Publishing Sector of the Society of Physicians of Vojvodina of the Medical Society of Serbia, Novi Sad, Vase Stajica 9

MEDICAL REVIEW

JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
THE FIRST ISSUE WAS PUBLISHED IN 1948

MEDICAL REVIEW is published bimonthly (six issues per year) with a circulation of 1.000 copies. The annual payment fee in 2018, for individuals from the territory of Serbia, is 3,000.00 dinars (the value-added tax included), 4,000.00 dinars for individuals from Serbia who are not members of the Society of Physicians of Vojvodina of the Medical Society of Serbia, 60 Euros for members outside the territory of Serbia, and 8,000.00 dinars (+ VAT) for institutions. The payment account is: 340-1861-70 or 115-13858-06, "Annual membership fee for Medical Review".

The manuscripts are submitted at: aseestant.ceon.rs/index.php/medpreg/. Editorial Office Address: Društvo lekara Vojvodine Srpskog lekarskog društva, 21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875, E-mail: dlv@sbb.rs; Website: www.dlv.org.rs

Printed by: Department for Joint Affairs of Provincial Authorities - Sector for Printing

Izdavačka delatnost Društva lekara Vojvodine Srpskog lekarskog društva, Novi Sad, Vase Stajića 9

MEDICINSKI PREGLED

ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
PRVI BROJ JE ŠTAMPAN 1948. GODINE.

MEDICINSKI PREGLED izlazi dvomesečno (šest dvobroja godišnje), u tiražu od 1000 primeraka. Pretplata za pojedince sa teritorije Srbije za 2018. godinu iznosi 3.000,00 dinara (sa uračunatim PDV-om), a 4.000,00 dinara za pojedince iz Srbije koji nisu članovi DLV-SLD, 60 eura za članove van Srbije, a za ustanove 8.000,00 dinara (uz dodavanje PDV-a). Uplate se vrše na račun broj 340-1861-70 ili 115-13858-06, s naznakom „Dodatna članarina za Medicinski pregled".

Prijem rukopisa vrši se u elektronskoj formi na stranici: aseestant.ceon.rs/index.php/medpreg/.
Adresa Redakcije: Društvo lekara Vojvodine Srpskog lekarskog društva,
21000 Novi Sad, Vase Stajića 9, Tel. 021/521-096; 063/81 33 875
E-mail: dlv@sbb.rs; Web: www.dlv.org.rs

Štamparija: Uprava za zajedničke poslove pokrajinskih organa - Odsek za poslove štamparije

M E D I C A L R E V I E W
JOURNAL OF THE SOCIETY OF PHYSICIANS OF VOJVODINA OF THE MEDICAL SOCIETY OF SERBIA
Novi Sad Vase Stajića 9 Serbia

Med Pregl 2018; LXXI (11-12): 343-430. Novi Sad: November-December.

CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

Med Pregl 2018; LXXI (11-12): 343-430. Novi Sad: novembar-decembar.

SADRŽAJ

ORIGINAL STUDIES
ORIGINALNI NAUČNI RADOVI

University of Novi Sad, Faculty of Medicine, Novi Sad[1]
Institute of Public Health of Vojvodina, Novi Sad
Center for Analysis, Planning and Organization of Health Care[2]
Health Promotion Center, Novi Sad[3]
Oncology Institute of Vojvodina, Sremska Kamenica[4]

Original study
Originalni naučni rad
UDK 613.84:613.955(497.113 Novi Sad)
https://doi.org/10.2298/MPNS1812349C

PREVALENCE OF TOBACCO SMOKING AND ELECTRONIC CIGARETTE USE AMONG THE HIGH SCHOOL STUDENTS IN NOVI SAD

PREVALENCIJA PUŠENJA DUVANA I ELEKTRONSKIH CIGARETA MEĐU UČENICIMA SREDNJIH ŠKOLA U NOVOM SADU

Sonja ČANKOVIĆ[1, 2], Snežana UKROPINA[1, 3], Vesna MIJATOVIĆ JOVANOVIĆ[1, 2], Tatjana TAMAŠ[4], Olja NIĆIFOROVIĆ ŠURKOVIĆ[1, 3] and Dušan ČANKOVIĆ[1, 3]

Summary

Introduction. Smoking is the leading preventable cause of mortality in the world. The World Health Organization estimates that annually more than seven million lives are lost worldwide due to smoking-related diseases. The aim of the study was to examine lifetime use of tobacco and electronic cigarettes, using patterns in the last 30 days, and to determine cigarette availability among the first grade high school students in Novi Sad. **Material and Methods.** The cross-sectional study was conducted among the first grade public high school students in Novi Sad. The study sample included 1.067 participants (587 girls and 480 boys), born in 2002. The survey used the questionnaire of the European School Project on Alcohol and other Drugs. **Results.** Of the examined sample, a total of 40.1% of high school students in Novi Sad have at least tried smoking cigarettes during their lifetime, girls significantly more often (p=0.001). With no gender difference, 20% of students smoked at least one cigarette in the month preceding the survey. Every seventh student (13.9%) tried their first cigarette at the age of 13 or younger, girls significantly more often (p=0.001). A little less than one fifth of the examinees (18.4%) tried electronic cigarettes. In the month preceding the survey, every twelfth examinee used electronic cigarettes (8.1%). **Conclusion.** Monitoring the prevalence of tobacco use in young people, especially new tobacco products, indicates the need for continuous and intensive activities in the field of health promotion with implementation of effective tobacco control measures.

Key words: Smoking; Electronic Nicotine Delivery Systems; Adolescent; Students; Schools; Tobacco Use; Adolescent Behavior

Sažetak

Uvod. Pušenje je vodeći uzrok mortaliteta u svetu koji se može prevenirati. Svetska zdravstvena organizacija je procenila da se na svetskom nivou godišnje izgubi više od sedam miliona života usled razvoja bolesti koje su povezane sa pušenjem. Cilj rada bio je da se ispita upotreba duvana i elektronskih cigareta u toku života i u poslednjih 30 dana, kao i dostupnost cigareta među učenicima prvih razreda srednjih škola i gimnazija u Novom Sadu. **Materijal i metode.** Istraživanje predstavlja studiju preseka sprovedenu na uzorku učenika prvih razreda državnih srednjih škola u Novom Sadu. Analizirani su podaci koji se odnose na ispitanike koji su rođeni 2002. godine, odnosno ukupno 1 067 ispitanika, od toga 480 mladića (45%) i 587 devojaka (55%). Kao instrument istraživanja korišćen je *European School Project on Alcohol and other Drugs* upitnik. **Rezultati.** Ukupno 40,1% učenika prvog razreda srednjih škola u Novom Sadu u toku života probali su da puše cigarete, značajno više devojke (p = 0,001). Bez razlike u odnosu na pol, 20% učenika je popušilo bar jednu cigaretu u mesecu koji je prethodio istraživanju. Svaki sedmi učenik (13,9%) probao je da puši cigarete sa 13 godina ili manje, značajno više devojke (p = 0,001). Nešto manje od petine ispitanika (18,4%) u toku života probalo je elektronske cigarete, a u mesecu koji je prethodio istraživanju, svaki dvanaesti ispitanik je koristio elektronske cigarete (8,1%). **Zaključak.** Praćenje prevalencije korišćenja duvana kod mladih, naročito novih duvanskih proizvoda, ukazuje na potrebu stalnog intenziviranja aktivnosti u oblasti promocije zdravlja a istovremeno i dosledne primene efikasnih mera kontrole duvana.

Ključne reči: pušenje; elektronske cigarete; adolescent; učenici; škole; upotreba duvana; adolescentsko ponašanje

Introduction

Tobacco addiction is considered to be the most common and problematic form of addiction world-

Acknowledgement
This study was part of the project "31 May - World No Tobacco Day, 2017" which was carried out by the Institute of Public Health of Vojvodina with financial support of the Secretariat for Health Care of the City of Novi Sad.

Corresponding Author: Doc. dr Dušan Čanković, Univerzitet u Novom Sadu, Medicinski fakultet, Institut za javno zdravlje Vojvodine, 21000 Novi Sad, Futoška 121, E-mail: dusan.cankovic@mf.uns.ac.rs

Abbreviations

WHO	– World Health Organization
US	– United States
FCTC	– Framework Convention on Tobacco Control
ESPAD	– European School Project on Alcohol and other Drugs

wide [1]. It is associated with a number of diseases, as one of the major risk factors for their development. Smoking is the leading cause of preventable mortality in the world. The World Health Organization (WHO) has estimated that tobacco kills more than 7 million people each year; more than 6 million of those deaths are the result of direct tobacco use, while almost 1 million deaths are the result of non-smokers being exposed to second-hand smoke [2]. The main chemical component of tobacco, present not only in cigarettes but also in other tobacco products, is nicotine. In unionized state, nicotine is readily absorbed across the epithelium of the lungs, oral mucosa, nose, and through the skin. Nicotine is responsible for tobacco dependence, but it also exerts its negative effects on a number of organs and organ systems [3]. Cigarette smoke contains more than 7,000 chemicals which include a number of toxic and carcinogenic substances (polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines; aromatic amines, formaldehyde, acetaldehyde, 1.3-butadiene and benzene, as well as various metals) [4].

Although cigarettes are the most common tobacco product, alternative methods of smoking are becoming increasingly common and popular such as electronic cigarettes (e-cigarette). E-cigarettes include a diverse group of devices that allow users to inhale an aerosol, which typically contains nicotine, flavourings, and other additives [5]. According to the United States (US) Surgeon General's Report sales of e-cigarettes in the US have risen rapidly since 2007 and the prevalence of current e-cigarette use (defined as use during at least 1 day in the past 30 days) among high school students increased dramatically to 16% by 2015 [5, 6].

The World Health Organization data show that in the period from 2007 to 2015, the prevalence of smoking among young people aged 15 years declined globally from 23.5% to 20.7% [7]. Data of the National Health Survey of the Republic of Serbia from 2013 showed that 19.2% of young people aged 15 - 19 were smokers, and 34.7% of the adult population smoke daily or occasionally (37.9% men and 31.6% women) [8]. Last survey that was conducted in Serbia in order to acquire a better insight into the prevalence of smoking among young people was in 2013. The results showed that at least one of 10 students smoked cigarettes (13%), girls more often (13.3%) than boys (12.7%) [9].

The research has shown that smoking typically begins with experimental use of cigarettes and that the transition to regular smoking can occur relatively quickly, after smoking about 100 cigarettes [10]. The study of Kendler et al. has shown that early nicotine exposure directly increases the level of later nicotine dependence [11].

In response to the global epidemic of smoking, in 2003, WHO has adopted the Framework Convention on Tobacco Control (FCTC). In order to assist implementation of this document and measures within its respective jurisdictions, in 2008 the WHO announced a package of six tobacco control measures called MPOWER, an acronym that includes the following measures: Monitor, Protect, Offer, Warn, Enforce, and Raise [7]. In accordance with the FCTC, the Government of the Republic of Serbia adopted a Tobacco Control Strategy with the ultimate aim of smoking prevention, particularly among the youth, by reducing the prevalence of smoking in the minors by 1% annually [12].

Assessment of the cigarette smoking prevalence and the onset of cigarette and other tobacco products use is of the utmost importance for monitoring the progress of tobacco control measures. The aim of the study was to examine the lifetime tobacco and electronic cigarette use, using patterns in the last 30 days, and to determine availability of cigarettes among the first grade high school students in Novi Sad.

Material and Methods

This cross-sectional study was carried out in November – December 2017, among first year students of public high schools in Novi Sad as the target population. A total of 1236 students were surveyed and the sample was stratified by the type of school (gymnasium or professional school - 3 and 4 year school programs, respectively) with class as sampling unit (19 high schools, 65 classes). The students who were at school on the day of the survey filled out the questionnaires anonymously during a lesson lasting 45 minutes. Besides students, only a research team member was in the classroom. All respondents were informed about the purpose of the study and agreed to participate. The survey instrument was a self-administered European School Project on Alcohol and other Drugs (ESPAD) questionnaire which was the sixth data-collection wave in 2015.

In order to provide comparison with the results of other studies that have used ESPAD methodology, questionnaires with more than 50% of missing answers and those with missing data about gender or year of birth were excluded from the database. The final analyzed sample included 1067 participants (587 girls and 480 boys) born in 2002.

The prevalence of tobacco as well as e-cigarette use was examined during lifetime, and in the last 30 days. Lifetime prevalence of smoking cigarettes was assessed through the question: "On how many occasions (if any) have you smoked cigarettes during your lifetime?" with answers being on a seven-point-scale ("0" to "40 or more times"). This variable was dichotomized to indicate any smoking in the lifetime (with those reporting 0 times classified as "No" (never tobacco smokers) and those report-

ing any occasion classified as "Yes". Respondents were asked how frequently they had smoked in the last 30 days, with answers on a seven-point-scale ranging from „not at all" to „more than 20 cigarettes per day". Analysis was done based on variables categorized as "not at all"; "less than 1 cigarette per day" and "1 or more cigarettes per day". Those reporting at least 1 cigarette smoked in the last 30 days were classified as current tobacco smokers. Ever tobacco smokers were respondents who smoked cigarettes during their lifetime, but not in the last month. Data about e-cigarette use were gathered by the question "Have you ever used e-cigarettes?" For our purposes, the obtained data were categorized into 3 categories: "Never";"Yes, but more than 30 days ago" (ever e-cigarette users) and " Yes, in the last 30 days" (current e-cigarette users). Early onset of tobacco and e-cigarette use was assessed by questions: "When (if ever) did you smoke your first cigarette/Use your first e-cigarette" and "When (if ever) did you start smoking cigarettes on a daily basis/Use e-cigarettes on a daily basis". Responses were grouped and analyzed as "Never"; "At the age of 13 or younger" and "At the age of 14 or older". In order to assess availability of tobacco the following question was put: "How difficult do you think it would be for you to get cigarettes if you wanted?" The possible answers were: impossible, very difficult and fairly difficult (coded as difficult); fairly easy and very easy (coded as easy); and I don't know.

A Chi-squared test was used to check whether differences between distribution of proportions of variables were statistically significant. The level of statistical significance was set at $p < 0.05$. All the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), 21.0 software package.

Results

The obtained results showed that a total of 40.1% of first year high school students in Novi Sad have tried cigarettes during their lifetime, girls significantly more (45.0%) compared to boys (34.1%). Every fifth student (20.0%) had smoked at least one cigarette during the 30 days preceding the survey, 18.2% of boys and 21.4% girls. During this period, 13.4% of the total has smoked on a daily basis (at least one cigarette per day). A total of 13.9% of students had smoked cigarettes at the age of 13 years or younger, girls significantly more (16.0% of girls vs. 11.3 % of boys). The percentage of students who began smoking cigarettes on a daily basis at the age of 13 or

Table 1. Prevalence of lifetime cigarette smoking and in the last 30 days, the age at which the first cigarette was smoked, and the availability of cigarettes among the first grade secondary school students in Novi Sad
Tabela 1. *Prevalencija pušenja cigareta tokom života i u poslednjih 30 dana, uzrast kada je popušena prva cigareta i dostupnost cigareta među učenicima prvih razreda srednjih škola u Novom Sadu*

Variable/*Varijabla*	Gender/*Pol*				Total/*Ukupno*		p
	Males/*Muški*		Females/*Ženski*				
	n	%	n	%	n	%	
Total/*Ukupno*	480	45.0	587	55.0	1067	100.0	
Lifetime cigarette smoking/*Pušenje cigareta tokom života*							
No/*Ne*	315	65.9	322	55.0	637	59.9	<0.001
Yes/*Da*	163	34.1	263	45.0	426	40.1	
Cigarette smoking in the last 30 days/*Pušenje cigareta u poslednjih 30 dana*							
Not at all/*Ne*	390	81.8	459	78.6	849	80.0	0.429
Less than 1 cigarette per day *Manje od 1 cigarete na dan*	28	5.9	42	7.2	70	6.6	
1 or more cigarettes per day/*1 ili više cigareta na dan*	59	12.4	83	14.2	142	13.4	
Age of first cigarette smoking/*Uzrast kada je popušena prva cigareta*							
Never/*Nikad*	317	66.3	322	54.9	639	60.1	
Age of 13 or younger/*Sa 13 godina ili mlađi*	54	11.3	94	16.0	148	13.9	0.001
Age of 14 or older/*Sa 14 godina ili stariji*	107	22.4	170	29.0	277	26.0	
Age of cigarette smoking on a daily basis/*Uzrast kada puši cigarete svakodnevno*							
Never/*Nikad*	411	86.2	470	81.2	881	83.4	
Age of 13 or younger/*Sa 13 godina ili mlađi*	16	3.4	22	3.8	38	3.6	0.079
Age of 14 or older/*Sa 14 godina ili stariji*	50	10.5	87	15.0	137	13.0	
Availability of cigarettes/*Dostupnost cigareta*							
Difficult/*Teško*	81	17.1	66	11.4	147	14.0	
Easy/*Lako*	297	62.5	392	67.8	689	65.4	0.029
Don't know/*Ne zna*	97	20.4	120	20.8	217	20.6	

Table 2. The prevalence of e-cigarette use, age of first e-cigarette use and use of e-cigarettes on a daily basis among first year high school students in Novi Sad
Tabela 2. *Prevalencija upotrebe e-cigarete (elektronske cigarete), uzrast prilikom prve upotrebe i svakodnevne upotrebe e-cigarete među učenicima prvih razreda srednjih škola u Novom Sadu*

Variable/*Varijabla*	Gender/*Pol*				Total *Ukupno*		p
	Males/*Muški*		Females/*Ženski*				
	n	%	n	%	n	%	
E-cigarette use/*Upotreba e-cigareta*							
Never/*Nikad*	349	79.3	461	83.4	810	81.6	<0.001
More than 30 days ago/*Pre više od 30 dana*	39	8.9	64	11.6	103	10.4	
In the last 30 days/*U poslednjih 30 dana*	52	11.8	28	5.1	80	8.0	
Age of first e-cigarette use/*Uzrast kada je upotrebljena e-cigareta prvi put*							
Never/*Nikad*	352	80.9	456	82.6	808	81.9	
Age of 13 or younger/*Sa 13 godina ili mlađi*	26	6.0	24	4.3	50	5.0	0.051
Age of 14 or older/*Sa 14 godina ili stariji*	57	13.1	72	13.0	129	13.1	
Age of e-cigarette use on a daily basis/*Uzrast kada koristi e-cigaretu svakodnevno*							
Never/*Nikad*	397	91.5	528	95.5	925	93.7	
Age of 13 or younger/*Sa 13 godina ili mlađi*	11	2.5	4	0.7	15	1.5	0.017
Age of 14 or older/*Sa 14 godina ili stariji*	26	6.0	21	3.8	47	4.8	

younger was 3.6% (3.4% of boys and 3.8% of girls). Over 60% of students replied that they could easily get cigarettes if they wanted to, girls significantly more (67.8%) compared to boys (62.5%) **(Table 1).**

Frequency distribution of e-cigarette use is shown in **Table 2**. A total of 183 participants (18.4%) had used e-cigarettes at least once. Boys (11.8%) were significantly more likely to have used e-cigarettes in the last 30 days than girls (5.1%). When examining at what age the respondents tried e-cigarettes for the first time, 5.1% reported that it was at the age of 13 years or younger. A total of 1.5% of students reported using e-cigarette on a daily basis since the age of 13 years or younger and 4.8% since the age of 14 years or older.

