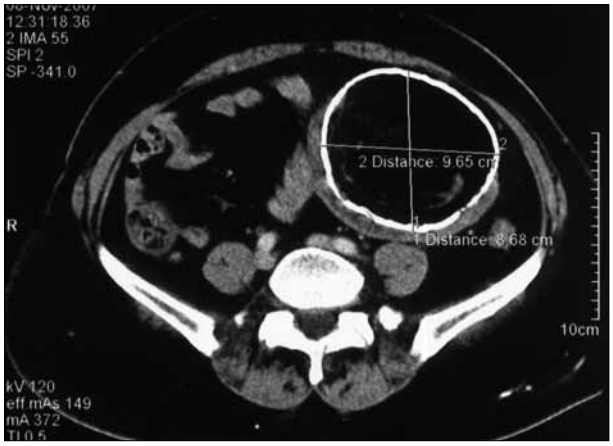

Figure 1. CT scan of the abdomen and pelvis – axial view

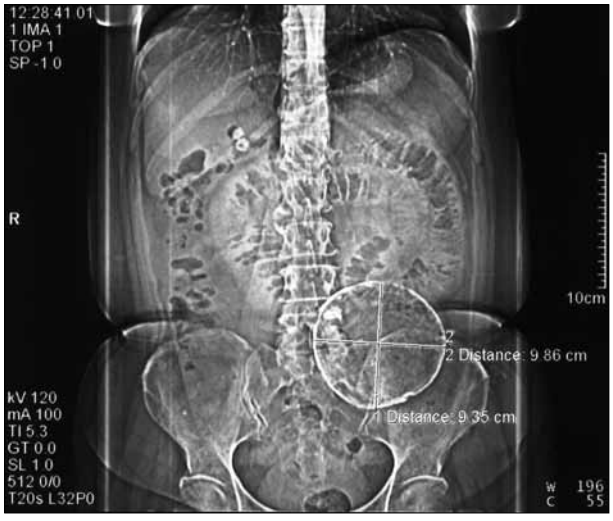

Figure 2. CT scan of the abdomen and pelvis – coronal view

DISCUSSION

Teratoma is the most commonly encountered germ cell tumor [4]. Extragonadal intraperitoneal teratomas are extremely rare, although there are almost 40 reports of teratomas arising from the greater and lesser omentum [4, 5, 6], and only 22 cases arising from mesentery and mesocolon [3]. The occurrence of mesenteric teratoma in males is less common than in females [3, 4]. These tumors more frequently occur in children than in adults, but rare cases of geriatric patients have also been reported [7–18]. Although cases with multiple masses have been described [14], mesenteric teratomas are usually solitary tumors with a diameter ranging 3–18 cm [12, 14, 17], located more often in the mesenterium than in the mesocolon [3].

Mesenteric teratomas may present with a variety of symptoms. These patients can be completely asymptomatic and just present with a nontender abdominal mass [8, 16, 17, 19], while on the other hand they can also show signs of intestinal obstruction [10, 12, 14, 15, 20], and cause abdominal pain [7, 14, 15, 18, 20–23].

Whereas these tumors are very rare, it is very difficult to establish a correct preoperative diagnosis with all available diagnostic imaging tools. The differential diagnosis of

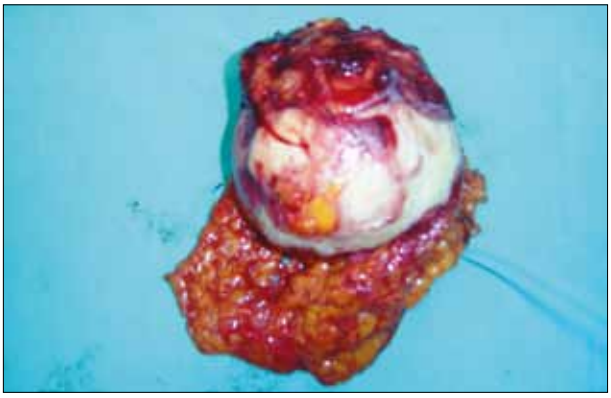

Figure 3. Gross finding of teratoma of the mesosigmoid with resected part of the great omentum

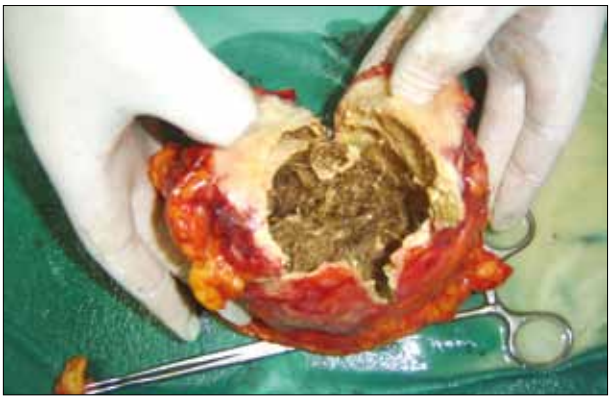

Figure 4. Gross finding of bisected teratoma of the mesosigmoid

intra-abdominal cystic masses includes mesenteric cysts, cystic teratoma, and cystic mesothelioma [24]. Since the definitive diagnosis can be made only on the basis of histopathological examination, complete surgical removal of the cyst is the only way in the treatment of these patients. Although laparoscopic surgery has been used in some cases [3, 19], the majority of authors, including our group, gave preference to a standard laparotomy approach [7–18, 20–23]. We chose laparotomy over laparoscopy for several reasons: the size of the tumor, intestinal obstruction with consecutive bowel distension and a possible interference of the tumor with adjacent blood vessels and organs.