Data on e-cigarette use in regard to tobacco use are shown in **Table 3**. Every fifth current cigarette smoker used e-cigarettes (21.7%), among ever tobacco smokers 9.1% were current e-cigarette users, while among students who have never smoked tobacco 3.8% were current e-cigarette users.

Discussion

This paper presents the findings of a survey which was conducted among the first year high school students in Novi Sad using ESPAD methodology. The results showed that a total of 40.1% of students have tried cigarettes during their lifetime, which is slightly more than in the previous survey conducted in 2013 in Novi Sad (37.7%) [13], but lower than the results from the survey performed at a national level in 2009 (46%) [14]. Data on lifetime prevalence of cigarette use from other countries in the region vary widely: Croatia (62%), Hungary (55%), Bulgaria (55%), Romania (52%), Slovenia (47%), Former Yugoslav Republic of Macedonia (38%) and Montenegro (34%) [15]. In the countries included in the ESPAD project the percentage of respondents who smoked at least once in their lifetime was reduced from 67% to 47% over the period 1995 - 2015 [16]. In our study, the prevalence was higher among girls (45.0% vs. 34.1%). However, across the

Table 3. E-cigarette use among first year high school students in Novi Sad in regard to the tobacco smoking status
Tabela 3. *Upotreba e-cigarete (elektronske cigarete) među učenicima prvih razreda srednjih škola u Novom Sadu u odnosu na pušački status*

	Never tobacco smokers *Nikad nisu pušili cigarete*		Ever tobacco smokers *Nekad su pušili cigarete*		Current tobacco smokers *Trenutno puše cigarete*		p
	n	%	n	%	n	%	
Never e-cigarette users *Nikad nisu koristili e-cigarete*	569	93.6	145	73.6	90	50.0	
Ever e-cigarette users *Nekad koristili e-cigarete*	16	2.6	34	17.3	51	28.3	<0.001
Current e-cigarette users *Trenutno koriste e-cigarete*	23	3.8	18	9.1	39	21.7	

ESPAD countries, boys were generally more likely to have tried cigarettes than girls. Countries with the largest gender differences, where higher rates were found in girls, are Monaco, Bulgaria and Malta [15].

According to the results of our study, a total of 20% of respondents have smoked at least one cigarette in the last 30 days, which is the same result like in 2011 ESPAD Report in Serbia [17]. Similarly, according to the 2015 ESPAD Report, on average, 21% of students in the ESPAD countries had used cigarettes during the last 30 days, while based on the results of a National Survey on Drug Use and Health in United States the prevalence of current cigarette smoking among youth 16 – 17 years of age was 13.6% [3, 15].

Results showed that the age of smoking initiation was 13 years or less in 13.9% of students. According to the literature, the age of smoking initiation is a significant factor for continuation of smoking. Khuder et al. concluded that men who started smoking before 16 years of age are at two times higher risk for not quitting smoking compared to those who started at a later age [18]. Adolescents who begin smoking earlier are more likely to become regular smokers at the age of 15 and are more likely to report multiple risk behaviours [19, 20].

There are two ways to obtain cigarettes; the first is to buy them from a store (commercially) and the second is to borrow, buy or steal them from other young people or adults (socially) [3]. Although in Serbia the Law on Tobacco prohibits selling tobacco products to minors, the results of Global Youth Tobacco Survey conducted in Serbia in 2013 among students 13 – 15 years old, showed that almost two thirds of students who smoke usually buy cigarettes from a supermarket, while a quarter of them get them from someone else [9, 21]. Consistent with previous findings, the results of our study showed that 65.4% of students considered that it would be easy to obtain cigarettes. This result is slightly higher compared to the average value of ESPAD countries (61%) [15].

This study also investigated the e-cigarette use among the first year high school students in Novi Sad. As described in the Surgeon General's Report, the patterns of tobacco use are changing recently, with more intermittent use of cigarettes and an increase in use of other products [3]. National Youth Tobacco Survey in the United States reports an increase in current use of e-cigarettes among high school students from 1.5% in 2011 to 16.0% in 2015 [5]. In our sample, the prevalence of current e-cigarette users is lower (8.0%). However, 18.4% are those who had tried e-cigarettes. This figure is lower than reported in Ireland among young people 16 - 17 years old (23.8%) and in Poland among students aged 15 - 19 years (62.1%) [22, 23]. In Finland, 17.4% of adolescents had tried e-cigarettes in 2013, and 25.0% in 2015 [24]. The prevalence of e-cigarette use in the last 30 days was significantly higher among young males than females. This corresponds with findings of other studies, which found that adolescent boys were more likely to use e-cigarettes than girls [22, 25]. Among current tobacco smokers the number of current e-cigarette users was significantly higher than in non-smokers. Tobacco smokers used e-cigarettes more frequently than non-smokers. However, among ever and never smokers, 9.1% and 3.8% were current e-cigarette users, respectively. Some authors suggested that e-cigarettes could recruite young non smokers to shift from e-cigarette to tobacco smoking once they are addicted to nicotine, who would otherwise be less susceptible to tobacco product use [26, 27].

Conclusion

Two-fifths of the first grade high school students in Novi Sad had tried smoking at least once during their life, significantly more girls. Every fifth student had smoked at least one cigarette in the month preceding the survey, and a total of 13.4% of respondents had smoked every day during this period. Every seventh student tried smoking cigarettes at the age of 13 or less, significantly more girls. Two-thirds of students believe they can easily get cigarettes, significantly more girls. Slightly less than one-fifth of respondents have tried e-cigarettes. In the month preceding the study 8% of students used e-cigarettes. Findings of this study indicate that the prevalence of tobacco smoking among young people needs to be carefully followed, especially e-cigarettes use as a relatively new tobacco product. These results point out the need for continuous increase of activities in the field of health promotion as well as a consistent implementation of effective tobacco control measures.

References

1. Baumeister RF. Addiction, cigarette smoking, and voluntary control of action: do cigarette smokers lose their free will? Addict Behav Rep. 2017;5:67-84.

2. The World Health Organization. Tobacco. Key facts [Internet]. [cited 2018 May 03]. Available from: http://www.who.int/en/news-room/fact-sheets/detail/tobacco.

3. U.S. Department of Health and Human Services. The health consequences of smoking: 50 years of progress. A report of the surgeon general [Internet]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014 [cited 2018 May 04]. Available from: https://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf.

4. U.S. Department of Health and Human Services. How tobacco smoke causes disease: the biology and behavioral basis for smoking-attributable disease: a report of the surgeon general [Internet]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2010 [cited 2018 May 04]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK53017/pdf/Bookshelf_NBK53017.pdf.

5. U.S. Department of Health and Human Services. E-cigarette use among youth and young adults: a report of the surgeon gen-

eral [Internet]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2016 [cited 2018 May 04]. Available from: https://www.cdc.gov/tobacco/data_statistics/sgr/e-cigarettes/pdfs/2016_sgr_entire_report_508.pdf.

6. Singh T, Arrazola RA, Corey CG, Husten CG, Neff LJ, Homa DM, et al. Tobacco use among middle and high school students - United States, 2011-2015. MMWR Morb Mortal Wkly Rep. 2016;65(14):361-7.

7. World Health Organization. WHO report on the global tobacco epidemic, 2017: monitoring tobacco use and prevention policies [Internet]. Geneva: World Health Organization; 2017 [cited 2018 Mar 16]. Available from: http://apps.who.int/iris/bitstream/handle/10665/255874/9789241512824-eng.pdf;jsessionid=5C31FFFD899DEDD7FDE9EBF5E39F0506?sequence=1.

8. Institut za javno zdravlje Srbije "Dr Milan Jovanović Batut". Rezultati istraživanja zdravlja stanovništva Srbije: 2013. godina [Internet]. Beograd: Institut za javno zdravlje Srbije "Dr Milan Jovanović Batut"; 2014 [cited 2016 Feb 15]. Available from: http://www.batut.org.rs/download/publikacije/IstrazivanjeZdravljaStanovnistvaRS2013.pdf.

9. Institut za javno zdravlje Srbije "Dr Milan Jovanović Batut". Globalno istraživanje upotrebe duvana među mladima 13-15 godina u Srbiji 2013. godine [Internet]. Beograd: Institut za javno zdravlje Srbije "Dr Milan Jovanović Batut"; 2014 [cited 2018 Mar 17]. Available from: http://www.batut.org.rs/download/izdvajamo/GYTS%20istrazivanje%202013.pdf.

10. U.S. Department of Health and Human Services. Preventing tobacco use among youth and young adults: a report of the surgeon general [Internet]. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2012 [cited 2018 Mar 16]. Available from: https://www.surgeongeneral.gov/library/reports/preventing-youth-tobacco-use/full-report.pdf.

11. Kendler KS, Myers J, Damaj MI, Chen X. Early smoking onset and risk for subsequent nicotine dependence: a monozygotic co-twin control study. Am J Psychiatry. 2013;170(4):408-13.

12. Vlada Republike Srbije. Strategija kontrole duvana. Službeni glasnik Republike Srbije. 2007;(8).

13. Radić I. Zloupotreba ilegalnih droga kod adolescenata u Novom Sadu [Specijalistički rad]. Novi Sad: Medicinski fakultet; 2013.

14. Ministarstvo zdravlja Republike Srbije, Institut za javno zdravlje. Evropsko istraživanje o upotrebi alkohola i drugih droga među mladima u Srbiji 2008. Beograd: Ministarstvo zdravlja Republike Srbije, Institut za javno zdravlje Srbije; 2009.

15. The European Monitoring Centre for Drugs and Drug Addiction, European School Survey Project on Alcohol and Other Drugs. ESPAD report 2015 results from the European School Survey Project on Alcohol and Other Drugs [Internet]. Luxembourg: European Monitoring Centre on Drugs and Drug Addiction; 2016 [cited 2018 Mar 17]. Available from: http://www.espad.org/sites/espad.org/files/ESPAD_report_2015.pdf.

16. The European Monitoring Centre for Drugs and Drug Addiction, European School Survey Project on Alcohol and Other Drugs. Trends across 25 countries [Internet]. ESPAD; 2017 [cited 2018 Mar 20]. Available from: http://www.espad.org/report/trends-1995-2015/trends-across-25-countries.

17. Hibell B, Guttormsson U, Ahlström S, Balakireva O, Bjarnason T, Kokkevi A, et al. The 2011 ESPAD report substance use among students in 36 European countries [Internet]. Stockholm: ESPAD; 2012 [cited 2018 March 20]. Available from: http://www.espad.org/sites/espad.org/files/The_2011_ESPAD_Report_FULL_2012_10_29.pdf.

18. Khuder SA, Dayal HH, Mutgi AB. Age at smoking onset and its effect on smoking cessation. Addict Behav. 1999;24(5):673-7.

19. Mazur J, Dzielska A, Małkowska-Szkutnik A. The relationship between tobacco smoking and risk behaviour syndrome among 15-year-old adolescents in Poland and other European countries. Przegl Lek. 2009;66(10):768-72.

20. O'Cathail SM, O'Connell OJ, Long N, Morgan M, Eustace JA, Plant BJ, et al. Association of cigarette smoking with drug use and risk taking behaviour in Irish teenagers. Addict Behav. 2011;36(5):547-50.

21. Law on tobacco. Official Gazette of RS. No. 18/2018.

22. Babineau K, Taylor K, Clancy L. Electronic cigarette use among Irish youth: a cross sectional study of prevalence and associated factors. PLoS One. 2015;10(5):e0126419.

23. Goniewicz ML, Gawron M, Nadolska J, Balwicki L, Sobczak A. Rise in electronic cigarette use among adolescents in Poland. J Adolesc Health. 2014 ;55(5):713-5.

24. Kinnunen JM, Ollila H, Lindfors PL, Rimpelä AH. Changes in electronic cigarette use from 2013 to 2015 and reasons for use among Finnish adolescents. Int J Environ Res Public Health. 2016;13(11):1114.

25. Ramo DE, Young-Wolff KC, Prochaska JJ. Prevalence and correlates of electronic-cigarette use in young adults: findings from three studies over five years. Addict Behav. 2015;41:142-7.

26. Wills TA, Knight R, Williams RJ, Pagano I, Sargent JD. Risk factors for exclusive e-cigarette use and dual e-cigarette use and tobacco use in adolescents. Pediatrics. 2015;135(1):e43-51.

27. Gostin LO, Glasner AY. E-cigarettes, vaping, and youth. JAMA. 2014;312(6):595-6.

Rad je primljen 31. VIII 2018.
Recenziran 10. IX 2018.
Prihvaćen za štampu 19.IX 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:349-354.

University of Novi Sad, Faculty of Medicine, Novi Sad[1]
Clinic of Dentistry of Vojvodina, Novi Sad[2]
Clinical Center of Vojvodina, Clinic for Infectious Diseases, Novi Sad[3]

Original study
Originalni naučni rad
UDK 616.311.2-002:[616.988:578.828
https://doi.org/10.2298/MPNS1812355G

EFFECTS OF PERIODONTAL TREATMENT ON SERUM INFLAMMATORY MARKERS AND CD4T-LYMPHOCYTE CELL COUNT IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

UTICAJ PARODONTOLOŠKE TERAPIJE NA KONCENTRACIJU SERUMSKIH INFLAMATORNIH MARKERA I BROJ CD4T-LIMFOCITA KOD PACIJENATA SA INFEKCIJOM VIRUSOM HUMANE IMUNODEFICIJENCIJE

Ivana GUŠIĆ[1,2], Milanko ĐURIĆ[1,2], Tanja VELJOVIĆ[1], Jelena MIRNIĆ[1], Duška BLAGOJEVIĆ[1,2] and Snežana BRKIĆ[1,3]

Summary

Introduction. Several studies have reported reduction in the serum concentration of systemic inflammatory markers upon completion of periodontal therapy. However, no studies have been conducted on the effects of periodontal therapy on systemic inflammation in human immunodeficiency virus-positive patients. The aim of this study was to investigate the effects of periodontal therapy on the serum levels of systemic inflammatory biomarkers and CD4T-lymphocyte cell count in human immunodeficiency virus-positive patients. **Material and Methods.** The study included 34 human immunodeficiency virus-positive patients with chronic periodontitis receiving antiretroviral therapy. Periodontal parameters (plaque index, gingival index, papilla bleeding index, probing depth and clinical attachment level) and serum samples, assessed for the levels of interleukin-1 β, tumor necrosis factor-α, and C-reactive protein, were evaluated at baseline, 1- and 3-months upon completion of the non-surgical periodontal therapy. The CD4T-lymphocyte count was measured at baseline and three months after treatment completion. **Results.** Significant reduction in the values of plaque index, gingival index, papilla bleeding index, and probing depth was noted ($p < 0.001$), whereas the reduction in the median clinical attachment level value did not reach a statistically significant level ($F = 1.321$; $p = 0.261$). Local inflammation reduction was accompanied by a significant decrease in serum C-reactive protein ($F = 5.765$; $p = 0.014$) and a CD4T-lymphocyte count increase ($t = 2.321$; $p = 0.027$). Serum interleukin-1β and tumor necrosis factor-α did not change significantly as a result of periodontal treatment. **Conclusion.** Periodontal therapy contributes to the reduction of C-reactive protein and improvement of general health in human immunodeficiency virus-positive patients receiving highly active antiretroviral therapy.

Key words: Periodontal Diseases; CD4-Positive T-Lymphocytes; Biomarkers; HIV Infections; C-Reactive Protein; Tumor Necrosis Factor-alpha; Interleukin-1beta; Treatment Outcome;

Sažetak

Uvod. U više naučnih radova objavljeno je da koncentracija serumskih inflamatornih markera značajno opada nakon parodontološke terapije. Međutim, do danas nema podataka u literaturi o uticaju lečenja parodontopatije na sistemsku inflamaciju kod pacijenata pozitivnih na virus humane imunodeficijencije. Cilj ovog istraživanja bio je da se ispita uticaj parodontološke terapije na serumsku koncentraciju markera sistemske inflamacije i broj CD4T-limfocita kod osoba inficiranih virusom humane imunodeficijencije. **Materijal i metode.** U istraživanje je uključeno 34 ispitanika pozitivna na virus humane imunodeficijencije koji primaju antiretroviralnu terapiju i boluju od hronične parodontopatije. Klinički parodontološki parametri (plak indeks, gingivalni indeks, indeks krvarenja papile, dubina sondiranja i nivo pripojnog epitela) i serumske koncentracije interleukina-1β, faktor nekroze tumora-α i C-reaktivni protein posmatrani su na početku, kao i jedan i tri meseca nakon završetka kauzalne parodontološke terapije. Broj CD4T-limfocita ispitan je na početku i tri meseca posle lečenja. **Rezultati.** Nakon parodontološkog tretmana došlo je do značajnog pada vrednosti plak indeksa, gingivalnog indeksa, indeksa krvarenja i dubine sondiranja ($p < 0,001$), dok pad srednje vrednosti nivoa pripojnog epitela nije dostigao statistički značajan nivo ($F = 1,321$; $p = 0,261$). Smirivanje lokalne inflamacije bilo je praćeno značajnim smanjenjem serumske koncentracije C-reaktivnog proteina ($F = 5,765$; $p = 0,014$) i značajnim porastom broja CD4T-limfocita ($t = 2,321$; $p = 0,027$). Koncentracije interleukina-1β i faktor nekroze tumora-α u serumu nisu se značajno promenile nakon lečenja parodontopatije. **Zaključak.** Klinički uspešna parodontološka terapija praćena je značajnim padom serumske koncentracije C-reaktivnog proteina i poboljšanjem opšteg zdravlja pacijenata pozitivnih na virus humane imunodeficijencije koji *k*oriste antiretroviralnu terapiju.

Ključne reči: paradontopatije; CD4+ T limfociti; biomarkeri; HIV infekcija; C reaktivni protein; tumor nekrozni faktor alfa; interleukin 1beta; ishod lečenja

Corresponding Author: Doc. dr Ivana Gušić, Klinika za stomatologiju Vojvodine, Medicinski fakultet, Univerzitet u Novom Sadu, 21000 Novi Sad, Hajduk Veljkova 12, E-mail: gusic.ivana@mf.uns.ac.rs

Abbreviations

HIV	– human immunodeficiency virus
TNF-α	– tumor necrosis factor-α
IL-1β	– interleukin-1β
CRP	– C-reactive protein
HAART	– highly active antiretroviral therapy
PI	– plaque index
GI	– gingival index
PBI	– papilla bleeding index
PD	– probing depth
CAL	– clinical attachment level

Introduction

Although it is known that human immunodeficiency virus (HIV) infection affects the periodontal condition, the inverse relationship, i. e., impact of periodontal disease on HIV, has only recently emerged as a topic of interest [1].

Few studies have shown that periodontal infection can cause reactivation of latent HIV in infected cellular reservoirs [2, 3].