It is recommended that teratomas should be classified as "mature" and "immature," replacing the terms "benign" and "malignant" [25]. While it is generally stated that an immature teratoma has a greater potential to metastasize than a mature one, increasing evidence indicates that other factors such as location of the tumor, patient's age and sex are also important factors in determining the potential of malignant behavior of these tumors [2]. Mesenteric teratomas are mostly benign [3, 7–14, 16–23], and for these patients surgery is a sufficient treatment option, whereas adjuvant chemotherapy for malignant mesenteric teratomas is required [15], as in gonadal and other extragonadal sites [2, 26].

Considering the fact that mesenteric teratomas are extremely rare tumors it is difficult to designate a general conclusion for an adequate treatment of these patients. Complete surgical excision is indicated in order to establish a correct histopathological diagnosis and to relieve the patients of symptoms.

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Бенигни цистични тератом сигмоидног мезоколона – приказ болесника

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КРАТАК САДРЖАЈ

Увод Екстрагонадални интраперитонеални тератоми су изузетно ретки, поготову они који су смештени у мезентеријуму или мезоколону. У савременој литератури објављено је и описано свега око 22 оваква случаја.

Приказ болесника Приказујемо случај болеснице у старосној доби од 52 године са бенигним цистичним тератомом сигмоидног мезоколона. Пацијенткиња је примљена на одељење хирургије са клиничким знацима цревне субоклузије. Компјутеризованом томографијом мале карлице и трбуха виђен је велики калцификовани тумор у доњем делу левог хемиабдомена величине 9,7 × 8,9 × 9,4 *cm*. Колоноскопијом је верификована екстралуминална опструкција на 35 *cm* од ануса. Као узрок екстралуминалне опструкције левог колона интраоперативно је нађен цистични калцификовани тумор сигмоидног мезоколона. Тумор је екстипиран уз парцијалну ресекцију приraслог великог оментума. Дефинитивним патохистолошким прегледом препарата постављена је дијагноза бенигног цистичног тератома.

Закључак С обзиром на чињеницу да су тератоми мезентеријума изузетно ретки тумори, врло је тешко донети опште закључке и поставити смернице за адекватан третман ових пацијената. Комплетна хируршка ексцизија је индикована у циљу постављања дефинитивне патохистолошке дијагнозе и ради отклањања последичних клиничких симптома.

Кључне речи: бенигни цистични тератом; сигмоидни мезоколон; цревна опструкција; хирургија дебелог црева

Примљен • Received: 14/12/2015 **Прихваћен • Accepted:** 29/06/2016

A rare localization of osteoid osteoma – Presentation of two cases

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SUMMARY
Introduction Osteoid osteoma is a benign osteoid-forming tumor generally localized to the long bones, is rarely localized in the hand and the major symptom is intermittent pain. This study aims to present two patients who were operated on for metacarpal osteoid osteomas.
Case Outline A 16-year-old female patient and an 18-year-old male patient were operated on for metacarpal osteoid osteomas. The major symptom was intermittent pain for both patients. After surgical excision of the niduses, the complaints resolved in both cases.
Conclusion In the case of high suspicion for osteoid osteoma, computed tomography or magnetic resonance imaging should be performed due to the risk of negative radiographic findings. Surgical excision is curative and a safe method of treatment.
Keywords: osteoid osteoma; metacarpal bone; surgical treatment

INTRODUCTION

Osteoid osteoma is a benign osteoid forming tumor [1]. The first description of osteoid osteoma term in medical literature was done by Jaffe in 1935 [2, 3]. It constitutes 10–12% of all benign bone tumors and is usually seen in children and young adults in the second and third decade of life [2, 3]. Approximately 50–60% of cases are seen in long bones, especially metaphysis of the femur and tibia. It is rarely localized in the hand, and the major symptom is intermittent pain. Sometimes it would be difficult to diagnose lesions in the hand due to the atypical pain pattern and different histologic features [1, 2]. This study aims to present two patients who were operated on for metacarpal osteoid osteomas. The written consent was obtained from the patients.

REPORTS OF CASES

Case 1

A 16 year-old female was admitted with nocturnally aggravated local tenderness and swelling on her fifth metacarpal bone in February 2011. The complaints had been present for three months, and relieved with salicylates. The laboratory analysis was normal. In plane X-ray, increased sclerosis in the middle third of the fifth metacarpal bone extending from cortical area to the medulla was observed (Figure 1). Magnetic resonance imaging (MRI) showed intracortical lesion with surrounding sclerotic tissue.

Under general anesthesia, an arm tourniquet was applied. A 3 cm dorsolateral incision was used for exposure. A nidus was completely excised during surgery, but the defect left after tumor resection was filled with allograft since the patient did not approve autografting. Histopathologic evaluation confirmed the diagnosis of osteoid osteoma. The patient's complaints were relieved in the immediate postoperative period, and during the four-year follow-up she did not express any complaints.

Case 2

An 18-year-old male was admitted to our clinic in January 2013 due to the pain in his left hand for one year. He was given rest and analgesics in the previous medical center but his com-

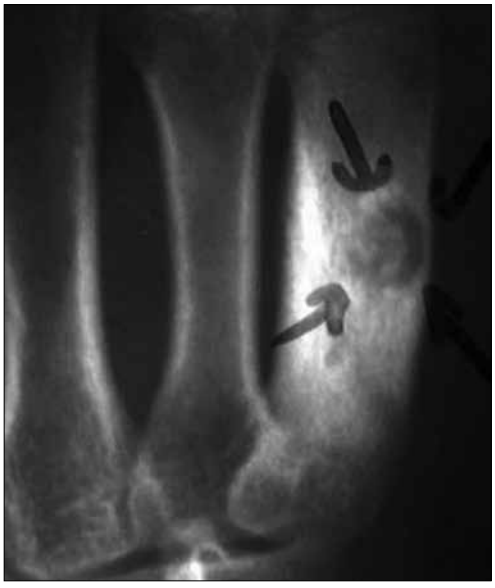

Figure 1. Plain radiograph of the first patient shows nidus and surrounding sclerotic bone

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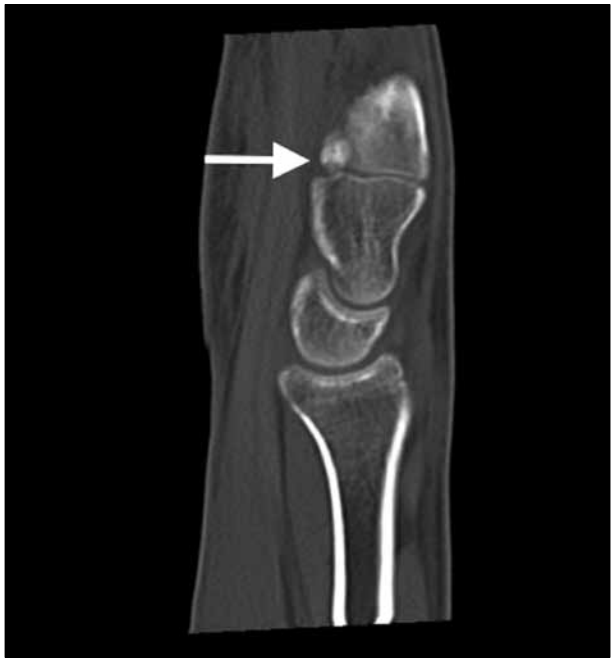

Figure 2. In the computed tomography section of the second patient, a sclerotic lesion was observed at the proximal palmar side of the third metacarpal

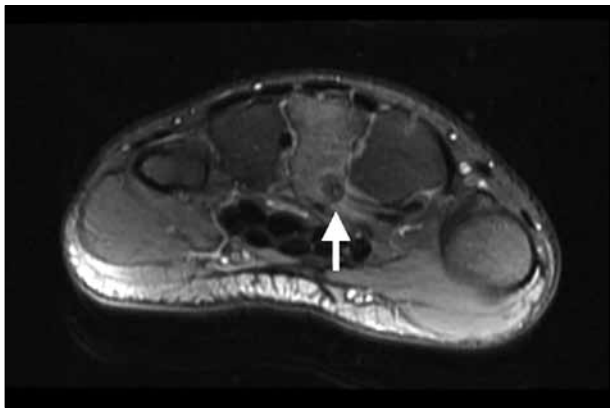

Figure 3. Magnetic resonance imaging of the second patient

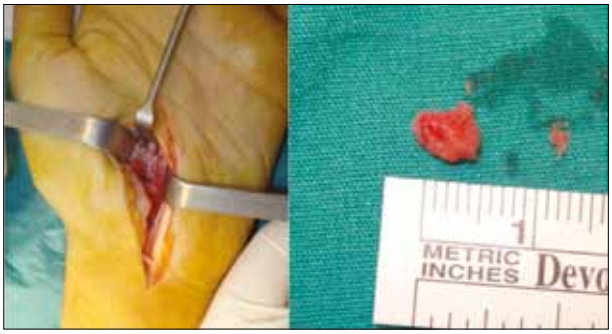

Figure 4. Intraoperative view of the lesion

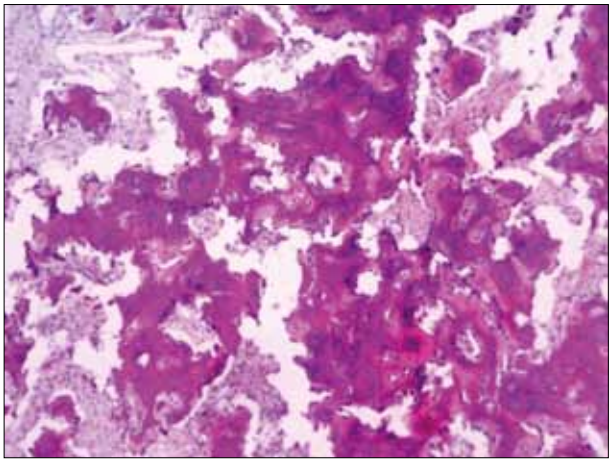

Figure 5. Histopathologic examination of the lesion in Case 2 – low magnification micrograph of nidus after H&E staining

plaints did not disappear. His pain was disturbing especially during nighttime and persisted during the day. The pain was reduced with non-steroidal anti-inflammatory drugs. On physical examination, tenderness was observed at the level of the proximal portion of his third metacarpal on palmar side of the left hand, but the finger and wrist range of motion were normal. A lesion which was 3 × 3 mm in diameter with well-defined margins in the proximal volar side of the third metacarpal was detected in the computed tomography (CT) sections (Figure 2). The MRI revealed the lesion and edema in the surrounding bone (Figure 3). The mass was accepted as osteoid osteoma, and it was decided to perform an excision.