While the focus of HIV-related research is increasingly shifting towards early diagnosis of comorbidities [4] and the development of strategies for controlling the residual systemic inflammation and immune activation [5], the aim of the present study was to test the hypothesis that non-surgical periodontal therapy may impact the levels of systemic proinflammatory cytokines, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) in HIV-positive patients receiving highly active antiretroviral therapy (HAART). These two cytokines were selected due to their link with the biological aspects of periodontal disease [6] and their role in the reactivation of the HIV virus in the latently infected cells [7]. We also monitored the C-reactive protein (CRP) serum levels, as this is a highly sensitive indicator of systemic inflammatory response. Furthermore, another objective was to evaluate the influence of periodontal therapy on CD4T-lymphocytes cell count and the HIV viral load.

Material and Methods

A prospective cohort intervention design was used. The study protocol was approved by the Ethical Committee of the Clinic of Dentistry of Vojvodina in Novi Sad, Serbia (Ethical number 02-3/15-2010).

Patients with HIV-infection were selected from a group of 158 individuals who visited the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina for routine check-up between December 2010 and November 2011. The inclusion criteria were as follows: (1) receiving HAART for more than 6 months, without change in the dose or medication protocols in the three preceding months; (2) having at least 20 natural teeth; (3) clinically diagnosed periodontal disease; and (4) volunteering for participation and a signed informed consent. Patients who met any of the following criteria were excluded from the study: (1) any systemic antibiotic therapy within the last 3 months, (2) use of anti-inflammatory drugs within the previous 3 months, (3) periodontal therapy within the preceding 6 months, and (4) diagnosis of any serious uncontrolled systemic conditions.

Table 1. Demographic data, smoking habit, HIV infection characteristics and antiretroviral parameters of the study group

Tabela 1. *Demografski podaci, navika pušenja, karakteristike infekcije virusom humane imunodeficijencije i parametri antiretroviralne terapije ispitanika*

Age (years), mean (SD)/*Starost (u godinama), srednja vrednost (SD)*	38.26 ±10.361
Sex (male/female)/*Pol (muški/ženski)*	31 (91.18%)/3 (8.82%)
Smokers (%)/*Pušači (%)*	22 (64.7%)
Years since HIV infection was diagnosed, mean (SD)/*Kada je dijagnostikovana infekcija virusom humane imunodeficijencije (u godinama), srednja vrednost (SD)*	5.37 ± 3.29
HIV risk behavior, n (%)/*Način inficiranja virusom humane imunodeficijencije, n (%)*	
MSM	25 (73.5%)
HS	7 (20.6%)
IVDU	1 (2.9%)
OR	1 (2.9%)
Duration of HAART (years)/*Dužina upotrebe HAART (u godinama)*	3.36 ± 2.58
Antiretroviral therapy/*Antiretroviralna terapija*	
NRTI + PI	17 (50%)
NRTI + NNRTI	16 (47.06%)
NRTI + NNRTI + PI	1 (2.94%)

Legend: MSM – men who have sex with men; HS – heterosexuals; IVDU – intravenous drug users; OR – other reasons; HAART – highly active antiretroviral therapy; NRTI – nucleoside analog reverse-transcriptase inhibitors; NNRTI – non-nucleoside reverse-transcriptase inhibitors; PI – protease inhibitors

Legenda: *MSM – muškarci koji imaju seksualne odnose sa muškarcima; HS – hetreroseksualna transmisija; IVDU – intravenski korisnici narkotika; OR – drugi način inficiranja, HAART – visoko aktivna antiretroviralna terapija; NRTI – inhibitori nukleozid-reverzne-transkriptaze; NNRTI – inhibitori non-nukleozid reverzne-transkriptaze; PI – inhibitori proteaza*

Table 2. Estimated marginal means of dependent variables
Tabela 2. *Procenjene marginalne sredine zavisnih kliničkih varijabli*

		EMM	SE	95% confidence interval/*95% inetrval poverenja*	
				lower limit/*donji limit*	upper limit/*gornji limit*
PI	Baseline/*Pre lečenja*	1.48	0.14	1.19	1.76
	1 month/*1 mesec posle*	0.72	0.10	0.51	0.93
	3 months/3 meseca posle	0.72	0.11	0.51	0.93
GI	Baseline/*Pre lečenja*	1.52	0.12	1.27	1.76
	1 month/*1 mesec posle*	0.57	0.07	0.42	0.71
	3 months/*3 meseca posle*	0.52	0.11	0.31	0.74
PBI	Baseline/*Pre lečenja*	1.90	0.17	1.55	2.25
	1 month/*1 mesec posle*	0.82	0.09	0.64	1.01
	3 months/*3 meseca posle*	0.81	0.11	0.58	1.05
PD	Baseline/*Pre lečenja*	2.14	0.10	1.93	2.34
	1 month/*1 mesec posle*	1.72	0.07	1.59	1.86
	3 months/*3 meseca posle*	1.83	0.08	1.67	2.00
CAL	Baseline/*Pre lečenja*	2.01	0.70	0.58	3.43
	1 month/*1 mesec posle*	1.33	0.21	0.89	1.76
	3 months/*3 meseca posle*	1.35	0.22	0.91	1.79

Legend: EMM – estimated marginal means; SE – standard error; PI – plaque index; GI – gingival index; PBI – papilla bleeding index; PD – probing depth; CAL – clinical attachment level
Legenda: EMM – procenjene marginalne sredine; SE – standardna greška; PI – plak indeks; GI – gingivalni indeks; PBI – indeks krvarenja papile; PD – dubina sondiranja; CAL – nivo pripojnog epitela

After the inclusion and exclusion criteria were applied, 39 individuals remained. However, five patients were subsequently excluded, due to the initiation of systemic antibiotic therapy during the period of investigation. Thus, the final sample included 34 subjects, all of whom completed the study. The clinical aspect of the study measured parameters that indicate the level of oral hygiene, inflammation and destruction of periodontal tissue, i.e., plaque index Silness-Löe (PI) [6], gingival index Löe-Silness (GI) [8], papilla bleeding index − Mühlemann (PBI) [9], probing depth (PD) and clinical attachment level (CAL). All dental variables were assessed at four sites (mesiobuccal, midbuccal, distobuccal, and midlingual) of each tooth, except the wisdom teeth. All assessments were carried out using the Williams periodontal probe. The definition of periodontitis used in this study was proposed by a work group with representatives from both the Centers for Disease Control and Prevention and the American Academy of Periodontology [10]. All clinical data were collected at baseline, at 1- and 3-months after periodontal treatment completion (allowing its efficacy to be assessed).

Blood analysis was conducted in order to determine CD4 lymphocyte count (quantified by flow cytometry analyzer, Becton Dickinson, USA) and HIV viral load (RT PCR Amplicor, Roche Diagnostics Corporation, Indianapolis, USA), both of which were assessed at baseline and three months after completion of therapy. Serums were separated from parts of blood samples and immediately stored at -70°C until required for analyses aiming to establish concentrations of IL-1β (R&D System, Quantikine HS, Human IL-1β Immunoassay, Minneapolis, MN, USA), TNFα (R&D System, Quantikine HS, Human TNFα Immunoassay, Minneapolis, MN, USA) and CRP (Immunoturbidimetric assay, Cobas Integra, Roche AG Diagnostics, Mannheim, Germany). All serum samples were blind tested at the end of the study.

The standard non-surgical, cause-related periodontal therapy was tailored to individual patient needs without any time limitations. Study participants were examined at baseline and at one and three months following the completion of periodontal therapy. The therapy included oral hygiene instructions, extraction of teeth with poor prognosis, the ultrasonic (miniPiezon by EMS, Electro Medical System S.A., Nyon, Switzerland) removal of supra- and sub-gingival plaque and calculus, and root planing under local anesthesia using an Gracey curettes (Kohler, Germany).

The clinical data significant for the study objectives were calculated for each parameter by computing the full-mouth mean scores. The analyses included repeated measures analysis of variance (RM ANOVA) in the general linear module of the Statistical package for the social sciences (SPSS) software package. The observed parameters PI, GI, PBI, PD and CAL, and serum concentrations of IL-1β, TNFα and CRP were used as dependent variables. The within-subjects factors were the three-stage assessments, performed at baseline, as well as at 1- and 3-months post-treatment follow-ups. When the Mauchly's test indicated that the assumption of sphericity had been violated (χ^2 (2) = 10.310, p =

0.006), the Greenhouse-Geisser $\varepsilon < 0.75$, Huynh-Feldt correction was used.

A t-test was used to determine changes in the CD4 cell count and the HIV viral load, which were analyzed at baseline and 3 months after therapy.

Results

The study sample included 34 HIV-positive patients, 31 male and only 3 female with a mean age 38.26 ± 10.36. The general characteristics of subjects and their respective HIV infection parameters are shown in **Table 1**.

At baseline, most participants showed a widespread inflammation, but limited periodontal tissue destruction. The estimated marginal means of periodontal indices at baseline, and at one and three months post-treatment are shown in **Table 2**. The RM ANOVA determined that the values of all periodontal indices were statistically significantly low (PI: $F = 54.192$; $p < 0.001$, GI: $F = 66.60$; $p < 0.001$, PBI: $F = 49.106$; $p < 0.001$, PD: $F = 30.673$; $p < 0.001$) with the exception of CAL ($F = 1.312$; $p = 0.26$).

The mean (± SD) values of IL-1β at baseline and one and 3 months after treatment were 0.37 (± 1.49) pg/ml; 0.09 ± 0.25 pg/ml and 0.08 ± 0.22 pg/ml, respectively. In addition, the mean (± SD) values of TNFα at baseline and one and 3 months after treatment were 1.13 (±0.87) pg/ml, 1.02 ±0.59 pg/ml, and 1.22 (±0.69) pg/ml, respectively. The RE ANOVA showed that the reduction in both parameters was not statistically significant (IL-1β: $F = 1.715$; $p = 0.200$; TNFα: $F = 0.530$; $p = 0.591$). On the other hand, reduction in periodontal inflammation was accompanied by a continued decline in the mean value of serum CRP (at baseline 4.51 (± 6.82) mg/l; one month after therapy: 2.83 ± 3.24 mg/l and 3 months later: 2.30 (± 2.03) mg/l) which reached a statistical significance ($F = 5.765$, $p = 0.014$).

Before the beginning of periodontal therapy, the CD4 lymphocyte values ranged from 92.00/ml to 1289.00/ml, corresponding to the mean of 575.41 ± 287.83/ml. At the 3-month follow-up, the mean CD4 lymphocyte values increased by 78.41 ± 196.99 on average, reaching 653.82 ± 278.10, which corresponded to a statistically significant improvement ($t = 2.321$; $p = 0.027$).

In the majority of patients that took part in the present study, the HIV viral load was adequately controlled. At baseline, detectable HIV viral load levels were noted in 6 patients (17.65%), whereas the remaining 28 (82.35%) participants had a non-detectable number of HIV RNA copies per ml of blood. More specifically, the initial mean HIV viral load was 537.00 ± 2701.42 copies /ml. Three months after completing the periodontal therapy, only four patients had a detectible HIV viral load, and the mean value declined to 65.51 ± 252.75 copies /ml. However, due to the extremely high standard deviation, the differences in the HIV viral load measured pre- and post-treatment were not statistically significant ($t = 1.101$ $p = 0.279$).

Discussion

To the best of our knowledge, thus far, no studies have been conducted on the impact of periodontal therapy on the level of systemic inflammation in population of HIV-positive patients. The aim of the present study was to address this gap in the extant knowledge by assessing the effects of causal periodontal therapy on serum concentrations of proinflammatory cytokines IL-1β and TNFα and serum levels of CRP, along with CD4 count and HIV viral load as the markers of HIV infection. Our results indicate that the serum IL-1β and TNFα values were not significantly changed by the periodontal treatment. These findings are consistent with those reported in systemically healthy patients by Ide [11] and Yamazaki et al. [12]. These authors also noted no statistically significant changes in serum CRP upon therapy completion, despite significant clinical improvements in the condition of periodontal tissues [11, 12]. Conversely, in our population of HIV-positive patients, periodontal treatment contributed to a significant decline in serum CRP.

A significant reduction in the serum CRP level following periodontal therapy was reported in numerous studies conducted in systemically healthy individuals [13 - 15]. However, a meta-analysis conducted by Demmer et al., showed that anti-infective periodontal treatment can reduce serum CRP by approximately 0.4 mg/l [16]. According to the findings yielded by the same study, periodontal therapy that includes use of antibiotics can produce a post-treatment decline in serum levels of CRP to about 0.75 mg/l [16]. In our study, however, we noted an unexpectedly large reduction in the mean CRP level after periodontal treatment, in particular, given the predominance of localized periodontal tissue destruction.

Studies have shown that, in HIV-positive patients, including those undergoing HAART treatment, CRP levels tend to be higher compared to HIV-negative individuals [17, 18]. Given that this is the first study on the effects of periodontal therapy on systemic inflammation in HIV-positive patients, it is not possible to compare our results with those reported in similar extant works. Nonetheless, empirical evidence suggests that, in patients with systemic comorbidities, such as diabetes mellitus, anti-infective periodontal treatment results in a somewhat higher CRP reduction in relation to that achieved in systemically healthy cohort. This argument is supported by the results reported by Erciyas et al. [19], who showed that, in patients with active rheumatoid arthritis, periodontal treatment alone (i. e., without any changes in anti-rheumatic therapy) resulted in a decline in CRP level, from 17.00 (6.52–27.30) mm/dl to 8.00 (3.54–12.50) mm/dl ($p < 0.001$). Thus, it can be assumed that periodontal therapy contributes to the reduction of systemic inflammation in HIV-positive patients receiving HAART.

One of the findings that emerged from our research pertains to a significant increase in the mean CD4 values three months after periodontal therapy. In addition,

we also noted a decline in the number of HIV RNA copies in the blood (however, it regard to the baseline values, this change was not statistically significant). Given that none of the study participants reported a change in systemic HIV-related drug therapy during the course of the study, we can suppose that the combination of periodontal and antiretroviral therapy has contributed to the significant overall improvement in the health of HIV-positive patients. Our results support those reported by Noro-Filho et al., who reported that, in their study sample, periodontal treatment was associated with a decline in HIV viral load and a significant increase in the CD4 lymphocyte count [20].

The results of this study indicate that adequate treatment to HIV-positive patients with chronic periodontal disease is important, as it can lead not only to significant local improvements, but also contribute to the overall enhancement of their systemic health. In addition, our findings emphasize the importance of treatment of periodontal disease in the overall health status and outcomes of HIV-positive patients.

Conclusion

Based on our findings, non-surgical periodontal treatment leads not only to a significant clinical improvement of periodontal disease status, but also contributes to the improvement in the general health of human immunodeficiency virus-positive patients receiving highly active antiretroviral therapy.

References

1. Pólvora TLS, Nobre AVV, Tirapelli C, Taba M Jr, Macedo LD, Santana RC, et al. Relationship between human immunodeficiency virus (HIV-1) infection and chronic periodontitis. Expert Rev Clin Immunol. 2018;14(4):315-27.

2. González OA, Ebersole JL, Huang CB. Oral infectious diseases: a potential risk factor for HIV virus recrudescence? Oral Dis. 2009;15(5):313-27.

3. Imai K, Ochiai K, Okamoto T. Reactivation of latent HIV-1 infection by the periodontopathic bacterium Porphyromonas gingivalis involves histone modification. J Immunol. 2009;182(6):3688-95.

4. Petrović SM, Jovičić Popovska B, Raković I, Čanović P, Nešić Lj, et al. The outcome of Pneumocystis jiroveci pneumonia complicated with ventilator - associated pneumonia in a patient suffering from acquired immunodeficiency syndrome - a case report. Med Pregl. 2017;70(9-10):319-23.

5. Mudd JC, Brenchley JM. Gut mucosal barrier dysfunction, microbial dysbiosis, and their role in HIV-1 disease progression. J Infect Dis. 2016;214 Suppl 2:S58-66.

6. Fentoğlu Ö1, Köroğlu BK, Hiçyılmaz H, Sert T, Özdem M, Sütçü R, et al. Pro-inflammatory cytokine levels in association between periodontal disease and hyperlipidaemia. J Clin Periodontol. 2011;38(1):8-16.

7. Poli G, Kinter AL, Vicenzi E, Fauci AS. Cytokine regulation of acute and chronic HIV infection in vitro: from cell lines to primary mononuclear cells. Res Immunol. 1994;145(8-9):578-82.

8. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand. 1964;22:121-35.

9. Saxer UP, Mühlemann HR. Motivation and education. SSO Schweiz Monatsschr Zahnheilkd. 1975;85(9):905-19.

10. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol. 2007;78 Suppl 7S:1387-99.

11. Ide M, McPartlin D, Coward PY, Crook M, Lumb P, Wilson RF. Effect of treatment of chronic periodontitis on levels of serum markers of acute-phase inflammatory and vascular responses. J Clin Periodontol. 2003;30(4):334-40.

12. Yamazaki K, Honda T, Oda T, Ueki-Maruyama K, Nakajima T, Yoshie H, et al. Effect of periodontal treatment on the C reactive protein and proinflammatory cytokine levels in Japanese periodontitis patients. J Periodontal Res. 2005;40(1):53-8.

13. Mysak J, Podzimek S, Vasakova J, Mazanek J, Vinsu A, Duskova J. C-reactive protein in patients with aggressive periodontitis. J Dent Sci. 2017;12(4):368-74.

14. de Souza AB, Okawa RTP, Silva CO, Araújo MG. Short-term changes on C-reactive protein (CRP) levels after non-surgical periodontal treatment in systemically healthy individuals. Clin Oral Invest. 2017;21(1):477-84.

15. Zuza EP, Barroso EM, Fabricio M, Carrareto AL, Toledo BE, Pires JR. Lipid profile and high-sensitivity C-reactive protein levels in obese and non-obese subjects undergoing non-surgical periodontal therapy. J Oral Sci. 2016;58(3):423-30.

16. Demmer RT, Trinquart L, Zuk A, Fu BC, Blomkvist J, Michalowicz BS, et al. The influence of anti-infective periodontal treatment on C-reactive protein: a systematic review and meta-analysis of randomized controlled trials. PLoS ONE. 2013;8(10):e77441.

17. Borato DC, Parabocz GC, Ribas SR, Kalva-Filho CA, Borba LM, Ito CA, et al. Changes of metabolic and inflammatory markers in HIV infection: glucose, lipids, serum Hs-CRP and myeloperoxidase. Metabolism. 2012;61(10):1353-60.

18. Wada NI, Jacobson LP, Margolick JB, Breen EC, Macatangay B, Penugonda S, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS. 2015;29(4):463-71.

19. Erciyas K, Sezer U, Üstün K, Pehlivan Y, Kısacık B, Şenyurt SZ, et al. Effects of periodontal therapy on disease activity and systemic inflammation in rheumatoid arthritis patients. Oral Dis. 2013;19(4):394-400.

20. Noro Filho GA, Salgado DM, Casarin RC, Casati MZ, Costa C, Giovani EM. Anti-infective periodontal therapy promoting improvement in systemic markers of HIV infection. AIDS Res Hum Retroviruses. 2013;29(7):1040-4.

Rad je primljen 30. XI 2018.
Recenziran 5. XII 2018.
Prihvaćen za štampu 11. XII 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:355-359.