Under general anesthesia, an arm tourniquet was applied. A 4 cm longitudinal incision parallel to the thenar crease was made. The transverse carpal ligament was cut. The median nerve and flexor tendons were retracted in order to reach the carpometacarpal joint. The joint capsule was opened and the lesion was exposed. The nidus was excised and the cavity was debrided (Figure 4). The skin and subcutaneous tissues were closed and compressive bandage was applied.

The histopathologic examination confirmed the diagnosis of osteoid osteoma (Figure 5). The pain disappeared after the excision. At the last control, the patient was pain free and working without limitation 22 months after the surgery.

DISCUSSION

Six to 13% of osteoid osteomas are localized in the hand. The phalanges are the most frequent sites for osteoid osteoma in the hand. The carpal bones and metacarpals are rarely affected [1, 2, 4]. In our patients, the lesions were located in the metacarpal bones. In their series of nineteen patients, Ambrosia et al. [5] reported only one patient whose metacarpal bone was affected.

The etiology of osteoid osteoma is unknown. Chromosomal abnormalities are suspected but have not been proven yet [6]. Kalil and Antunes [7] reported two brothers that had osteoid osteoma with similar properties, like onset of symptoms and localization. Another suspect for etiology of osteoid osteoma is trauma [1, 2]. Uda et al. [8] and Baron et al. [9] reported their cases of osteoid osteoma and claimed that the lesions were presented after injury. Our patient had neither family history nor trauma.

Pain is the main complaint in patients with osteoid osteoma. It's nocturnal and increases with activity. Salicylates

or other non-steroidal anti-inflammatory agents typically cause relief of the symptoms [10]. Basu et al. [11] reported a metacarpal osteoid osteoma without pain. Swelling can also be observed in superficially localized lesions [12]. Our patients were admitted to our clinic due to nocturnal pain which was being relieved with non-steroidal anti-inflammatory agents. The patient from the first case had a swelling on the dorsal side of her hand, but we did not observe any swelling in the second case. This may be related to the deep localization of the osteoid osteoma in the second case. In our opinion, the nocturnal pain is the most important clinical symptom for osteoid osteoma. Patients' response to painkillers should also be noted.

The diagnosis of a metacarpal osteoid osteoma can be challenging and can be made using clinical and radiologic findings. In plain radiographs, a radiolucent area surrounded with a sclerotic rim can be observed. However, 15–25% of lesions cannot be detected in plain radiographs [13, 14]. For the lesions located in the hand, due to small diameters, central radiolucent nidus may not be seen. Instead, a sclerotic area can draw attention [1]. On the other hand, in some cases neither lytic nidus nor reactive sclerosis can be seen [15]. In case of suspicion, CT, bone scintigraphy or MRI may be helpful [1, 2, 15]. In Case 1, the lesion was obvious in plain radiographs, but in Case 2 the X-rays were not helpful. Therefore, MRI and CT were ordered. We prefer to obtain MRI or CT in addition to plain X-rays despite obvious radiographic findings. This helps surgeons not only in reaching diagnosis, but also during surgical planning.

Although excision of the nidus is the gold standard treatment modality for osteoid osteoma, there are few reports of spontaneous regression and good results with medical treatment [16]. Radio-frequency percutaneous ablation is a popularized method with very low morbidity [1]. Schmidt et al. [17] reported their series of 23 patients and gave the success rate as 100%. Mylona et al. [18] reported their cases who had been operated on for osteoid osteoma localized at technically challenging locations such as spine or intraarticular positions. The primary clinical success was 91.3%, and 100% for the second procedures. In our opinion, this method can be preferred if it is available in a healthcare institution, as it has lower morbidity compared to surgical excision. The success rate is high, but the traditional method also works well if it is perfomed right.

Despite its rare localization in the hand, osteoid osteoma has to be considered in the differential diagnosis. In the case of high suspicion, we advise obtaining CT or MRI examinations due to the risk of negative radiographic findings. In our opinion, surgical excision is curative and a safe method of treatment, but radio-frequency percutaneous ablation would be an alternative modality in such cases.

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Ретка локализација остеоидног остеома – приказ два болесника

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КРАТАК САДРЖАЈ

Увод Остеоидни остеом (ОО) јесте бенигни тумор који формира остеоид, који се најчешће јавља на дугим костима. Локализација на шаци је ретка и главни симптом је повремени бол. Циљ овог рада је приказ два болесника над којима је извршена операција услед метакарпалних остеоидних остеома.

Прикази болесника Шеснаестогодишња болесница и осамнаестогодишњи болесник оперисани су услед метакарпалних остеоидних остеома. Код оба пацијента је главни симптом био повремени бол. У оба случаја су се симптоми повукли после хируршке ексцизије нидуса.

Закључак У случају изражене сумње, потребно је извршити снимање компјутеризованом томографијом или магнетном резонанцом због ризика да радиографски налази буду негативни. Хируршка ексцизија је куратива и безбедан метод лечења.

Кључне речи: остеоидни остеом; метакарпална кост; хируршко лечење

Примљен • Received: 11/12/2015 **Ревизија • Revision:** 14/04/2016 **Прихваћен • Accepted:** 18/04/2016

Vojislav Arnovljević described "Sézary syndrome" ten years before Sézary and Bouvrain

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SUMMARY

In 1938 Sézary and Bouvrain reported on a patient with a set of symptoms which later began to carry an eponymous designation "Sézary syndrome." Ten years previously, Vojislav Arnovljević had described a patient with exactly the same set of symptoms, as well as physical, laboratory, autopsy, and histopathology findings. Unfortunately, his contribution remained unnoticed, not only by the international but Serbian audience as well.

In 1928, in the *Serbian Archives of Medicine*, in Serbian, Vojislav Arnovljević published an article titled "The chronic lymphoid leukaemia with skin lymphomatosis," in which he described a 43-year-old man with a two-year history of progressive development of a diffuse erythroderma with itching and hair loss over the entire torso, leukemia of 240,000/mm^3, 91% of which lymphocytes and 5% eosinophils, who soon after admission developed a bronchopulmonary infection and died. The autopsy showed a pronounced lymphadenopathy in axillae, chest, and abdomen, enlarged liver and spleen with multiple infiltrates and thick skin. The histology confirmed a profound lymphocyte infiltration of axillar, mediastinal and abdominal lymph nodes, as well as liver, spleen and skin, while "the reaction of the other parts of the lymph and blood systems was relatively weak."

There is more than enough clinical, laboratory, autopsy and histological evidence to support that the patient Arnovljević described in 1928 had a syndrome that ten years later was described by Sézary and Bouvrain, which now bears the eponymous designation of Sézary syndrome.

Keywords: erythroderma; itching; lymphadenopathy; lymphoid leukemia; eosinophilia; hepatosplenomegaly

INTRODUCTION

In 1938 Sézary and Bouvrain described a triad of symptoms consisting of erythroderma, leukemia with circulating mononuclear cells containing large atypical mononuclear cells with a large, convoluted, single cerebriform nucleus, and enlarged lymph nodes infiltrated by these cells [1]. They were later named Sézary cells, which further turned out to be T-lymphocytes. At present, apart from the aforementioned triad, in order to establish a diagnosis of Sézary syndrome, one of the following criteria is required: an absolute Sézary cells count of at least 1,000 mm^3; an expanded CD4+ T-cell population resulting in a CD4+/CD8+ ratio of more than 10 and/or loss of one or more T-cells or more antigens [2]. Eosinophilia and increased levels of immunoglobulin E may also be present as a consequence of possible Sézary cell-induced heightened secretion of IL-4 and IL-5 [3], which could lead to terminal organ insufficiency [4, 5].

The aim of the report is to show that Arnovljević had described exactly the same syndrome ten years before Sézary and Bouvrain.

ARNOVLJEVIĆ'S REPORT OF A PATIENT

Dr. Vojislav Arnovljević was a clinical assistant to Professor Radenko Stanković, head of the Internal Propedeutic Clinic of the School of Medicine of Belgrade, Serbia (Figure 1).

Arnovljević's description of "Chronic lymphoid leukaemia with skin lymphomatosis" was published in the *Serbian archives of Medicine* in 1928 [6]. Two years prior to admission, a 43-year-old male exhibited initial symptoms of a burning sensation and itching, as well as a minor red and glossy skin lesion on his right lumbar area. The redness and edema gradually spread from the lesion over the waist, then to the back, towards the left shoulder, lastly upwards and downwards, until it enveloped the entire torso. Hair loss from the head (scalp and moustache), armpits, and pubic region followed. The abdomen was getting progressively enlarged and the patient tired quickly. He reported no pain.

On examination, the skin of the face, neck, shoulders, arms, and legs was atrophic, thin, glossy, tense, and pale, while the skin on the thorax, abdomen, scrotum, and the upper half of the thighs was livid-red, dry, very hard and thickened, with creases, folds, and cuts as deep as seen in elephantiasis, particularly conspicuous on the side, under the armpits and in the groin (Figure 2). On the transition from the infiltrated towards the atrophic part of the skin, especially on the thorax, lesser infiltrative nodes in the form of larger and smaller papules could be observed. Hair was missing from under the armpits and the pubic area. Hair on

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Figure 1. Dr. Vojislav Arnovljević, first on the left; Professor Radenko Stanković in the middle; picture taken approximately at the time of presentation

Figure 2. The skin of the thorax, abdomen, scrotum, and the upper half of the thighs was livid-red, dry, very hard and thickened, with creases, folds and cuts as deep as seen in elephantiasis, particularly conspicuous on the side, under the armpits and in the groin [6] (reproduced with permission of the *Serbian Archives of Medicine* (*Srpski arhiv za celokupno lekarstvo*)