University of Novi Sad, Faculty of Medicine, Novi Sad

Original study
Originalni naučni rad
UDK 616.89:159.94.072
https://doi.org/10.2298/MPNS1812360R

INITIAL INVESTIGATION OF SOMATIZATION IN THE GENERAL POPULATION OF SERBIA: PREVALENCE, MANIFESTATIONS AND PREDICTORS

INICIJALNO ISTRAŽIVANJE SOMATIZACIJE U SRPSKOJ OPŠTOJ POPULACIJI: PREVALENCIJA, MANIFESTACIJE I PREDIKTORI

Nikola ROKVIĆ

Summary

Introduction. Somatization is one of the most prevalent current health issues affecting the well-being and quality of life in the general population. Many psychological constructs influence somatization and its outcomes. It was our aim to assess the features and prevalence of somatization in general population of Serbia by using the Patient Health Questionnaire-15 instrument, as well as to determine its relations with personality traits, factors of psychological distress and well-being. **Material and Methods.** Two studies were performed: Study 1 (N = 714) aimed to determine the relations between the Big Five personality traits, alexithymia and somatization, and Study 2 (N = 807) investigated the relationship between factors of psychological distress such as depression, anxiety and stress, factors of well-being such as life satisfaction and subjective vitality with somatization. **Results.** In Study 1, Neuroticism and Toronto Alexithymia Scale-20 Factor 1, difficulty identifying feelings, strongly correlated with somatization, and the measured constructs explained 33.4% of somatization variance. In Study 2, anxiety and stress had the strongest correlation indices from the measured constructs and Study 2 regression model explained 44.7% of the variance. The most prevalent symptoms measured by the Patient Health Questionnaire-15 were tiredness, back pain and headaches. **Conclusion.** Somatization levels were slightly higher than those previously reported in general population. However, they were still well under those reported in the clinical populations. Symptom prevalence was compatible with previous findings in the general population, whereas Neuroticism and anxiety were most closely associated with somatization. Further research is needed to define other factors that contribute to the development of somatization.

Key words: Somatoform Disorders; Affective Symptoms; Neuroticism; Anxiety; Mental Disorders; Personal Satisfaction; Personality; Signs and Symptoms; Surveys and Questionnaires

Sažetak

Uvod. Somatizacija je jedna od najprevalentnijih pretnji za zdravlje, subjektivno blagostanje i kvalitet života današnje opšte populacije. Mnogi psihološki konstrukti utiču na somatizaciju i njene ishode. Naš cilj je da utvrdimo kvalitet i prevalentnost somatizacije u opštoj populaciji Srbije koristeći *Patient Health Quearionnaire*-15 upitnik i da odredimo njene relacije sa crtama ličnosti i faktorima psihološkog distresa i blagostanja. **Materijal i metode.** Izradili smo dve studije. Studija 1 (N = 714) ima za cilj da odredi relacije između *Velikih pet crta ličnosti*, aleksitimije i somatizacije. Studija 2 (N = 807) imala je za cilj da utvrdi odnos faktora psihološkog distresa, odnosno depresivnosti, stresa i anksioznosti, zadovoljstva životom i subjektivne vitalnosti sa somatizacijom. **Rezultati.** U studiji 1 *Neuroticizam* i faktor 1 *Toronto Alexithymia Scale*, otežana identifikacija osećanja, najjače koreliraju sa somatizacijom i konstrukti mereni u ovoj studiji objašnjavaju 33,4% varijanse somatizacije. Anksioznost i stres koreliraju najsnažnije sa stepenom somatizacije u studiji 2. Regresioni model u studiji 2 ukazuje da mereni konstrukti objašnjavaju 44,7% varijanse. Najprevalentniji simptomi mereni *Patient Health Questionnaire-15* su umor, bolovi u leđima i glavobolja. **Zaključak.** Nivoi somatizacije su malo iznad najviših merenih u opštoj populaciji, ali ispod nalaza merenja na kliničkoj populaciji. Prevalencija simptoma je u skladu sa dosadašnjim nalazima. Neuroticizam i anksioznost su tesno povezani sa somatizacijom. Dalja istraživanja treba da odrede dodatne faktore koji utiču na razvoj somatizacije.

Ključne reči: somatoformni poremećaji; afektivni simptomi; neuroticizam; anksioznost; mentalni poremećaji; lično zadovoljstvo; ličnost; znaci i simptomi; istraživanja i upitnici

Introduction

Somatization, presence of physical symptoms that lack a demonstrable organic basis, represents one of the major problems of today's health care systems and it also leads to significant disruptions in daily functioning of individuals suffering from it [1]. An analysis of trends in European Union (EU) countries from 2011, which was focused on what authors classified as mental disorders, has shown that up to 38.9% of the population suffered from at least one of the 27 disorders which were the focus of the screening [2]. To further illustrate the problem, a recent study has shown that 63.1% of the population in Europe over the age 65 years reported having three or more somatoform disorders during their lifetime [3].

Corresponding Author: Psiholog Nikola Rokvić, Univerzitet u Novom Sadu, Medicinski fakultet, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: nikola.rokvic@mf.uns.ac.rs

Abbreviations

PHQ – Patient Health Questionnaire
DASS – Depression Anxiety and Stress Scale
BFI – Big Five Inventory
SVS – Subjective Vitality Scale

This report suggests that, at any time, about 20.4 million people in Europe suffer from some manifestations of somatoform disorders [2]. The screening has also shown that about one third of all patients that sought treatment from a medical practitioner did not receive an adequate explanation for their symptoms [4]. Another study showed that from 20% to 50% of patients in primary care seek help due to symptoms that can be characterized as medically unexplained [5]. Such somatoform disorders negatively influence comorbid psychiatric disorders, quality of life and cause functional impairment [6]. Measuring the level of somatization, primarily in the clinical populations, represents a diagnostic problem that is a focus of extensive research. The Patient Health Questionnaire-15 (PHQ-15) has been developed as a clinical tool to be used in the population of in- and out-patients. That is why it contains only 15 items covering 90% of complaints that patients usually have and 14 out of 15 most prevalent somatization disorders as defined by the Diagnostic and Statistical Manual (DSM) IV classification [7]. This scale has later been validated in the general populations [1] making it suitable for our research.

The subject of "medically unexplained symptoms" has been much researched because of its impact on patient care and well-being. A study has found that there is a great discrepancy in the incidence of these symptoms when examined from the perspective of patients and medical practitioners [8]. And also, there is a great variability between reported incidences [9]. While the PQH-15 scale has not been precisely designed to detect what should be considered "medically unexplained symptoms", it was highly associated with determining the prevalence of somatic disorder symptoms [10, 11]. These symptoms fluctuate over the time [11] and can be attributed to a large number of causes such as meteoropathy [12].

Depression, anxiety and somatization are the most common mental health issues in primary care [13]. General practitioner is usually the first person from whom a patient with overlapping psychological and physical problems seeks help [14]. Looking into a large sample of outpatients with severe depression in primary care, one can notice that severe anxiety and high levels of somatization overlap to a great extant; depression and anxiety account for 49% of the somatization variance, while anxiety itself accounts for 35% of the variance in healthy but obese population [13, 15]. Anxiety and its association with somatization has specifically been a target of much research but it still receives less attention than depression in primary care settings [15]. Only between 15% and 36% of patients with anxiety disorders, such as general anxiety, social anxiety and panic disorder, are recognized by general practitioners [16].

In general and primary care populations, the correlation between somatization and depression has been reported to be one of the highest among all the measured constructs [17, 18], and the above mentioned research has entirely proven its importance in studying somatization. While specific forms of stress-related disorders, such as Post Traumatic Stress Disorder (PTSD) and their relation with somatization are thoroughly researched, we have found little data on the influence of stress, as part of general psychological distress, on somatization. In that respect, the use of the Depression Anxiety and Stress Scale (DASS)-21 questionnaire was the best for our study, because not only does it measure the levels of most common constructs related to somatization such as depression, stress and anxiety, but it is also a measure of general psychological distress [19]. Furthermore, all three constructs are shown to overlap with somatization in large populations [20]. Life satisfaction, as part of well-being, was found to correlate significantly with somatization and related constructs [1], and is therefore included in our research.

Other psychological constructs, apart from those pertaining to the concept of well-being, influence somatization. Personality traits also have a significant impact on the phenomenon in non-clinical and clinical populations, and are responsible for mediating outcomes in many illnesses designated as psychosomatic, especially Neuroticism, know also as emotional stability [21, 22]. Apart from the traditional Big Five personality traits, it has also been shown that alexithymia – inability to identify, perceive and value emotions – has a significant effect on the overall health of the population and treatment outcomes [22–24]. In light of this, we have decided to include this construct in our study of somatization.

The aim of this study was to determine the level and quality of somatization in the Serbian general population, as well as its relationship with factors of psychological distress, well-being and personality traits. Also, we determined the predictive value of these constructs on somatization levels.

Material and Methods

This investigation included two separate studies conducted from 2016 to 2017. Both surveys were web-based and the questionnaires were distributed on social media. The PHQ-15 questionnaire was used in both studies, along with different additional tests to gain greater insight into the relation between somatization and other constructs in the Serbian general population and to determine the predictive value of those constructs. Study 1 included 714 participants, 537 (75.2%) female, with a mean age of 29.61 (min = 18; max = 59). Study 2 included 807 participants, 585 (72.5%) of them were female, with a mean age of 27.73 years (min = 18; max = 66; SD = 7.01).

To determine the overall level and quality of life in somatization, we have summed up the results of the PHQ-15 instrument from both studies so that this extended sample could give us a more compre-

hensive understanding. The combined sample consisted of 1521 participants, 1122 (73.8%) of them were females, with a mean age of 28.61 years (min = 18; max = 66; SD = 7.23).

The PHQ-15 questionnaire has been developed as a clinical tool for measuring the level of somatization. Its items analyze symptoms or symptom clusters that account for more than 90% of physical complaints, except the upper respiratory tract, reported in the outpatient settings [7]. The translations of the questionnaire were further validated for the use in the primary care patients [25], and further validated for the use in the general population [26]. This instrument was used both in study 1 and 2 and in the combined sample.

The Toronto Alexithymia Scale [TAS-20] is one of the most commonly used instruments to measure levels of alexithymia. The scale was translated into Serbian [27] but after further validation of its factor structure, item 20 was removed in order to retain its original factor structure [24]. This instrument consists of 3 subscales; factor 1 – difficulty identifying feelings, factor 2 – difficulty describing feelings, and factor 3 – externally-oriented thinking. The instrument showed good internal consistency (α = .84) as a whole and at the level of subscales with expected shortcomings of the factor 3 subscale (α = .83; α = .82; α = .53). This instrument was used in Study 1.

The Big Five Inventory (BFI) contained 44 items because of its relative shortness and proven reliability. The Big Five personality traits are Extraversion, Agreeableness, Conscientiousness, Neuroticism and Openness, and alpha values for all subscales are .83, .74, .82, .84, .85, respectively. This instrument was used in Study 1.

DASS-21 questionnaire is a 21 item short form scale used for measuring levels of depression, stress and anxiety, being a general measure of psychological distress. The DASS-21 scale has been expertly translated into Serbian and the psychometric properties have proven to be satisfactory [28]. Internal consistency of depression, stress and anxiety subscales was high, α = .87, α = .81, α = 79, respectively. This instrument was used in Study 2.

Satisfaction with Life Scale (SWLS) is a measure of global life satisfaction. It measures life satisfaction compared to the participant's own criteria and represents the cognitive element of subjective well-being [29]. The psychometric properties of the Serbian translation have been validated and proven satisfactory [30] and the scale's internal consistency in our sample was good (α = .85). This instrument was used in Study 2.

Subjective Vitality Scale (SVS) was proposed [31] as a seven item self-report instrument. Later, its psychometric properties have been revised [32] and item 2 was eliminated. At present, both seven- and six-item versions are being used, although the six-item version is more prevalent in research, and this variation has therefore been used in our study. The internal consistency for the SVS was within acceptable margins (α = .82). This instrument was used in Study 1.

The gathered data were processed by using descriptive statistics, correlation and regression statistical methods. These operations were performed by using International Business Machines' (IBM) Statistical Package for the Social Sciences (SPSS) software. All used instruments are in the public domain. The PHQ-15 questionnaire was translated by bilingual experts, and no permission is required for its usage and translation.

Results

The correlations between the measured constructs and somatization are represented by the PHQ-15 scores in **Table 1**. The multiple regression analysis was also performed. In the model, BFI personality traits and the general alexithymia scores were used as independent variables. After controlling for age, the model F we proposed explained 33.4% of the variance (7, 706) = 50.59, p < .001. Values for individual variables are also presented in **Table 1**.

The correlations between measured constructs and the PHQ-15 somatization scores in Study 2 are presented in **Table 2**. The regression model with these variables controlled for the participants' age

Table 1. Correlation coefficients between PHQ-15 score and other measured constructs in Study 1, and β values and significance of individual variables in the regression model
Tabela 1. *Koeficijenti korelacije između PHQ-15 skora i drugih merenih konstrukata u studiji 1, i β-vrednosti i značajnost pojedinačnih varijabli u regresionom modelu*

	Correlation with PHQ-15 score/*Korelacije sa PHQ-15 skorom*	β	Sig.
Factor 1/*Faktor 1*	.524**	/	/
Factor 2/*Faktor 2*	.321**	/	/
Factor 3/*Faktor 3*	.011	/	/
TAS-20 score/*TAS-20 skor*	.419**	.22	.000
Extraversion/*Ekstraverzija*	-.310**	-.057	.129
Agreeableness/*Prijatnost*	-.227**	.013	.716
Conscientiousness/*Savestnost*	-.274**	-.062	.079
Neuroticism/*Neuroticizam*	.528**	.418	.000
Openness/*Otvorenost*	-.074*	.132	.000

PHQ – Patient Health Questionnaire; TAS – *Toronto Alexithymia Scale*

Table 2. Correlations between measured constructs and PHQ-15 score in Study 2, and β values and significance of individual variables in the repression model
Tabela 2. *Koeficijenti korelacije između PHQ-15 skora i drugih merenih konstrukata u studiji 2, i β-vrednosti i značajnost pojedinačnih varijabli u regresionom modelu*

	Correlation with PHQ-15 score/*Korelacije sa PHQ-15 skorom*	β	Sig.
Subjective Vitality Scale (SVS) *Subjektivna vitalnost (SVS)*	-.36**	-.14	.000
Satisfaction With Life Scale SWLS) *Zadovoljstvo životom (SWLS)*	-.29**	.017	.611
Depression (DASS-21) *Depresivnost (DASS-21)*	.483**	-.012	-.272
Stress (DASS-21)/*Stres (DASS-21)*	.547**	.201	.000
Anxiety (DASS-21) *Anksioznost (DASS-21)*	.633**	.468	.000

PHQ – Patient Health Questionnaire; TAS – *Toronto Alexithymia Scale*

Table 3. Distribution of participants by symptoms and their severity
Tabela 3. *Distribucija participanata po simptomu i njihovoj težini*

Symptom *Simptom*	Not bothered at all (0 points) – N *Nikakve smetnje*	Bothered a little (1 point) – N *Male smetnje*	Bothered a lot (2 points) – N *Velike smetnje*	Mean score by symptom (0 - 2)/*Prosečan skor po simptomu*
Feeling tired or having low energy *Osećaj umora ili manjka energije*	390 (25.6%)	827 (54.4%)	304 (20%)	.94
Back pain/*Bolovi u leđima*	537 (35.3%)	811 (53.3%)	173 (11.4%)	.76
Headaches/*Glavobolja*	637 (41.9%)	733 (48.2%)	151 (9.9%)	.68
Menstrual cramps or other problems with periods (Female participants only)/*Menstrualni grčevi ili drugi problemi sa menstruacijom (samo za ispitanike ženskog pola)*	360 (32.1%)	534 (47.6%)	228 (20.3%)	.88
Trouble sleeping *Problemi sa spavanjem*	745 (49%)	537 (38%)	198 (13%)	.64
Nausea, gas or indigestion *Mučnina, nadutost ili žgaravica*	744 (48.9%)	667 (43.9%)	110 (7.2%)	.58
Abdominal pain/*Bolovi u stomaku*	745 (48.9%)	684 (45%)	92 (6%)	.57
Pain in the arms, legs, or joints (knees, hips, etc.)/*Bolovi u rukama, nogama i zglobovima (kolena, laktovi i sl.)*	899 (59.1%)	543 (35.7%)	79 (5.2%)	.46
Constipation, loose bowels, or diarrhea/*Zatvor, dijareja ili drugi problemi sa stolicom*	929 (61.1%)	543 (35.7%)	90 (5.9%)	.45
Feeling your heart pound or race *Osećaj lupanja srca*	1037 (68.2%)	426 (28%)	58 (3.8%)	.38
Shortness of breath/*Osećaj da ostajete bez daha, da ne možete da nadišete*	1152 (75.7%)	302 (19.9%)	67 (4.4%)	.29
Dizziness/*Vrtoglavica*	1134 (74.6%)	346 (22.7%)	41 (2.7%)	.28
Chest pain/*Bolovi u grudima*	1221 (80.3%)	267 (17.6%)	33 (2.2%)	.22
Pain or problems during sexual intercourse/*Bol ili problemi tokom polnog odnosa*	1292 (84.9%)	202 (13.3%)	27 (1.8%)	.17
Fainting spells *Povremeni gubitak svesti*	1391 (91.5%)	202 (7.4%)	27 (1.8%)	.1

was also calculated. The model F we proposed in Study 2 explained 44.7% of the variance (6, 798) = 107.49, $p < .001$. Values for individual variables are also presented in **Table 2**.

Combined Sample

The average PHQ-15 score was 7.15 (min = 0; max = 24; SD = 4.38), 7.59 in females (min = 0; max = 24; SD = 4.46) and 5.91 in males (min = 0; max = 21; SD = 3.89). We found that score levels were significantly higher in female participants ($p > .001$). PHQ-15 score values were stratified into 4 groups. Minimal somatization, scores from 0 to 4, was detected in 455 (29.9%) participants. Low levels of somatization, 5 to 9 points, were detected in 675 (44.4%) of the participants. Medium and high levels of somatization, from 10 to 14 and from 15 to 30, were detected in 283 (18.6%) and 108 (7.1%) participants, respectively. Analysis of variance (ANOVA) test showed that there were no differences between age groups of examinees with somatoform disorders. The Chi square test showed that there was a statistically significant difference in gender distribution between somatization categories, c^2 (3, n = 1521) = 45.48, $p > .001$, Cramer's V = .173, $p > .001$, in favor of female participants being more distributed in higher somatization categories. There was a significant difference between genders in somatization levels in favor of female participants, but there was no significant correlation between participants' age and somatization scores. The distribution of participants by symptoms and their severity is presented in **Table 3**.

Discussion

The main difficulty in interpreting the results is the lack of normative data for the Serbian population and differences between available normative data with regards to respective examined populations. There are two main groups of populations being examined in the literature – the general and the clinical population. The clinical population is then subdivided into primary care patients, psychiatric outpatients, chronic disease patients and participants with similar disorders. The large samples of clinical population, especially when defined as primary care outpatients, represent a very diverse group. Our sample represents the cross-section of the Serbian society, irrespective of their current health status, in order to provide an insight into levels and quality of somatization and to determine pilot normative data.