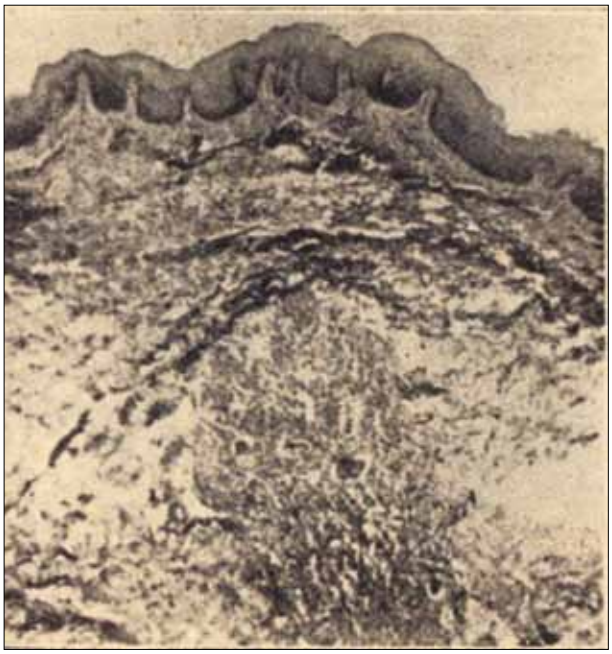
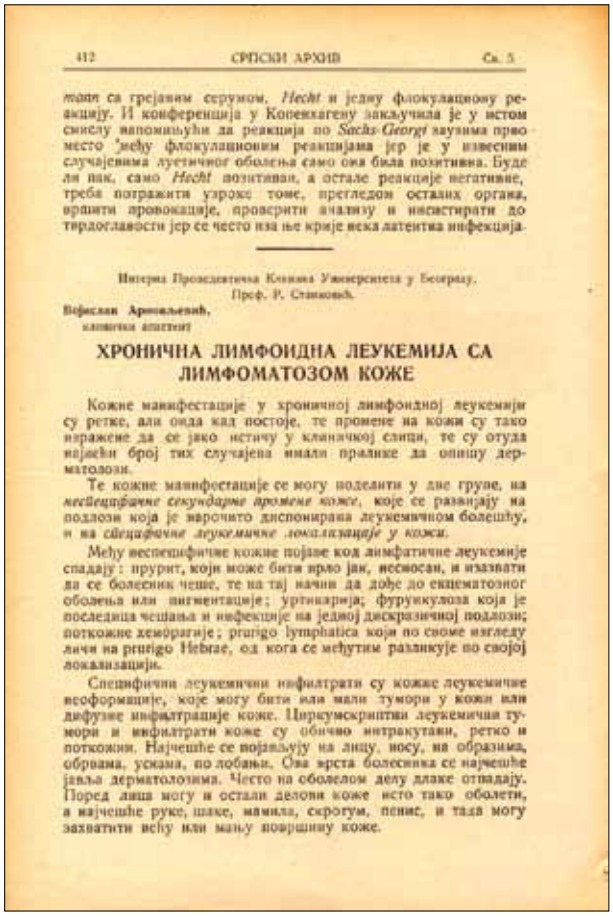


Figure 3. Histology of the skin showing thickness which was "due to deposits of small, round cells, most prevalent in the lower parts of the dermis, around the papillary bodies, where they are particularly grouped around and along blood vessels, forming the proper follicles" [6] (reproduced with permission of the *Serbian Archives of Medicine* (*Srpski arhiv za celokupno lekarstvo*)

the scalp was very thin, hairs small and "moustache very sparse and falling off." Submandibular and neck lymph nodes were not enlarged, while the axillar and inguinal ones could not be palpated because of the thickened skin. The tonsils were of normal size. The infiltrated and thick skin allowed no palpation or percussion of liver or spleen.

The blood analyses were as follows: hemoglobin 50%; erythrocytes 3,000,000 mm^3; leukocytes 280,000 mm^3. The leukocyte formula showed 91% lymphocytes (254,800 mm^3), 5% eosinophilic leukocytes (14,000 mm^3), and 4% polymorphonuclear neutrophilic leukocytes (11,206 mm^3). Erythrocyte sedimentation rate after one

doi: 10.2298/SARH1610557C



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тапп са грејаним серумом. Hecht и једну флокулационну реакцију. И конференција у Копенхагену закључила је у истом смислу напомињући да реакција по Sachs-Georgi заузима прво место међу флокулационим реакцијама јер је у извесним случајевима луетичног оболења само она била позитивна. Буде ли пак, само Hecht позитиван, а остале реакције негативне, треба потражити узроке томе, прегледом осталих органа, вршити провокације, проверити анализу и инсистирати до тврдоглавости јер се често иза ње крије века латентна инфекција.

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ХРОНИЧНА ЛИМФОИДНА ЛЕУКЕМИЈА СА ЛИМФОМАТОЗОМ КОЖЕ

Кожне манифестације у хроничној лимфоидној леукемији су ретке, али онда кад постоје, те промене на кожи су тако изражене да се јако истичу у клиничкој слици, те су отуда најећи број тих случајева имали праве да опишу дерматозоли.

Те кожне манифестације се могу поделити у две групе, на *неспецифичне секундарне промене коже*, које се развијају на подлози која је првочито диспонирана леукемичном болешћу, и на *специфичне леукемичне локализације у кожи*.

Међу неспецифичне кожне појаве код лимфатичне леукемије спадају: прурит, који може бити врло јак, несносан, и изазвати да се болесник чеше, те на тај начин да дође до екцематозног оболења или пигментације; уртикарија; фурункулоза која је последица чешања и инфекције на једној дискразичној подлози; поткожне хеморагије; prurigo lymphatica која по своме изгледу личи на prurigo Hebrae, од кога се међутим разликује по својој локализацији.

Специфични леукемични инфилтрати су кожне леукемичне неоформације, које могу бити или мали тумори у кожи или дифузне инфилтрације коже. Циркумскрипти леукемичи тумори и инфилтрати коже су обично интракутани, ретко и поткожни. Најчешће се појављују на лицу, носу, на образима, обрвама, ускама, по лобањи. Ова врста болесника се најчешће јавља дерматолозима. Често на оболелом делу длаке отпадају. Поред лица могу и остали делови коже исто тако оболети, а највешће руке, шаке, мамила, скротум, пенис, и тада могу захватити већу или мању површину коже.

Figure 4. The first page of Arnovljević's paper [6] (reproduced with permission of the *Serbian Archives of Medicine* (*Srpski arhiv za celokupno lekarstvo*).

hour was 40 (Westergren). The Bordet–Wassermann reaction was negative.

On the third day after admission, the patient developed a fever of up to 39.5°C and started coughing, initially without sputum. In the following days, he manifested signs of bronchitis and the sputum became purulent. The fever lowered to 38°C, but a state of "cardiac adynamia occurred" and the patient died on the sixth day after admission.