Study 1 was conducted during 2016, while Study 2 was performed during 2017. The common denominator between these studies is the usage of the PHQ-15 questionnaire and the fact that they were both web-based surveys relying on participation of social network users. The PHQ-15 results of the two studies were combined to be used as a single sample. We believe that by doing this, we were able to provide a more complete overview of somatization in the Serbian population, and provide more accurate data representative of the population.

In Study 1, we attempted to determine the relationship between somatization measured by the PHQ-15 questionnaire and various personality determinants. We found an expected strong correlation between somatization and alexithymia [33], especially for factor 1 – difficulties identifying feelings. Surprisingly, factor 3, externally oriented thinking, showed no significant correlation. At present, we cannot explain this finding, so it should be a starting point for further research. Out of the Big Five personality traits, as expected, Neuroticism has the strongest correlation index with somatization [34]. Although we expected that Openness has a negligible correlation index with somatization, we were surprised by the fact that it contributed in a unique way to the Study 1 regression model and it accounts for 22% of the model variance. Personality traits explained 33.4% of the variance, but because we could not find corresponding data in the literature, we cannot compare our findings with other studies.

In Study 2, we examined the relationship of somatization with parameters of psychological well-being and distress. In our study we analyzed the correlation indices and created a regression model controlling the age of participants. Correlation levels between depression and somatization measured by the PHQ-15 vary to a great extent between available studies but they are always significant. In the general population, coefficients vary from .4 [17] to .75 [23], while a value of .72 was reported in the population of primary care patients [11]. Our results occupy the middle ground between reported values but given the large discrepancy in reported findings, a significant conclusion cannot be made.

We have a clearer picture when examining the relationship between anxiety and somatization. General population surveys report a .47 and .54 correlation index between anxiety and PHQ-15 scores [17, 10], while the correlation coefficient between these two constructs is .67 in the primary care patients population [11]. Correlation levels detected in Study 2 are closer to the clinical end of the spectrum. Different instruments were used to measure anxiety levels in the aforementioned studies and anxiety itself is a complex multifaceted phenomenon. Detection of elevated correlation coefficient between anxiety and somatization in our sample bears further scrutiny, specifically to determine if it is a result of our study or a trait of the Serbian population as such.

There is little information about the correlation between stress levels and somatization, causing difficulties in interpretation. Correlation coefficient values are much higher in our sample (r = .55) than in the available literature (.44), and this finding is further validated because both our study and the study in question were performed in the general population [17]. When analyzing the relationship between life satisfaction and somatization the results correspond to those found in the literature with reported correlation indexes of r = -.37 and r = -.36 in

Study 2. Negative correlation between vitality and somatization has been reported [1], but there are some reservations whether different scales measured the same psychological construct. Still, we can say that in this respects, our findings are expected.

Life satisfaction, subjective vitality, depression, anxiety and stress account for 44.7% of somatization variance in the Study 2. Further examination showed that only anxiety, stress and subjective vitality significantly explain somatization variance, regardless of high correlation levels detected with other constructs. This is contrary to what was expected. Depression has been reported to be the strongest predictor of somatization levels [16]. Also, depression and anxiety have been reported to account for 46% of the somatization variance in primary care patient sample [11]. It may be assumed that anxiety and depression have been measured by different tools than in our study, but somatization levels have been evaluated by the PHQ-15 questionnaire. Still, the fact that anxiety is the most prominent single contributor to the regression model may be linked with stronger correlation between anxiety and somatization than expected, making anxiety the leading factor influencing somatization levels in our study.

We have performed an analysis of the somatization levels and symptom prevalence in the complete sample. The original guidelines for interpreting PHQ-15 scores define a score of 1 – 4 as minimal, 5 – 9 as low, 10 – 14 as medium, and 15 – 30 as high. Also female participants have higher levels of somatization than males [7]. This research has been performed in primary care patients, whereas normative data for the general population have been created several times for different cultures. Standard mean values of somatization in the general population were reported to be 4.3 for females and 3.4 for males [1], 5.63 for females and 3.71 for males [10], 7.18 for females and 5.25 for males [17]. All studies found significant gender differences. When interpreting our results, we found that our participants have slightly higher somatization levels than the highest so far reported in general population.

Also, the mean score of 6.3 was found in participants without an anxiety disorder, while means for those with depression and anxiety disorder are 15.2 and 13.9, respectively [11]. A mean score of 13 was found in participants with generalized anxiety, while it was 12 in participants with social anxiety [13]. Mean scores above 11.5 have been found in Korean psychiatric outpatients and therefore we can associate PHQ-15 mean score with a possibility of an anxiety and a depressive disorder. Our sample is above the normative data in the general population, but within low somatization levels given by the original research, and also beneath mean values found in participants suffering from some type of anxiety and depressive disorder. We can conclude that while there is some cause for alarm somatization levels in the population, they are still under pathological levels.

Sleep deprivation, back pain, tiredness, low energy and abdominal pain have the highest mean item scores. On the other hand, tiredness and sleeping problems have the highest incidence in subjects that are experiencing major difficulties with those disorders. This can perhaps be associated with the fact that anxiety has the highest correlation with the PHQ-15 general score and that anxiety levels account for much of its variance. In the female population, 20.3% of participants have severe difficulties with menstrual cramps and other period related symptoms, making it the fourth most prevalent symptom. Due to the association between anxiety and menstrual symptoms, as well as cultural beliefs with reporting menstrual symptoms [34], further investigation is needed. Comparing the severity of individual symptoms with other research is difficult because of the difference in mean scores, but we can compare the most and least prominent symptoms in the population. In general population, sleeping problems, back pain and low energy were the top three symptoms participants complained about, whereas pain during sexual intercourse, blackouts and chest pain were the three least reported symptoms [9]. This roughly corresponds to our findings.

This research has several limitations. In order to get as large a sample as possible, we have reduced the number of socio-demographic questions, providing the participants an increased sense of anonymity. Although this has yielded a large sample, the lack of more in depth socio-demographic data somewhat limits the study. As in all web-based surveys, the male to female ratio tilted towards the female population, but the number of male participants was still significant to perform valid statistical comparisons. We believe that at this preliminary stage it would be inappropriate to read more into the possible psychological origins of somatization. More sophisticated psychological instruments are needed to make such conclusions on appropriate samples to perform a facet by facet analysis of various personality constructs and their relationships with somatization. This study was conceptualized as a pilot study, only to provide an overview and light the way for future investigation of the concept of somatization and its psychological origins in the Serbian cultural area.

Conclusion

Somatization levels in the Serbian general population are slightly higher, in both male and female participants, than the accepted standards, but still well under pathological findings. Tiredness, low energy, headaches and back pain are most prominent symptoms with menstrual difficulties gaining a prominent position among symptoms. Neuroticism and anxiety have the highest correlation indices with somatization and should be the focus of further research into this mater. Stress, depression and alexithymia should follow closely. Factors of psychological well-being and distress are better predictors of somatization than personality traits, explaining the larger part of the variance in the regression model. More large scale research with different instruments designed to measure somatization are

needed for further investigation of the particularities of this phenomenon in the Serbian population, but we believe we have created a starting point for further development in this area of research.

References

1. Kocalevent DR, Hinz A, Brahler E. Standardization of a screening instrument (PHQ-15) for somatization in the general population. BMC Psychiatry. 2013;13:91.

2. Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B, et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol. 2011;21(9):655-79.

3. Dehoust MC, Schulz H, Härter M, Volkert J, Sehner S, Drabik A, et al. Prevalence of somatoform disorders in the elderly: results of a European study. Int J Methods Psychiatr Res. 2017;26(1):e1500.

4. Marple RL, Kroenke K, Lucey CR, Wilder J, Lucas CA. Concerns and expectations in patients presenting with physical complaints. Arch Intern Med. 1997;157(13):1482-8.

5. Van Ravesteijn H, Wittkampf K, Lucassen P, van de Lisdonk E, van den Hoogen H, van Weert H, et al. Detecting somatoform disorders in primary care with the PHQ-15. Ann Farm Med. 2009;7(3):232-8.

6. Han C, Pae CU, Patkar AA, Masand PS, Kim KW, Joe SH, et al. Psychometric properties of the Patient Health Questionnaire-15 (PHQ-15) for measuring the somatic symptoms of psychiatric outpatients. Psychosomatics. 2009;50(6):580-5.

7. Kroenke K, Spiltzer RL, Williams JB. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. Psychosom Med. 2002;64(2):258-66.

8. Schumacher S, Rief W, Brähler E, Martin A, Glaesmer H, Mewes R. Disagreement in doctor's and patient's rating about medically unexplained symptoms and health care use. Int J Behav Med. 2013;20(1):30-7.

9. Steinbrecher N, Koerber S, Frieser D, Hiller W. The prevalence of medically unexplained symptoms in primary care. Psychosomatics. 2011;52(3):263-71.

10. Hinz A, Ernst J, Glaesmer H, Brähler E, Rauscher FG, Petrowski K, et al. Frequency of somatic symptoms in the general population: normative values for the Patient Health Questionnaire-15 (PHQ-15). J Psychosom Res. 2017;96:27-31.

11. Claassen-van Dessel N, van der Wouden JC, Hoekstra T, Dekker J, van der Horst HE. The 2-year course of Medically Unexplained Physical Symptoms (MUPS) in terms of symptom severity and functional status: results of the PROSPECT cohort study. J Psychosom Res. 2018;104:76-87.

12. Žikić M, Rabi Žikić T. Meteoropathy and meteorososensitive persons. Med Pregl. 2018;71(3-4):131-5.

13. Fergus TA, Limbers CA, Griggs JO, Kelley LP. Somatic symptom severity among primary care patients who are obese: examining the unique contributions of anxiety sensitivity, discomfort intolerance, and health anxiety. J Behav Med. 2008;41(1):43-51.

14. Lowe B, Spitzer RL, Williams JB, Mussell M, Schellberg D, Kroenke K. Depression, anxiety and somatization in primary care: syndrome overlap and functional impairment. Gen Hosp Psychiatry. 2008;30(3):191-9.

15. Kroenke K, Spitzer RL, Williams JB, Monahan PO, Lowe B. Anxiety disorders in primary care: prevalence, impairment, comorbidity, and detection. Ann Intern Med. 2007;146(5):317-25.

16. Lowe B, Grafe K, Zipfel S, Spitzer RL, Herrmann-Lingen C, Wittel S, et al. Detecting panic disorder in medical and psychosomatic outpatients: comparative validation of the Hospital Anxiety and Depression Scale the Patient Health Questionnaire, a screening question and physicians diagnosis. J Psychosom Res. 2003;55(6):515-9.

17. Hyphantis T, Kroenke K, Papatheodorou E, Paika V, Theocharopolous N, Ninou A, et al. Validity of the Greek version of the PHQ 15-item Somatic Symptom Severity Scale in patients with chronic medical conditions and correlations with emergency department use and illness perceptions. Compr Psychiatry. 2014;55:1950-9.

18. Nordin S, Palmquist E, Nordin M. Psychometric evaluation and normative data for a Swedish version of the Patient Health Questionnaire 15-item Somatic Symptom Severity Scale. Scand J Psychol. 2013;54(2):112-7.

19. Henry JD, Crawford JR. The short-form version of the Depression Anxiety Stress Scales (DASS-21): construct validity and normative in a large non-clinical sample. Br J Clin Psychol. 2005;44(Pt 2):227-39.

20. Bener A, Al-Kazaz M, Ftouni D, Al-Harthy M, Dafeeah EE. Diagnostic overlap of depressive, anxiety, stress and somatoform disorders in primary care. Asia Pac Psychiatry 2013;5(1):E29-38.

21. Koh JS, Ko HJ, Wang SM, Cho KJ, Kim JC, Lee SJ, et al. The association of personality trait on treatment outcomes in patients with chronic prostatitis/chronic pelvic pain syndrome: an exploratory study. J Psychosom Res. 2014;76(2):127-33.

22. De Gucht V. Stability of neuroticism and alexithymia in somatization. Compr Psychiatry. 2003;44(6):466-71.

23. Kajanoja J, Scheinin NM, Karlsson L, Karsslon H, Karukivi M. Alexithymia as a health risk and resilience factor: response to Dr. Davydov. J Psychosom Res. 2017;101:135-6.

24. Roković N, Jovanović T. Alexithymia measured with the TAS-20 questionnaire: determining the validity of the factor structure and its relation of life satisfaction and the Big Five personality traits. Psihološka istraživanja. 2018;21(1):23-40.

25. Interian A, Allen LA, Gara MA, Excobar JI, Diaz-Martinez AM. Somatic complaints in primary care: further examining the validity of the Patient Health Questionnaire (PHQ-15). Psychosomatics. 2006;47(5):392-8.

26. Kocalevent RD, Hinz A, Brahler E. Standardization of the depression screener Patient Health Questionnaire (PHQ-9) in the general population. Gen Hosp Psychiatry. 2013;35(5):551-5.

27. Trajanović NN, Djurić V, Latas M, Milovanović S, Jovanović AA, Djurić D. Serbian translation of the 20-item Toronto Alexithymia Scale: psychometric properties and the new methodological approach in translating scales. Srp Arh Celok Lek. 2013;141(5-6):366-70.

28. Jovanović V, Gavrilov-Jerković V, Žuljevic D, Brdarić D. Psychometric evaluation of the Depression Anxiety Stress Scales-21 (DASS-21) in a Serbian student sample. Psihologija. 2014;47(1):93-112.

29. Diener E, Emmons RA, Larsen RJ, Griffin S. The satisfaction with Life Scale. J Pres Assess. 1985;49(1):71-5.

30. Jovanović V. The validity of the satisfaction with Life Scale in adolescents and a comparison with a single item life satisfaction measures: a preliminary study. Qual Life Res. 2016;25(12):3173-80.

31. Ryan RM, Frederick C. On energy, personality, and health: subjective vitality as a dynamic reflection of well-being. J Pers. 1997;65(3):529-65.

32. Bostic TJ, McGartland Rubio D, Hood M. A validation of the Subjective Vitality Scale using structural equation modeling. Soc Indic Res. 2000;52(3):313-24.

33. Lockenhoff CE, Sutin AR, Ferrucci L, Costa PT. Personality traits and subjective health in later years: the association between NEO-PI-R and SF-36 in advanced age is influenced by health status. J Res Pers. 2008;42:1334-46.

34. Sigmon ST, Dorhofer DM, Rohan KJ, Boulard NE. The impact of anxiety sensitivity, bodily expectations, and cultural beliefs on menstrual symptom reporting: a test of the menstrual reactivity hypothesis. J Anxiety Disord. 2000;14(6):615-33.

Rad je primljen 3. VIII 2018.
Recenziran 10. X 2018.
Prihvaćen za štampu 29. X 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:360-367.

Primary Health Center Banja Luka, Republic of Srpska, Bosnia and Hercegovina[1]
Medical Faculty, University of Banja Luka, Bosnia and Hercegovina[2]
University Clinical Center of Republic of Srpska[3]

Original study
Originalni naučni rad
UDK 616-001-02-053.9:616.89-008.454
UDK 616-001-02-053.9:616.894
UDK 613.98:614.821
https://doi.org/10.2298/MPNS1812368S

EFFECTS OF DEPRESSION AND DEMENTIA ON THE RISK OF FALLS IN THE ELDERLY TREATED AT THE HEALTH CENTER BANJA LUKA

UTICAJ DEPRESIJE I DEMENCIJE NA RIZIK OD PADA KOD STARIJIH OSOBA LEČENIH U DOMU ZDRAVLJA BANJA LUKA

Kosana STANETIĆ[1, 2], Vesna KEVIĆ[1] and Mirko STANETIĆ[2, 3]

Summary

Introduction. The number of people over 65 is increasing in the world, and falls are rather common among them. The objective of this research was to examine the impact of depression, dementia, the number of chronic diseases and the number of used medications in the risk of falls in the elderly. **Material and Methods.** We carried out a prospective study during the period from March 20 to May 20, 2016. The patients were interviewed, and data were also obtained from medical records. The data collection included a socio-demographic questionnaire, the Tinetti Gait and Balance Instrument, Beck Depression Inventory and the Folstein Mini-Mental State test to assess cognitive functions. **Results.** The study included 208 patients older than 65 years, 81 men, 127 women. The Tinetti Gait and Balance tool revealed that one-third (63) of patients were at a high risk of falls, 35 patients at a moderate risk, and 110 patients presented with a low risk of falls. The results of our study showed a statistically significant association between dementia (p = 0.000) and depression (p = 0.000) as the risks of falling. Patients with some chronic diseases and patients continuously taking various drugs were also at higher risk of falls. **Conclusion.** Timely detection and treatment of depression and dementia may contribute to reducing the risk of falls in the elderly. Patients´ medication lists should be reviewed to decrease the number of medications or modify the dose. Family physicians play a major role in preventing falls in the elderly.

Key words: Depressive Disorder; Dementia; Accidental Falls; Risk Factors; Cognition; Walk Test; Postural Balance; Chronic Disease; Therapeutics

Sažetak

Uvod. U svetu kontinuirano raste broj osoba preko 65 godina, a povećava se i broj padova kod ovih osoba. Cilj rada je ispitati uticaj depresije, demencije, broja hroničnih bolesti i broja korišćenih lekova na rizik od pada kod starijih osoba. **Materijal i metode.** Istraživanje je obavljeno po tipu prospektivne studije i sprovedno je u periodu od 20. marta do 20. maja 2016. godine. Pacijenti su anketirani, a uzimani su i podaci iz zdravstvenih kartona. Kao instrumenti za prikupljanje podataka korišteni su: socio-demografski upitnik, *The Tinetti Gait and Balance Instrument*, *Beck Inventory* za procenu depresivnosti i kratki orijentacijski test *Folstein Mini Mental State* za procenu kognitivnih funkcija. **Rezultati.** Istraživanjem je obuhvaćeno 208 pacijenata starijih od 65 godina, muškaraca 81, žena 127. *Tinetti* upitnik za procenu hoda i ravnoteže pokazao je da jedna trećina pacijenata ima visok rizik od pada (63), umeren rizik ima 35, a nizak rizik 110 pacijenata. Rezultati našeg istraživanja pokazuju statistički značajnu povezanost između demencije (p = 0,000) i depresije (p = 0,000) sa rizikom od pada. Pacijenti sa većim brojem hroničnih bolesti i koji koriste veći broj lekova u kontinuiranoj terapiji su u većem riziku od pada. **Zaključak.** Blagovremeno otkrivanje i lečenje depresije i demencije može doprineti smanjenju rizika od pada kod starijih osoba. Pacijentima bi trebalo pregledati lekove koje koriste sa ciljem smanjenja broja korištenih lekova ili modifikacije doze. Doktor porodične medicine ima veliku ulogu u prevenciji padova kod starijih osoba.

Ključne reči: depresivni poremećaji; demencija; slučajni padovi; faktori rizika; kognicija; test hoda; ravnoteža; hronično oboljenje; terapija

Introduction

Old age (third age) is a stage of life, not a disease. Aging is a biological process associated with maturation, a continuing process that lasts a lifetime. It is related with decline in all vital functions that makes it susceptible to diseases and injuries. The United Nations defined the elderly as people who are > 60 years old. Most developed countries have adopted a definition that the elderly are people aged 65 and over. In addition to age, as a criterion for the definition of the elderly, there are also changes related to old age: changes in social roles (e.g. working methods, adult children, menopause), and impairment of abilities (disability, senility, mental changes). The changes in social roles are the most important criterion for the definition of old age [1, 2].

The population of elderly is growing much faster than the general population. In the general population, percentage of the elderly has increased from 9.2% in

Corresponding Author: Dr Kosana Stanetić, Centar za primarnu zdravstvenu zaštitu Banja Luka, Univerzitet u Banjoj Luci, 51000 Banja Luka, V. Momčila 9/a, Republika Srpska, Bosna i Hercegovina, E-mail: stanetic.kosana@gmail.com

Abbreviations

MMSE – Mini Mental State Examination
ACE – angiotensin-converting enzyme
AD – Alzheimer's disease

1990 to 11.7% in 2013, and it is expected to grow to 21.15% by 2050 (from 841 million in 2013 to two billion in 2050). In developed countries, the number of older persons has already exceeded the number of children, and it is considered that this number will double by 2050. Currently, two-thirds of the elderly population in the world lives in developing countries. Considering that the older population grows faster in less-developed countries, it is expected that in 2050 eight in ten older people will live in developing or less developed countries. The elder population is dominated by women. People over 80 years of age account for 14% of the elder population, and it is believed that in 2050 19% will be older than 80 [3].

Falls are more common among the elderly than in the general population. More than one-third of people older than 65 falls each year, and half of them are repeated falls [4, 5]. Approximately one in ten falls results in severe injuries, such as hip fracture, other fractures, subdural hematoma, other serious soft tissue injuries or head injuries [6–8].

The most common mental disorders in the elderly are depression and dementia, which are a significant public health problem, although they are diagnosed and treated in little less than 20% [9].

According to the previous researches worldwide, the prevalence of depression in the elderly population varies significantly and estimates that can be found in the literature range from 3% to 35% [10, 11]. Depressive disorder is the fourth leading cause of the global disease burden and it is the second most common health problem in women. The number of persons suffering from depressive disorder is in constant rise since the beginning of the last century to the present day in all industrialized countries, so that 20% of women and 10% of men develop at least one depressive episode during their lifetime [12].

Depression is not an unavoidable consequence of aging. The Croatian Society for Gerontology and Geriatrics has confirmed that loneliness is a major problem in the elderly. The elderly are prone to social isolation due to permanent immobility and it is consequently associated with depression. Most geriatric patients rarely contact their family doctor in relation to this issue, and members of their close family consider that depressive mood is a normal part of getting older. Older age is associated with several health problems, financial difficulties, stress, alienation, dissatisfaction, retirement, loss of a spouse, family members or friends, being left by children, reduced need and motivation for activities that used to be a normal part of life, so it all makes the elderly susceptible to depression [13]. Depression in the elderly can be treated successfully, and the elderly respond to treatment as well as the young [14].

Dementia is a syndrome of global and progressive impairment of acquired cognitive abilities, caused by organic disease of the central nervous system, with preserved consciousness, which particularly damages memory, learning, abstract thinking, orientation and understanding of visual-spatial relations [15]. Dementia is a disorder of mental functions in at least three of the following five mental activity domains: cognition, memory, language, visuospatial skills and personality [16]. It is a clinical syndrome characterized by deterioration of previously acquired intellectual functions, which lead to reduction or inability to perform daily activities. It is also an important factor for development of other geriatric problems such as falls, incontinence and others. Considering that the number of older adults, who are at higher risk of falls, is continuously increasing in our country, our goal was to study the impact of depression, dementia and other factors on the risk of falls in the elderly.

Material and Methods

A prospective study was carried out during the period from March 20 to May 20, 2016. The patients were interviewed, and data were obtained from electronic or written medical records. The study included outpatients aged 65 and older who were treated by family physicians in Banja Luka. Their medical histories were reviewed to obtain data on patients who had falls in the past five years and whether falls were associated with any fracture.

The study included 208 randomly selected patients from three teams of family physicians of the Health Center Banja Luka. Using the patient registry, every third patient who was ≥ 65 years old, regardless of gender, was included in the study. Out of a total of 724 patients older than 65, registered in these three teams, 241 patients were asked to participate in the study. Of these 241 patients, the research included 208 patients (response rate 86.3%). Patients who did not take part in the study were unable to respond or did not want to participate in the study. All patients were informed about the research objectives and signed informed consent forms. The study was conducted in compliance with the Helsinki Declaration on medical research and the principles of good scientific practice. A consent was also obtained from the Ethics Committee of the Health Center in Banja Luka.

The instruments for data collection were a general questionnaire on socio-demographic data, conditions of residence, education level, marital status, chronic diseases and current therapy, and the Tinetti Gait and Balance Instrument [17] which was designed to determine the risk of falls in the elderly in the next year. We conducted a physical examination of patients and set up a state of their balance for which we calculated a special record, as well as stroke patients for which we have also calculated the score and eventually got the final score based on which the risk of falls in the next year was assessed. Beck Depression Inventory was used to evaluate depression [18]. The patients' task was to read multiple-choice questions carefully, and choose the statement that best described

how they felt that week and that day. Each claim was assigned to appropriate number. The obtained score was a basis to determine the presence or absence of depression. A short, Folstein Mini Mental State Examination (MMSE) [19] was used for the assessment of cognitive impairment. This questionnaire tested orientation of the patient, current memory, attention and mental calculation, memory and language. By summing all the individual scores we obtained the final score, indicating the patients' cognitive level.

After entering the gathered data into the Excel database, they were statistically analyzed. The results were analyzed using the Statistical Package for the Social Sciences (SPSS) 11.5 on several levels. Data were processed using different statistical tests: the descriptive analysis of frequencies and percentages for the whole reviewed sample and each question individually. Differences between particular categories of subjects (age, gender, household size, qualifications, etc.), scales (mental status, depression and the risk of falling) were analyzed using Chi-square test. A t-test was used to compare the average value of two groups. Significance levels were set at 0.05 and 0.01.

Results

The study included 208 patients older than 65 years. There were 38.9 % of male and 61.1% of female subjects. The average age of patients was 73.7 years. Most patients (36.5%) were 65 to 70 years old, and 14.9% patients were 81 years old or older. Most subjects (61.1%) were married, and 35.1% of patients were widows/widowers. Of the total number, 82.7% of patients lived in urban, and 58.2% lived in rural area. A small number of subjects lived alone (27.4%), 41.8% lived with a spouse, and one-third of the subjects lived in large families with three or more family members. The households were generally (86.5%) with no children under 18 years of age. Most patients had secondary education (40.4%), 7.2% of patients had no formal schooling, while only 5.8% of patients had an academic title (**Table 1**).

In the past five years, 110 (52.9%) patients had no falls, and 98 (47.1%) had one or more falls. Most patients (N = 59) reported one fall (28.4%), two or three falls were reported by 14 patients (6.7%), five falls were reported by six patients (2.9%), and six falls were reported by 3 patients.

The Tinetti gait and balance test revealed that the one-third (63) of patients were at high risk of falling (30.3%), 35 patients were at a moderate risk (16.8%), and low risk was observed in 110 (52.9%) patients.

Results of the MMSE showed that most of the subjects had normal cognitive functions 149 (71.6%), 44 (21.2%) patients had borderline cognitive functions, and 15 (7.2%) patients had impaired cognition.

The Beck Depression Inventory found that almost half of patients - 102 (49.0%) had elements of depression, mild depression was observed in 55 (26.4%), and moderate in 51 (24.5%) patients.

The results of our study showed a statistically significant association between dementia and the risk of falling ($p = 0.000$). Most patients (86.4%) at a low risk of falling also had normal cognitive functions, 13.6% of patients had borderline cognitive functions, and in this group, there were no patients with impaired cognition. In the group of patients at high risk of falling, 28 patients were with normal cognitive functions, 22 were with borderline, and 13 patients were with impaired cognitive functions (**Table 2**).

The degree of depression was statistically significantly associated with the risk of falling ($p = 0.000$). Patients at high risk of falls showed moderate depression (58.8%), and only 16.7% of patients had no depression. In the group of patients at low risk of falling, 45.5% had mild depression, and 15.7% had moderate depression (**Table 3**).

During the study, we examined the impact of the number of chronic diseases and the number of medications used continuously on the risk of falling. There was a statistically significant correlation ($p = 0.000$) between the number of chronic diseases and the risk of falling; chronic diseases were related with a larger risk of falling. The most common chronic diseases were hypertension in 192 patients (92.3%), congestive heart failure (CHF) in 41 (19.7%), osteoarthrosis (OA) in 56 (26.9%), diabetes mellitus in 49 (23.6%), and visual disorders in 63 (30.3%) patients. In the group of patients at high risk of falling, 52.5% patients were suffering from three or more diseases, 20.8% were suffering from three concurrent diseases, and 13.3% of patients had up to two diseases. In the group of patients at low risk of falling, 69.3% of patients had up to two, 58.5% suffered from three concurrent diseases, and 33.8% of patients had four or more disease (**Table 4**).

The number of medications used continuously significantly affected the risk of falling ($p = 0.000$), i.e., the greater the number of medications, the higher the risk of falls. The most commonly used medications were angiotensin-converting enzyme (ACE) inhibitors by 135 (64.9%), ACE inhibitor + diuretics by 69 (33.2%), beta-blockers by 72 (34.6%), calcium-channel blockers by 52 (25.0%), nitrates by 43 (20.7%), diuretics by 37 (17.8%), oral antihyperglycemic agents by 37 (17.8%), insulin by 14 (6.7%), benzodiazepines by 64 (30.8%) and nonsteroidal anti-inflammatory drugs (NSAIDs) by 72 (34.6%) patients. In the group of patients at high risk of falls, 50.5% used four or more drugs, while in the group of patients at low risk of falls 69.0% used two or fewer drugs (**Table 5**).

Discussion

Our research shows that the degree of depression has a statistically significant influence on the risk of falls ($p = 0.000$). It suggests that the greater the degree of depression, the greater the risk of falls, and it is in agreement with numerous studies that have been conducted around the world.

Kamel et al. [20] conducted a study in Egypt including 340 subjects confirming that the level of

Table 1. Socio-demographic data of the participants
Tabela 1. *Sociodemografski podaci ispitanika*

Variable *Varijabla*	Number of patients (N = 208) *Broj pacijenata*	Percentage (%) *Procenat*
Gender/*Pol*		
Male/*Muški*	81	38.9
Female/*Ženski*	127	61.1
Age/*Dob* (Years/*Godine)*		
65 - 70	76	36.5
71 - 75	56	26.9
76 - 80	45	21.6
≥ 80	31	14.9
Marital status/*Bračno stanje*		
Married/*U braku*	127	61.1
Single/*Neoženjen/neudata*	5	2.4
Divorced/*Razveden/razvedena*	3	1.4
Widowed/*Udovac/udovica*	73	35.1
Place of living/*Mesto življenja*		
Village/*Selo*	36	17.3
City/*Grad*	172	82.7
Dwelling house/*Mjesto stanovanja*		
Apartment building/*Zgrada*	87	41.8
House/*Kuća*	121	58.2
Number of persons in the household/*Broj osoba u domaćinstvu*		
One person/*Jedna osoba*	57	27.4
Two persons/*Dve osobe*	87	41.8
Three and more/*Tri i više osoba*	64	64.8
Number of children in the family/*Broj dece u porodici*		
Childless/*Bez dece*	180	86.5
One child/*Jedno dete*	14	6.7
Two children/*Dvoje dece*	13	6.3
Three and more children/*Troje i više dece*	1	0.5
Education level/*Stepen obrazovanja*		
No formal schooling/*Bez škole*	15	7.2
Four grades of elementary school/*Četiri razreda osnovne škole*	25	12.0
Eight grades of elementary school/*Osam razreda osnovne škole*	44	21.2
High school/*Srednja i viša škola*	112	53.9
University degree/*Fakultet*	12	5.8

depression and the risk of falling were significantly associated. The same results were also confirmed by Dubljanin-Raspopović et al. [21] in their study, noting that more than 90% of subjects also had some degree of cognitive impairment.

The depressive symptoms that are statistically significantly associated with risk of falls in the elderly are also reported by Eggermont et al. [22] in a sample of 722 adults, aged 78.3 years on average.

Kwan et al. [23] conducted a two-year study in five randomly selected villages in Taiwan. The study included 260 people aged 65 to 91, the average age 74.9. None of the subjects used antidepressants. Of the total of 260 subjects, 174 (66.9%) experienced no fall, 51 (19.6%) fell once, and 35 (13.5%) fell twice or more. Depressive symptoms were significantly more commonly reported among the subjects who fell several times (40.0%), those with one fall (27.5%) and in subjects without falling these symptoms were reported only by 16.1% patients. It was concluded that depressive symptoms are often present in old Taiwanese that are significantly associated with the risk of falling.

Also, an extensive study was conducted in Japan in 2010. It was carried out by Tanaka et al. [24]. The study included 563 subjects aged 65 and over, villagers of Kumamoto Prefecture in Japan. To establish the level of depression and risk of falls, Geriatric Depression Scale - Short Form and the Simple Screening Test for Risk of Falls were used. Also, different factors were considered, including age, gender, chronic diseases, use of hypnotics, cognitive

Table 2. Association between dementia and the risk of falls
Tabela 2. *Povezanost između demencije i rizika od pada*

Risk of falls *Rizik od pada*	Folstein Mini-Mental State Examination /*Folstajnov mini kognitivni test*			χ^2 test	p*
	Normal/*Normalno* N (%)	Borderline/*Granično* N (%)	Impaired/*Oslabljen* N (%)		
High/*Visok*	28 (44.4)	22 (34.9)	13 (20.6)		
Moderate/*Umeren*	26 (74.3)	7 (20.0)	2 (5.7)	42.255	0.000
Low/*Nizak*	95 (86.4)	15 (13.6)	0 (0.0)		

*Statistically relevant difference at $p < 0,05$

Tabela 3. Association between depression and the risk of falls
Tabela 3. *Povezanost između depresije i rizika od pada*

Risk of falls *Rizik od pada*	Beck Inventory/*Beckov indicator depresije*			χ^2 test	p*
	No depression *Nema depresije* N (%)	Mild depression *Blaga depresija* N (%)	Moderate depression *Umerena depresija* N (%)		
High/*Visok*	17 (16.7)	16 (29.1)	30 (58.8)		
Moderate/*Umeren*	8 (7.8)	14 (25.5)	13 (25.5)	53.359	0.000
Low/*Nizak*	77 (75.5)	25 (45.5)	8 (15.7)		

*Statistically relevant difference at $p < 0.05$

functions and their association with falls. Of all, 395 subjects had a significant cognitive impairment, and a statistically significant relationship between depression and risk of falls.

In Boston, an extensive study was conducted including 763 patients aged 70 and over. It was done by Quach et al. [25] in the period from 2005 to 2009. The entry criteria were at least 70 years of age, being able to communicate in English, walk independently around the small room, either with or without a cane or a walker and no significant cognitive impairment, i. e. Mini-Mental State Examination scores ≥ 18. The results of this study showed that depression increases the risk of falling both inside and outside the home. This was confirmed by the research of Kelsey et al. [26].

In our study, 71.6% of patients had normal cognition, 7.2% had impaired cognition, and 26.4% of patients showed signs of mild depression, and 24.5% had elements of moderate depression. Slightly more than half of the patients showed evidence of depression. In their study, carried out at the Health Center Ćuprija in 2010, including 100 patients aged 65 - 84 years, Urošević et al. [27] showed that older people who were treated in primary care presented with a high percentage (55.0%) of depression. There is accumulating evidence to suggest that depression may be a risk factor for the development of dementia [28].

The results of our study showed a statistically significant association between dementia and the risk of falling ($p = 0.000$). There was no patient at low risk of falling and impaired cognition.

By studying the correlation of mental status and the risk of falling Kamel et al. [20] found that 47.1% of the surveyed population had impaired cognitive functions, and 32.3% of them have experienced a fall. The statistically significant association between cognitive impairment and the risk of falling was observed, being in agreement with our results.

However, in a study conducted in Poland, Kaminska et al. [29] have not found a statistically significant association between dementia and risk of falls.

Eggermont et al. [22] carried out an extensive 18-month long study, including 722 patients aged 70 and over, in Boston and five surrounding cities not further than 5 miles in radius. The average age of patients was 78.3, and their mental status was measured using the Mini-Mental State Examination. The results of the study showed that the mental status of patients was significantly associated with the risk of falling.

Gleason et al. studied the risk of falls in older people who showed less severe cognitive impairment at a multimedia test in comparison to the control group and showed that the risk of falling increases with higher cognitive impairment. The study included 172 elderly patients over a period of 12 months [30].

Borges et al. examined the fear of falls and falls in older patients with secondary cognitive impairment and Alzheimer's disease (AD) and found a large number of falls in this group of patients compared to the control group. Elderly patients with cognitive impairment were more likely to report fear of falling than those with AD [31].

In their study, Muir et al. demonstrated a strong association between dementia in the elderly and the risk of falls [32].

Stark et al. conducted a prospective cohort study over 12 months in 125 patients, mostly women, 96% of them were Caucasian and the average age of 74.4. The phenomenon of falling in people who probably suffered from preclinical AD was observed. It has

Table 4. The impact of the number of chronic diseases on the risk of falls
Tabela 4. *Uticaj broja hroničnih bolesti na rizik od pada*

Risk of falls *Rizik od pada*	Number of chronic diseases/*Broj hroničnih bolesti*			Total *Ukupno* N (%)	χ^2 test	p*
	≤ two diseases ≤ *dve bolesti* N (%)	three diseases *tri bolesti* N (%)	more than three diseases *više od tri bolesti* N (%)			
High/*Visok*	10 (13.3)	11 (20.8)	42 (52.5)	63 (30.3)		
Moderate/*Umeren*	13 (17.3)	11 (20.8)	11 (13.8)	35 (16.8)	32.378	0.000
Low/*Nizak*	52 (69.3)	31 (58.5)	27 (33.8)	110 (52.9)		

*Statistically relevant difference at $p < 0.05$

been shown that the preclinical form of AD was a likely risk of falling in the elderly [33].

Out of a total of 208 patients who participated in our study, most were patients suffering from three or more diseases – 80 (38.5%), while 75 (36.1%) suffered from two or less diseases. There is a statistically significant correlation ($p = 0.000$) between the number of chronic diseases and the risk of falling; the higher the number of chronic diseases, the larger the risk of falling. In the group of patients at high risk of falls, 42 (52.5%) were patients suffering from three or more diseases, and only 10 (13.3%) patients were with up to two diseases. In the group of patients at low risk of falling, 52 (69.3%) were patients suffering from up to two, 31 (58.5%) were suffering from 3 concurrent diseases, and 27 (33.8%) suffered from three or more diseases.

The analysis of chronic diseases in the study group showed that congestive heart failure ($p = 0.001$), depression ($p = 0.035$), cerebrovascular diseases ($p = 0.009$), osteoporosis ($p = 0.017$), and osteoarthritis ($p = 0.000$) significantly increased the risk of falls. It also showed that hypertension, diabetes mellitus and vision problems of the elderly do not affect significantly the risk of falls.