The autopsy findings are as follows: the skin on the thorax and abdomen was hypertrophic, an average thickness of 1–1.5 centimeters. About 1 liter of clear fluid was found in the abdominal cavity. The liver and spleen were enlarged, the liver weighing 2,700 g, and the spleen weighing 2.040 g, with 28 × 17 × 7 cm in size. On the spleen transection "numerous diffusely scattered focal sites paler in hue were found, ranging in size from a grain of millet to a grain of corn." "On section, the lungs were reddish and hyperemic, a purulent-foamy liquid exited the bronchi and the alveoli were dilated."

"The thoracic and mesenteric lymph nodes were much enlarged, hard in consistency, yellowish-red in color on the section with a much hypertrophied lymphatic tissue. The reaction of the other parts of the lymph and blood systems was relatively weak."

Histopathology of the skin showed thickness which was "due to deposits of small, round cells, most prevalent in the lower parts of the dermis, around the papillary bodies, where they are particularly grouped around and along blood vessels, forming proper follicles" (Figure 3).

Arnovljević states that "both the clinical and the pathoanatomical features in this case are characteristic of chronic lymphoid leukemia", but that "the pronounced leukemic skin lesions stand out so much that in the majority of similar cases they were described under the joint term of skin leukemia (leukemia cutis, lymphadenosis cutis, or lymphodermia)". Arnovljević rather disputes this terminology "because according to these designations one might assume that the skin condition was the basis of the disease. These manifestations are, however, only an additional sign of an illness that at its foundation has typical changes in the hematopoietic system that underlie all other disease related pathology. Therefore, it is much more accurate to emphasize in the name of the disease its most important feature, and that is leukemia. Besides this defining element, it is also important to note its particular clinical aspect, and that is the lymphocytic infiltration of the skin. Having all this in mind, we have named this specific case chronic lymphoid leukemia with skin lymphomatosis."

The author states that "it is very rare that a skin leukemic infiltration spreads across the whole of the torso, abdomen, back, hips and upper part of the thighs, all the while being of such intensity that it creates skin folds seen only in elephantiasis." He further notes that the leukemic infiltrates, or leukemic lymphomas, were most prominent on the skin, followed by changes in the spleen, liver and several axillary and mesenteric lymph nodes. These pathological findings were considerably less expressed on the other parts of the lymph and blood systems. Finally, the author calls to attention the striking peripheral blood eosinophilia amounting to 5% of total leukocyte count, which "has not been described in any of the cases of lymphoid leukemia so far." For this finding Arnovljević had no clear explanation.

DISCUSSION

Sézary syndrome is a very rare disease comprising less than 5% of all cutaneous T-cell lymphomas [7]. It is predominant in men, more often those older than 60 years [2]. Being a leukemia, in its advanced phase, Sézary syndrome could affect all organs, while bone marrow stays preserved the longest [2].

The most frequent symptoms inciting patients to seek medical help are erythroderma and generalized lymphadenopathy. Less often there are itching, alopecia, plantar or palmar hyperkeratosis, onychodystrophy, and ectropion [2].

An increased frequency of cutaneous and systemic malignancies seen in these patients is attributed to reduced immunity caused by the loss of normal circulating CD4+ T-cells [8].

The disease has a poor prognosis. Even with modern therapy, only 10–20% of patients survive more than five years [7].

The main causes of death are opportunistic infections [2]. Prognosis tends to be worse with the larger numbers of Sézary cells in the peripheral blood and with larger lymphadenopathy [9, 10].

When comparing the original and modern descriptions of Sézary syndrome with the one from Arnovljević, what is obvious is an almost complete match of accounts. Arnovljević's male patient had a characteristic triad (erythroderma with itching, leukemia with circulating mononuclear lymphocytes and axillar, thoracic, and abdominal lymph node enlargement with leukemic cell infiltration) while the infiltration of other parts was „considerably less expressed on the other parts of the lymph and blood systems." The description of skin changes is also characteristic of the disease. Disseminated erythroderma and hair loss from almost the entire body, including hair from the scalp and moustache, are typical as well.

Peripheral blood findings unequivocally indicated leukemia with 91% of lymphocytes, as well as elevated counts of eosinophils of 5%, which is also frequent in Sézary syndrome. Unfortunately, we lack a more detailed account of the appearance of lymphocytes in peripheral blood. As the symptoms developed for two years, there was more than enough time for the pathologic changes to occur within the liver and the spleen, which were undoubtedly infiltrated with abnormal lymphocytes, today known as Sézary cells.

Patients with Sézary syndrome have a marked tendency towards developing opportunistic infections, which are the leading cause of death in this population. This was indeed the case with Arnovljević's patient, who died of lung infection on the third day since the symptoms first appeared (coughing soon followed by expectoration and high grade fever). The rapidity of the unfavorable outcome was not only due to lack of antibiotic therapy at the time, but also as a consequence of reduced immunity, a typical occurrence in these patients. It is a possibility that eosinophilia could have also contributed to the speed at which death occurred as well. It was possible that the pronounced eosinophilia had contributed to the rapid death outcome.

Although the detailed description of pathologic lymphocytes in peripheral blood is lacking, the account provided by the author offers such an abundance of information that it could be said with certainty that the disease was, in fact, Sézary syndrome. Arnovljević correctly understood that the pathologic changes in the skin are a secondary manifestation of a generalized illness, which he described as chronic lymphoid leukemia.