In their study, Stanetić et al. [34] showed that hypertension and diabetes, as isolated diseases, have no significant effects on the risk of falls in the elderly, but considering that patients suffering from these diseases are mainly associated with comorbidity, the risk of falling increases with age. All patients suffering from diabetes mellitus also had hypertension. In their study, the greatest risk of falling was found in patients suffering from cardiovascular diseases, such as cardiac arrhythmia, coronary artery disease and heart failure. One chronic disease was diagnosed in 21 patients (14.0%), two were diagnosed in 36 patients (24.0%), three in 45 (30.0%), four in 24 (16.0%) and five chronic diseases were diagnosed in 14 (9.3%) patients. Using Pearson's coefficient of linear correlation, it has been proven that patients suffering from several chronic diseases at the same time have a higher risk of falls that was also confirmed in our study.

Kamel et al. [20] pointed out that the most common chronic diseases in Egyptian patients who had experienced falls were as follows: one-third of patients (30.0%) had diabetes mellitus, one-third (29.7%) had hypertension and 18.5% patients had osteoarthritis.

Lo Alexander et al. [35] pointed out that in patients who participated in the study in Alabama it was confirmed that the increasing number of chronic diseases increased the risk of falls.

Dubljanin-Raspopović et al. [21] found that the largest number of patients who were hospitalized due to a fall-related hip fracture, suffered from depression 161 (47.1%), followed by vascular diseases 61 (18.0%), chronic obstructive pulmonary disease 22 (6.5%), arthritis 8 (2.4%), neurological diseases (cerebrovascular accident with neurologic deficit - Parkinson's disease) 45 (13.16%) and visual impairment 199 (58.2%). In our research, vision problems had no significant effect on the risk of falling. This can be explained by the fact that patients who have a vision problem cautiously move in their environment.

Yu PL et al. examined the incidence of falls in 1,512 persons during one year. They examined the influence of various chronic diseases and the occurrence of falls in patients older than 60 years. The research has shown that people suffering from de-

Table 5. The impact of the number of used medications on the risk of falls
Tabela 5. *Uticaj broja korištenih lekova na rizik od pada*

Risk of falls *Rizik od pada*	Number of medications/*Broj lijekova*			Total *Ukupno* N (%)	χ^2 test	p*
	≤ two drugs ≤ *dva leka* N (%)	three drugs *tri leka* N (%)	four and more drugs *četiri i više lekova* N (%)			
High/*Visok*	8 (11.3)	9 (19.6)	46 (50.5)	63 (30.3)	35.461	0.000
Moderate/*Umeren*	14 (19.7)	6 (13.0)	15 (16.5)	35 (16.8)		
Low/*Nizak*	49 (69.0)	31 (67.4)	30 (33.0)	110 (52.9)		

*Statistically relevant difference at $p < 0.05$

mentia, depression and who have more than two chronic diseases are at higher risk of falls [36].

A study conducted in Korea in 2011 investigated the risk factors of falls in the elderly Koreans showed that patients suffering from diabetes mellitus, osteoarthritis, osteoporosis, urinary incontinence, cataracts, depression and stroke were at high risk of falls. People with hypertension were at low risk of falls [37].

Out of a total of 208 patients in our study, most of them - 91 (43.8%) took 4 or more medications, and 71 (34.1%) used two or fewer drugs. The results of our research suggest that the number of medications that patients use in continuous treatment significantly affect the risk of falls ($p = 0.000$), i.e. the greater the number of drugs, the higher the risk of falls. In the group of patients at high risk of falls, 46 (50.5%) used four or more drugs, 9 patients (19.6%) used three drugs, and only 8 (11.3%) patients used two or fewer drugs. In the group at low risk of falls, there were 49 (69.0%) patients using two or fewer drugs, and 30 (33.0%) patients used four or more drugs.

The analysis of the impact of certain medications used by patients in the study group showed that diuretics ($p = 0.000$), nitrates ($p = 0.011$), benzodiazepines ($p = 0.000$) and NSAIDs ($p = 0.000$) statistically significantly increase the risk of falls.

Stanetić et al. [34] pointed out that the greatest risk of falls was associated with diuretics and NSAIDs that coincides with our results. Benzodiazepines were used by 50 (33.3%) patients, and it did not affect significantly the risk of falls, which is opposite to our results. In their study, more than half of the subjects used more than three drugs continuously, which certainly increased the risk of falls.

In their study, Leipzig et al. [38] confirmed that older people who use more than three or four drugs are at higher risk of falls.

Matović et al. [39] conducted a study at the Community Health Center Foča and found that eight out of 300 patients, who reported at least one fall during the 12 months, used more than three drugs. A large number of authors have confirmed that using multiple drugs carries a risk of adverse interactions that may increase the risk of falls [40–42].

Dubljanin-Raspopović et al. performed a research at the Department of Orthopedic Surgery, Clinical Center of Serbia, found that more than a third of patients 124 (36.3%) who sustained a hip fracture after a little trauma, used more than four drugs [21].

Quach et al. conducted a study including 763 patients aged 70 years or more, reported that the risk of falls increased in patients taking antidepressants compared to those without depression and using no drugs for depression [25].

Kamel et al. conducted a study in Egypt including 340 patients, aged 60 years and over; 78.5% of them used medications and 51.2% of them had at least one fall. This could be explained by drug interactions that affected mental alertness and motor coordination. About a third of the studied population used NSAIDs and half of them hypoglycemic agents [20]. Ziere et al. pointed out that the risk of falls increases with the number of daily used drugs. Using more than three drugs per day increases the risk of falls in the elderly [43].

Many studies, including studies conducted by Wang and de Vries et al., have shown a significant association between the use of benzodiazepines and falls or fractures. The risk of falls is highest with the initial doses and after a prolonged usage of drugs [44, 45].

Benzodiazepines are still the most frequently prescribed hypnotic sedatives in the elderly. Our study found that one-third of the patients used benzodiazepines, and in the group of those who have experienced falls one third used benzodiazepines. These drugs should be prescribed for a short time, not more than a few weeks, but the research shows that nearly 80% of old people used them continuously for more than two years [46].

The important role of a family physician is early diagnosis and treatment of patients with depression and dementia. A large number of authors made recommendations for prevention of falls in older people, because those are the most effective and efficient interventions. The preventive measures include health promotion, review of medications that patients use, physical exercise and creation of security at home and in the home environment. Other authors recommend professional training for establishing gait and balance, a gradual reduction in the use of psychotropic substances (benzodiazepines, hypnotics, neuroleptics, antidepressants) and modification of risky behavior. Specific recommendations in the literature include wearing safe and comfortable shoes, slippers (slip resistant), using light at night and making additional steps. All older patients should periodically review their medications, including over-the-counter medications, reduce the number of drugs or modify the doses. The family physician has a significant role in prevention of falls in the elderly [47, 48].

Conclusion

In the studied group of patients, 30.3% were at high risk of falls (measured using Tinetti questionnaire). The Folstein Mini-Mental Status test revealed that 7.2% of patients had impaired cognitive functions. Beck Inventory showed that 26.4% of patients had mild depression and 24.5% had moderate depression. Dementia and depression significantly affect the risk of falling in the elderly.

References

1. World Health Organization. Proposed working definition of an older person in Africa for the MDS Project [Internet]. 2013 [cited 2015 Apr 12]. Available from: http://www.who.int/healthinfo/survey/ageingdefnolder/en/.

2. Milanović P, Milošević DP, Davidović M. Padovi u starosti. In: Milošević DP, Davidović M, editors. Medicinska gerontologija. Beograd: Davidović M, Milošević DP; 2007. p. 81-90.

3. United Nations. World population ageing 2013 [Internet]. New York: United Nations; 2013 [cited 2015 May 18]. Available from: https: www.un.org/.../population/.../ageing/WorldPopulationAgeingReport2013.shtml.

4. Tinetti ME. Clinical practice. Preventing falls in elderly persons. N Engl J Med. 2003;348(1):42-9.

5. American Geriatric Society, British Geriatrics Society, American Academy of Ortopaedic Surgeons Panel on Falls Prevention. Guideline for the prevention of falls in older persons. J Am Geriatr Soc. 2001;49(5):664-72.

6. Parker MJ, Gillespie LD, Gillepsie WJ. Hip protectors for preventing hip fractures in the elderly. Cochrane Database Syst Rev. 2001;2:CD001255.

7. Stevens M, Holman CD, Bennett N. Preventing falls in older people: impact of an intervention to reduce environmental hazards in the home. J Am Geriatr Soc. 2001;49(11):1442-7.

8. Jovanović Z. Mehanizmi neurodegeneracije kod Alchajmerove bolesti. Med Pregl. 2012;65(7-8):301-7.

9. Woolcott JC, Richardson KJ, Wiens MO, Patel B, Marin J, Khan KM, et al. Meta-analysis of the impact of 9 medication classes on falls in elderly persons. Arch Intern Med. 2009;169(21):1952-60.

10. Scheffer AC, Schuurmans MJ, van Dijk N, van der Hooft T, de Rooij SE. Fear of falling: measurement strategy, prevalence, risk factors and consequences among older persons. Age Ageing. 2008;37(1):19-24.

11. Cole MG, Bellavance F, Mansour A. Prognosis of depression in elderly community and primary care populations: a systematic review and meta-analysis. Am J Psychiatr. 1999;156(8):1182-9.

12. Maj M, Sartorius N, editors. Depressive disorders [monograph on the Internet]. Chichester: John Wiley& Sons; 2002 [cited 2015 May 18]. Available from: http://www.al-edu.com/wp-content/uploads/2014/05/MajSartorius-eds-Depressive-Disorders.pdf.

13. Martinčević-Ljumanović R. Zdrava i sretna starost: suvremena gerontologija i gerijatrija. Zagreb: Spektar; 1985.

14. Blazer DG. Depression in late life: a review and commentary. J Gerontol A Biol Sci Med Sci. 2003;58(3):249-65.

15. Brayne C, Gao L, Dewey M, Matthews FE. Dementia before death in ageing societies - the promise of prevention and the reality. Plos Med. 2006;3(10):e397.

16. Pavlović DM, Pavlović AM. Demencije. Engrami. 2002;24(3-4):9-28.

17. Tinetti ME, Doucette J, Claus E, Morottoli RA. Risk factors for serious injury during falls by older persons in the community. J Am Geriatr Soc. 1995;43(11):1214-21.

18. Beck AT, Guth D, Steer R, Ball R. Screening for major depression disorders in medical inpatients with the Beck Depression Inventory for Primary Care. Behav Res Ther. 1997;35(8):785-91.

19. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

20. Kamel MH, Abdulmajeed AA, Ismail Sel-S. Risk factors of falls among elderly living in urban Suez-Egypt. Pan Afr Med J. 2013;14:26.

21. Dubljanin-Raspopović E, Denić Marković Lj, Tulić G, Grajić M, Tomanović S, Kadija M, et al. Prevencija preloma kuka u gerijatrijskoj populaciji – neiskorišćena prilika. Vojnosanit Pregl. 2012;69(5):420-4.

22. Eggermont LH, Penninx BW, Jones RN, Leveille SG. Depressive symptoms, chronic pain and falls in older community-dwelling adults: the MOBILIZE Boston Study. J Am Geriatr Soc. 2012;60(2):230-7.

23. Kwan MM, Lin SI, Close JC, Lord SR. Depressive symptoms in addition to visual impairment, reduced strength and poor balance predict falls in older Taiwanese people. Age Ageing. 2012;41(5):606-12.

24. Tanaka M, Kusaga M, Ushijima K, Watanabe C. Association between depression and fall risk among elderly community residents. Nihon Ronen Igakkai Zasshi. 2012;49(6):760-6.

25. Quach L, Yang MF, Berry SD, Newton E, Jones RN, Burr JA, et al. Depression, antidepressants and falls among community-dwelling elderly people: the MOBILIZE Boston Study. J Gerontol A Biol Sci Med Sci. 2013;68(12):1575-81.

26. Kelsey JL, Berry SD, Procter-Gray E, Quach L, Nguyen US, Li W, et al. Indoor and outdoor falls in older adults are different: the maintenance of balance, independent living, intellect and Zest in the Elderly of Boston Study. J Am Geriatr Soc. 2010;58(11):2135-41.

27. Urošević J, Davidović M, Odović G, Alexopoulos CG, Mitrović Lj. Učestalost depresije kod starih osoba. PONS medicinski časopis. 2010;7(4):144-9.

28. Lovretić V, Mihaljević-Peleš A. Je li poremećaj kognitivnih funkcija u depresiji put u demenciju. Socijalna psihijatrija. 2013;41(2):109-17.

29. Kamiska MS, Brodowski J, Karakiewicz B. Fall risk factors in community-dwelling elderly depending on their physical function, cognitive status and symptoms of depression. Int J Environ Res Public Health. 2015;12(4):3406-16.

30. Gleason CE Gangnon RE, Fischer BL, Mahoney JE. Increased risk for falling associated with subtle cognitive impairment: secondary analysis of a randomized clinical trial. Dement Geriatr Cogn Disord. 2009;27(6):557-63.

31. Borges Sde M, Radanovic M, Forlenza OV. Fear of falling and falls in older adults with mild cognitive impairment and Alzheimer's disease. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn. 2015;22(3):312-21.

32. Muir SW, Gopaul K, Montero Odasso MM. The role of cognitive impairment in fall risk among older adults: a systematic review and meta-analysis. Age Ageing. 2012;41(3):299-308.

33. Stark SL, Roe CM, Grant EA, Hollingsworth H, Benzinger TL, Fagan AM, at al. Preclinical Alzheimer disease and risk of falls. Neurology. 2013;81(5):437-43.

34. Stanetić K, Marković B, Tešanović G, Savić S, Šukalo M, Kremenović Z. Assessment of risk for falls in elderly. Opšta medicina. 2014;20(1-2):25-34.

35. Lo AX, Brown CJ, Sawyer P, Kennedy RE, Allman RM. Life-space mobility declines associated with incident falls and fractures. J Am Geriatr Soc. 2014;62(5):919-23.

36. Yu PL, Qin ZH, Shi J, Wu ZL, Sun ZQ. Study on the relationship between chronic diseases and falls in the elderly. Zhonghua Liu Xing Bing Xue Za Zhi. 2009;30(11):1156-9.

37. Choi EJ, Kim SA, Kim NR, Rhee JA, Yun YW, Shin MH. Risk factors for falls in older Korean adults: the 2011 Community Health Survey. J Korean Med Sci. 2014;29(11):1482-7.

38. Leipzig RM, Cumming RG, Tinetti ME. Drugs and falls in older people: systematic review and meta-analysis: II. Cardiac and analgesic drugs. J Am Geriatr Soc. 1999;47(1):40-50.

39. Matović J, Pejović V, Račić M. Sveobuhvatna gerijatrijska procjena pacijenata u ambulantama porodične medicine Doma zdravlja Foča. Biomedicinska istraživanja. 2013;4(1):13-25.

40. Berdot S, Bertrand M, Dartigues JF, Fourrier A, Tavernier B, Ritchie K, et al. Inappropriate medication use and risk of falls - a prospective study in a large community-dwelling elderly cohort. BMC Geriatr. 2009;9:30.

41. Fick DM, Cooper JW Wade WE, Waller JL, Maclean JR, Beers MH. Updating the Beers criteria for potentially inappropriate medication use in older adults: results of a US consensus panel of experts. Arch Intern Med. 2003;163(22):2716-24.

42. Laroche ML, Charmes JP, Merle L. Potentially inappropriate medications in the elderly: a French consensus panel list. Eur J Clin Pharmacol. 2007;63(8):725-31.

43. Ziere G, Dieleman JP, Hofman A, Pols HA, van der Cammen TJ, Stricker BH. Polypharmacy and falls in the middle age and elderly population. Br J Clin Pharmacol. 2006;61(2):218-23.

44. Wang PS, Bohn RL, Glynn RJ, Mogun H, Avorn J. Hazardous benzodiazepine regimens in the elderly: effects of half-life, dosage and duration on risk of hip fracture. Am J Psychiatry. 2001;158(6):892-8.

45. de Vries OJ, Peeters G, Elders P, Sonnenberg C, Muller M, Deeg DJ, et al. The elimination half-life of benzodiazepines and fall risk: two prospective observational studies. Age Ageing. 2013;42(6):764-70.

46. Passaro A, Volpato S, Romagnoni F, Manzoli N, Zuliani G, Fellin R. Benzodiazepines with different half-life and falling in a hospitalized population: the GIFA study. Gruppo Italiano di Farmacovigilanza nell'Anziano. J Clin Epidemiol. 2000;53(12):1222-9.

47. Stanetić K, Tešanović G. Influence of age and length of service on the level of stress and burnout syndrome. Med Pregl. 2013;66(3-4):153-62.

48. Stanetić K. Padovi kod starih. U: Tešanović G, Stanetić K, Petrović V, Savić S. Porodična medicina. Banja Luka: Medicinski fakultet; 2014.

Rad je primljen 27. II 2018.
Recenziran 24. IV 2018.
Prihvaćen za štampu 29. XI 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:368-376.

Clinical Center Vojvodina, Novi Sad
Clinic of Otorhinolaryngology and Head and Neck Surgery[1]
University of Novi Sad, Faculty of Medicine, Novi Sad[1]
Clinical Center Vojvodina, Department of Infectious Diseases, Novi Sad[2]
Clinical Center Vojvodina, Department of Internal Medicine, Novi Sad[3]

Original study
Originalni naučni rad
UDK 616-097 i UDK 616.98-079.4
https://doi.org/10.2298/MPNS1812377T

PROLIFERATION OF B-LYMPHOCYTES IN INFLAMMATORY AND HEMATOLOGICAL DISEASES

PROLIFERACIJA B-LIMFOCITA U INFLAMATORNIM I HEMATOLOŠKIM BOLESTIMA

Tamara TEŠIĆ[1], Dajana LENDAK[2,3], Ivana UROŠEVIĆ[2,4], Igor MITIĆ[2,4] and Vanja ANDRIĆ[2]

Summary

Introduction. A proliferation-inducing ligand is a membrane binding protein that represents one of the main survival factors for immature, naive and activated B-cells, and is involved in the global immune response. The objective of this study was to determine whether plasma levels of a proliferation-inducing ligand may be used to assess the proliferation of B-lymphocytes in patients with bacterial infections, B-cell malignancies and autoimmune inflammatory disorders. **Material and Methods.** The study included 91 patients divided into three groups and 30 blood donors assigned to the control group. Group 1 included 34 patients with bacterial infections confirmed by microbiology and/or radiology diagnostic tests; group 2 included 32 patients with B-cell malignancies; and group 3 included 25 patients with autoimmune inflammatory diseases. All plasma samples were assayed for a proliferation-inducing ligand using enzyme-linked immunosorbent assay. The differences between groups were examined by one-way analysis of variance test and post hoc analysis. **Results.** One way analysis of variance test showed a statistically significant difference in concentrations of a proliferation-inducing ligand in the examined groups. The highest mean value of a proliferation-inducing ligand was found in patients with established bacterial infections ($\bar{x}$ = 8,294 ng/ml). Post hoc analysis showed that a proliferation-inducing ligand levels in the plasma samples of patients with bacterial infections were significantly higher than in healthy controls, and patients with hematological and autoimmune diseases, respectively. **Conclusion.** B-cell proliferation was increased in patients with bacterial infections in regard to patients with other disorders. Therefore, a proliferation-inducing ligand can be used to differentiate bacterial infections from other inflammatory disorders and may be helpful in decision making whether to start antibiotic treatment or not.