CONCLUSION

Notwithstanding a deficient depiction of the aforementioned lymphocytes, especially of their nuclei, all of the other information strongly advocates in favor of Sézary syndrome. It is unfortunate that this case, reported ten years prior to that of Sézary and Bouvrain, was published in Serbian and not in international scientific literature, because it would have certainly not been unnoticed. Surprisingly, it has not been recognized even by the Serbian hematologists so far.

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Војислав Арновљевић је описао Сезаријев синдром десет година пре Сезарија и Буврена

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[3]Клинички центар Србије, Клиника за хематологију, Београд, Србија

Сезари и Буврен су 1938. године описали болесника са групом симптома која је по Сезарију касније добила име. Десет година раније, Војислав Арновљевић је описао болесника са потпуно истом клиничком сликом, али је тај рад у литератури остао незапажен.

У Српском архиву за целокупно лекарство од 1928. године, Војислав Арновљевић је објавио чланак под називом „Хронична лимфоидна леукемија са лимфоматозом коже", приказавши 43 године старог болесника са две године дугом анамнезом болести, који је имао еритродермију коже целог трупа, праћену сврабом, опадањем длака скоро са целог тела, леукемију са 240.000 леукоцита у mm^3, од чега су 91% били лимфоцити и 5% еозинофили, увећане лимфне жлезде у пазушним јамама, грудном кошу и абдомену, увећану јетру и нарочито слезину са макроскопски видљивим инфилтратима, који је трећег дана по пријему на клинику постао високофебрилан, почео да кашље и искашљава и који је после два дана летално завршио од плућне инфекције, што је потврђено обдукционим налазом, при којој је хистолошки нађена масивна инфилтрација лимфоидним ћелијама коже и лимфних жлезда пазушних јама, грудног коша и абдомена, јетре и слезине, док је остали део лимфног и крвног система био углавном сачуван.

Иако има неких малих мањкавости, има више него довољно клиничких, лабораторијских, аутопсијских и хистолошких доказа да је Арновљевић описао болесника са синдромом који су десет година касније описали Сезари и Буврен, а који је по Сезарију добио име.

Кључне речи: еритродермија; пруритус; лимфаденопатија; лимфоидна леукемија; еозинофилија; хепатоспленомегалија

Примљен • Received: 25/05/2016 **Ревизија • Revision:** 16/08/2016 **Прихваћен • Accepted:** 19/08/2016

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво може одложити објављивање рада или чак одбити његово публиковање. Стога се ауторима и потенцијалним сарадницима саветује да пажљиво прочитају ово упутство пре него што приступе припреми рукописа за штампу у „Српском архиву за целокупно лекарство“.

ОПШТА УПУТСТВА. Оригинални и стручни радови се у целини достављају на енглеском језику са сажетком на енглеском и српском. Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ ентера, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, IL-6, O_2, $Б_{12}$, CD8).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања.

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања која се односе на једну здравствену интервенцију или на више њих, а ради испитивања утицаја на здравствени исход. Регистарски број истраживања треба да се наведе у последњем реду Кратког садржаја.

ЕТИЧКА САГЛАСНОСТ. Рукописи о хуманим медицинским истраживањима или историјама болести пацијената треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с локалним правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог рада, наравно, уз писани пристанак.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

КРАТАК САДРЖАЈ. Уз оригинални рад, саопштење, преглед литературе, приказ болесника, рад из историје медицине, рад за рубрику „Језик медицине“ и рад за праксу, на другој по реду страници документа треба приложити кратак садржај рада обима 100–250 речи. За оригиналне радове кратак садржај треба да има следећу структуру: Увод, Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. За приказе болесника кратак садржај треба да има следеће делове: Увод, Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Кратког садржаја навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити кратак садржај (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан кратак садржај на енглеском језику. Превод појмова из стране литературе треба

да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад обавезно треба да има следеће поднаслове: Увод, Циљ рада, Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (референце у којима је био први аутор или коаутор рада) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ болесника чине: Увод, Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од седам аутора.

Прилоге (табеле, графиконе, слике итд.) сместити на крај рукописа, а у самом телу текста назначити место које се односи на дати прилог. Крајња позиција прилога пре штампања рада зависиће од прелома текста.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у кратком садржају, али ако су неопходне, сваку скраћеницу поново објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5±3.8), а у тексту на српском језику са зарезом (нпр. 12,5±3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм – *kg*, литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РУКОПИСА. Целокупни рукопис рада – који чине насловна страна, кратак садржај, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, саопштење, рад из историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.

Провера броја речи у документу може се извршити у програму *Word* кроз подмени *Tools–Word Count* или *File–Properties–Statistics*.

ТАБЕЛЕ. Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

СЛИКЕ. Слике се означавају арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати као *Grayscale* (или у боји) у резолуцији 300 *dpi* и снимити их у оригиналној величини.

Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

ГРАФИКОНИ. Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона.

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

СХЕМЕ (ЦРТЕЖИ). Схеме цртати у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE.*

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не треба да буде старија од пет година. Избегавати коришћење апстракта као референце, а апстракте старије од две године не цитирати. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html.* Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И ПРЕТПЛАТА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва), док први аутор мора бити и претплатник на часопис, за годину у којој се рад предаје Уредништву. Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату, као доказ о уплатама. Аутори из иностранства нису дужни да буду чланови Српског лекарског друштва, нити претплатници на часопис за текућу годину. Додатне информације о чланарини и претплати могу се добити на телефоне 011/3245-149 и 011/3346-963, односно имејлом (*srparhiv@bvcom.net*) и на интернет-страници часописа (*http://www.srpskiarhiv.rs*).

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ISSN 0370-8179
ISSN Online 2406-0895

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ISSN Online 2406-0895

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