Key words: B-Lymphocytes; Bacterial Infections; Autoimmune Diseases; Hematologic Diseases; Tumor Necrosis Factor Ligand Superfamily Member 13; Biomarkers; Diagnosis, Differential

Sažetak

Uvod. Ligand induktor proliferacije je transmembranski protein koji predstavlja jedan od glavnih faktora preživljavanja nezrelih, naivnih i aktiviranih B-limfocita kao i jedan od regulatora celokupnog imunoodgovora. Cilj ovog rada bio je upoređivanje proliferacije B-limfocita, pomoću plazmatskih koncentracija liganda induktora proliferacije kod bolesnika sa bakterijskim infekcijama, autoimunim inflamatornim oboljenjima i B-ćelijskim malignitetima. **Materijal i metode.** Istraživanje je obuhvatilo 91 ispitanika koji su podeljeni u tri grupe i 30 dobrovoljnih davalaca krvi koji su činili kontrolnu grupu. U prvoj grupi nalazilo se 34 bolesnika sa dokazanim bakterijskim infekcijama; u drugoj 32 bolesnika sa B-ćelijskim malignitetima; a treću grupu činilo je 25 bolesnika sa autoimunim inflamatornim oboljenjima. Svim ispitanicima određene su plazmatske koncentracije liganda induktora proliferacije tehnikom enzimski vezanog imunosorbetnog testa. Srednje vrednosti koncentracija ligand induktora proliferacije u posmatranim grupama upoređene su jednosmernom analizom varijanse i post hok analizama. **Rezultati.** Jednosmernom analizom varijanse uočena je statistički značajna razlika u koncentracijama liganda induktora proliferacije među grupama. Najviše vrednosti ligand induktora proliferacije nađene su kod pacijenata sa dokazanim bakterijskim infekcijama ($\bar{x}$ = 8,294 ng/ml). Post hok analizom uočava se da su plazmatske koncentracije liganda induktora proliferacije kod pacijenata sa dokazanim bakterijskim infekcijama značajno više nego kod zdravih ispitanika, bolesnika sa hematološkim oboljenjima i bolesnika sa autoimunim oboljenjima. **Zaključak.** Proliferacija B-limfocita je veća kod bakterijskih infekcija nego kod autoimunih inflamatornih oboljenja i B-ćelijskih maligniteta. Prema tome, ligand induktora proliferacije može biti upotrebljen u razlikovanju bakterijskih infekcija od drugih upalnih oboljenja i samim tim pomoći pri donošenju odluke o započinjanju antibiotske terapije.

Ključne reči: B-limfociti; bakterijske infekcije; autoimune bolesti; hematološke bolesti; transmembranski protein APRIL; biomarkeri; diferencijalna dijagnoza

Introduction

Recent studies have shown that determination of serum concentrations of a proliferation-inducing ligand (APRIL) may be useful in differentiation of bacterial infections and nonbacterial inflamma-

Acknowledgement
The study "Non-invasive Markers in Diagnosis and Prognosis of Critical Conditions" was funded by the Provincial Secretariat for Science and Technological Development of the APV through the Decision No. 114-451-1921 / 2011-01, for the period 2011-2015. The Project Manager was Aleksandra Novakov-Mikić, Clinic of Gynecology and Obstetrics, Faculty of Medicine, Novi Sad

Corresponding Author: Dr Tamara Tešić, Univerzitet u Novom Sadu, Medicinski fakultet, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: tamara.tesic@mf.uns.ac.rs; ttesic@yahoo.com

Abbreviations

APRIL – a proliferation-inducing ligand
ELISA – enzyme-linked immunosorbent assay
R & D – Research and Development
CCV – Clinical Center of Vojvodina
BCMA – B cell maturation antigen
IgM – immunoglobulin M

tions. APRIL induces proliferation of B-cells in autoimmune and malignant diseases, as well as a response to bacterial infections [1]. The objective of this study was to determine whether plasma levels of APRIL can be used to assess the proliferation of B-lymphocytes in patients with bacterial infections, B-cell malignancies and autoimmune inflammatory disorders.

B-lymphocytes produce immunoglobulins as a response to infection, cytokines as immunoregulatory cells, and as antigens they participate in T-B cooperation [2, 3]. Elevated concentrations of cytokines and markers of the B-lymphocyte function are present in various autoimmune diseases (rheumatoid arthritis, systemic lupus, Sjogren's Syndrome) [4, 5], as well as in B-cell malignancies [6–8], while the role of B-lymphocytes as regulatory immune cells in response to bacterial infections remains the object of many studies. Newer researches both in animal models [6] and in human population [2, 3] highlighted individual forms of B-lymphocytes as one of the main regulators of the immune response to infectious agents. The main regulators of B-lymphocytes' function and activity are tumor necrosis factor alpha (TNF-α) superfamily members [9–11]. APRIL, as one of the superfamily members, is a type II transmembrane protein which releases as a trimer in a soluble form, and it is produced by monocytes, tissue macrophages and dendritic cells. APRIL exhibits physiological effects by binding to two B-lymphocyte receptors, transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA) which promote B-cell proliferation, stimulate production of immunoglobulin M (IgM) antibodies and survival of plasma cells, stimulate lymphocyte maturation and antibody production [12–14]. Considering that APRIL plays an important role in the survival, proliferation and maturation of B-lymphocytes, it is assumed that determination of this biomarker's concentration may serve as a predictor of bacterial infections severity [1].

Material and Methods

The study included 91 patients who were hospitalized at the Clinic of Infectious Diseases, Clinic of Hematology and the Clinic of Nephrology and Clinical Immunology of the Clinical Center of Vojvodina (CCV). The control group included 30 healthy blood donors. Determination of serum concentration of APRIL is currently available for research purposes only, so there are no defined reference values for healthy persons. Blood samples from the control group were collected at the Blood Transfusion Institute of Vojvodina. Patients were divided into three groups. The first group included 34 patients with bacterial infections confirmed by microbiology and/or radiology diagnostic tests. The bacterial infection origin was identified by physical examination, radiological procedures and microbiological analysis of various samples obtained on admission that were sent to the Microbiology Center of the Institute of Public Health of Vojvodina. The second group included 32 patients with B-cell malignancies in remission (multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma) diagnosed at the Clinic of Hematology of the CCV. The third group included 25 patients with inactive autoimmune in-

Table 1. Average age among the groups
Tabela 1. *Prosečna starost unutar grupa*

	N	$\bar{x}$	SD	Min	Max
Control group/*Kontrolna grupa*	30	34.43	11.67	19	55
Bacterial infections/*Bakterijske infekcije*	34	59.50	12.13	38	84
B-cell malignancies/*B-ćelijski maligniteti*	32	52.48	14.30	24	72
Autoimmune inflammatory disorders *Autoimuni inflamatorni poremećaji*	25	55.32	18.56	19	81

Table 2. Gender distribution in the groups
Tabela 2. *Distribucija po polovima unutar grupa*

	N	Males/*Muški pol*		Females/*Ženski pol*		p
		N	%	N	%	
Control group/*Kontrolna grupa*	30	17	56.67	13	43.33	0.465
Bacterial infections/*Bakterijske infekcije*	34	20	59%	14	41%	0.303
B-cell malignancies/*B-ćelijski maligniteti*	32	22	69%	10	31%	0.073
Autoimmune inflammatory disorders *Autoimuni inflamatorni poremećaji*	25	4	16%	21	84%	0.000

Table 3. Plasma level of APRIL in plasma samples (ng/ml)
Tabela 3. *Koncentracija APRIL-a u uzorcima plazme (ng/ml)*

	N	x̄	SD	Median/*Medijana*	Interval/*Raspon*
Control group/*Kontrolna grupa*	30	2.2232	0.51970	2.00	1.50-3.75
Bacterial infection/*Bakterijske infekcije*	34	8.2941	6.85606	5.75	1.25-39.50
B-cell malignancies/*B-ćelijski maligniteti*	32	1.6875	0.47519	1.75	1.00-3.25
Autoimmune inflammatory disorders *Autoimuni inflamatorni poremećaji*	25	2.0900	0.65701	2.00	1.00-4.00

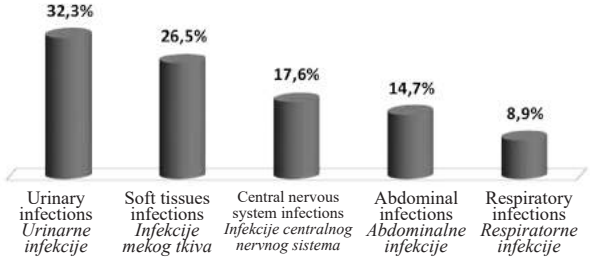
APRIL - a proliferation inducing ligand/*ligand induktor profliferacije*

flammatory diseases (systemic lupus and autoimmune vasculitis) diagnosed at the Clinic of Nephrology and Clinical Immunology of the CCV.

Demographic data, age and sex, were collected from medical records of all patients included in the study, while the concentration of APRIL was done after sampling additional 5 ml of venous blood and prepared plasma samples according to manufacturer's instructions. The concentration of APRIL was determined using the enzyme-linked immunosorbent assay (ELISA) technique of commercial reagents manufactured by Research and Development (R&D) Systems Europe. Reading was performed automatically on a spectrophotometer - Chemwell, USA. Reagents for the determination of APRIL concentrations were provided through the Project of the Provincial Secretariat for Science and Technological Development: "Noninvasive Markers in Diagnostics and Prognosis of Critical Conditions". Statistical data processing was done using the software program Statistical Package for Social Sciences (SPSS) 21.0.

Results

The average age of patients in the first group was 59.50 ± 12.13 years, in the second 52.48 ± 14.30 years, and in the third group 55.32 ± 18.56 years. One-way ANOVA showed that there was no statistically significant difference in patient age among the groups (F = 1.514, p = 0.226), while the mean age of voluntary blood donors in the control group was 34.43 ± 11.67 and it was statistically significantly lower than in other groups (p = 0.000, p = 0.000, p = 0.000). The results are shown in **Table 1**.



Graph 1. The origin of infection
Grafikon 1. *Ishodište infekcije*

Persons in the control group were significantly younger, so in order to determine the possible correlation between the APRIL concentrations and the age of patients, a correlation analysis was conducted. There was no statistically significant association between APRIL concentrations and age (ρ = -0.032, p = 0.727), indicating that age differences did not affect differences in APRIL concentrations.

The gender distribution is shown in **Table 2**. A statistically significant difference between genders was found in the group of autoimmune inflammatory diseases, by using Hi-square test (p = 0.000). By comparing mean values of APRIL in men (4.27 ± 5.59 ng/ml) and women (3.27 ± 3.36 ng/ml), Student's t-test showed that gender did not affect APRIL concentrations (t = 1.183, p = 0.240).

The origin of the bacterial infection was identified by microbiology and/or radiology tests in all patients in the first group. The most frequent origin was the urinary tract in 11 (32.3%) patients and soft tissues in 9 (26.5%), while respiratory infections were the least present. The results are shown in **Graph 1**.

Plasma levels of APRIL in groups are given in **Table 3**. One-way ANOVA showed a statistically significant difference in APRIL concentrations among groups. Post-hoc analysis showed that plasma concentrations of APRIL in patients with diagnosed bacterial infections were significantly higher than in healthy subjects, patients with hematological disorders and patients with autoimmune disorders (p = 0.000, p = 0.000, p = 0.000). Although the mean concentrations of APRIL in patients with autoimmune and hematological diseases were slightly lower than in the healthy population, post-hoc analyses showed that the difference was not statistically significant (p > 0.05).

Discussion

The results of our study showed that there was no statistically significant difference in age among the examined groups of patients. Healthy blood donors were significantly younger than the remaining examined patients. There was no correlation between the age and plasma concentration of APRIL between examined subjects. There are no available data on the effects of patients' age on APRIL concentrations in the literature. According to previous studies, men have been shown to be more prone to infection [14] and that was also confirmed in our

sample. Even though our study included more male subjects with B-cell malignancy, it was not found that men statistically more often suffer from this disease, which is consistent with previously conducted studies [8, 15]. Women are statistically more susceptible to developing autoimmune inflammatory diseases [16], as in our research. The explanation for this phenomenon was obtained through many studies [16, 17] that highlight the effect of sex hormones on humoral immunity, i. e. that androgens have immunosuppressive and estrogens immunostimulatory effects. The results of our research show that there is no statistically significant difference regarding concentration of APRIL in relation to sex. There are no available data on this matter in current literature.

The results of our study show that the most common source of bacterial infection is the urinary tract in contrast to literature data reporting that the respiratory tract is the leading source. This result is expected, since Vojvodina has a specialized institution for the treatment of respiratory tract infections, the Institute of Lung Diseases in Sremska Kamenica and those patients are rarely hospitalized at the Clinic of Infectious Diseases, mainly based on unclear evaluation of febrile conditions.

A proliferation-inducing ligand is an indicator of the function of our immune system and its concentrations in body fluids are not routinely determined, but only for research purposes. There are no clearly defined reference values of APRIL in healthy population, therefore plasma concentrations of this parameter were measured in 30 healthy individuals. The mean value of APRIL concentration in the healthy population of our sample was 2.22 ng/ml, ranging from 1.50 ng/ml to 3.75 ng/ml. Similar results were obtained by other researchers using the R&D Systems [18], and researchers who used tests of other manufacturers Plantinum ELISA eBioscience, Austria and Bender Med Syst Vienna [7, 8].

A proliferation-inducing ligand concentrations were significantly elevated in patients with confirmed bacterial infections compared to healthy individuals, similar to the research of Roderburg et al. [1]. Elevated values of this biomarker in subjects with bacterial infection are expected, given the role of APRIL in B-lymphocytes homeostasis, as well as their role in pathogenesis of bacterial infections.

In the group of patients with B-cell malignancy, the mean APRIL value was 1.69 ng/ml, ranging from 1.00 ng/ml to 3.25 ng/ml. These values, although somewhat lower, did not show a statistically significant difference in relation to the average APRIL values in healthy subjects. Bolkun et al. pointed out that APRIL values at all stages of multiple myeloma were elevated in contrast to the control group [7]. Similar results were also obtained by Chiu et al. in their study in which they noted elevated APRIL values in subjects with Hodgkin's lymphoma compared to healthy population [14]. On the other hand, Haiat et al. found that the APRIL concentrations were lower in their subjects with chronic lymphocytic leukemia and follicular lymphoma [6]. The fact that in our group the majority of patients had follicular lymphoma explains the correlation between our results and the results provided by Haiat et al., as opposed to studies that predominantly included patients with multiple myeloma and Hodgkin's lymphoma.

In our study, the mean value of APRIL in the group suffering from autoimmune disease was 2.09 ng/ml. These values were slightly lower than median APRIL in healthy subjects, without a statistically significant difference. These findings are inconsistent with the majority of previous studies showing that APRIL values in autoimmune disorders are elevated in contrast to those in healthy population [5, 14]. In contrast, Vincent et al. revealed that APRIL values were lower than those in healthy population in some forms of systemic lupus, which may indicate certain differences in the serum level of APRIL within the autoimmune group [4]. Different studies show variations in APRIL concentration in healthy population depending on their selection. Vincent et al. included only patients with renal and neurological manifestations of systemic lupus [4], while Chiu et al. included only patients in the active phase of the disease [14]. It is assumed that research on a large number of subjects, which would include patients both in acute and remission phases of the disease, as well as patients with various manifestations, might provide more precise data.

The results of our study show a statistically significant difference in serum APRIL values in patients with confirmed bacterial infection in relation to healthy population, patients with hematological disorders and patients with autoimmune disorders. Currently, there are not many papers that compared APRIL as a biomarker of immune system function in such groups, but Roderburg et al. suggested that APRIL concentrations were significantly higher in septic patients compared to those with other causes of systemic inflammatory response [1, 19, 20]. Our new data show that APRIL serves as a sensitive and specific marker for septic patients [21].

Conclusion

The plasma concentrations of a proliferation-inducing ligand are significantly higher in patients with confirmed bacterial infections than in healthy subjects, patients with B-cell malignancies and those with autoimmune inflammatory disorders. Therefore, a proliferation-inducing ligand can be used to differentiate bacterial infections from other inflammatory diseases and hence help in decision making on the initiation of antibiotic therapy.

References

1. Roderburg C, Koch A, Tacke F, Nieuwenhuijsen L, Bruensing J, Vargas Cardenas D, et al. Serum concentrations of A Proliferation-Inducing Ligand (APRIL) are elevated in sepsis and predict mortality in critically ill patients. J Crit Care. 2013;28(5):882-e1-11. Martin GS. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther. 2012;10(6):701-6.

2. de Pablo R, Monserrat J, Prieto A, Alvarez-Mon M. Role of circulating lymphocytes in patients with sepsis. Biomed Res Int. 2014;2014:671087.

3. Vaughan AT, Roghanian A, Cragg MS. B cells-masters of the immunoverse. Int J Biochem Cell Biol. 2011;43(3):280-5.

4. Vincent FB, Northcott M, Hoi A, Mackay F, Morand EF. Association of serum B cell activating factor from the tumour necrosis factor family (BAFF) and a proliferation – inducing ligand (APRIL) with central nervous system and renal disease in systemic lupus erythematosus. Lupus. 2013;22(9):873-84.

5. Koyama T, Tsukamoto H, Miyagi Y, Himeji D, Otsuka J, Miyagawa, et al. Raised serum APRIL levels in patients with systemic lupus erythematosus. Ann Rheum Dis. 2005;64(7):1065-7.

6. Haiat S, Billard C, Quiney C, Ajchenbaum-Cymbalista F, Kolb JP. Role of BAFF and APRIL in human B-cell chronic lymphocytic leukaemia. Immunology. 2006;118(3):281-92.

7. Bolkun L, Lemancewicz D, Jablonska E, Kulczynska A, Bolkun-Skornicka U, Kloczko J, et al. BAFF and APRIL as TNF superfamily molecules and angiogenesis parallel progression of human multiple myeloma. Ann Hematol. 2014;93(4):635-44.Lemancewicz D, Bolkun L, Jablonska E, Kulczynska A, Bolkun-Skornicka U, Kloczko J, et al. Evaluation of TNF superfamily molecules in multiple myeloma patients: correlation with biological and features. Leuk Res. 2013;37(9):1089-93.

8. Rawlings DJ, Schwartz MA, Jakson SW, Meyer-Bahlburg A. Integration of B cell responses through Toll-like receptors and antigen receptors. Nat Rev Immunol. 2012;12(4):282-94.

9. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 2005;115 (1):1-20.

10. Aggarwal BB, Gupta SC, Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood. 2012;119(3):651-65.

11. Vincent FB, Saulep-Easton D, Figgett WA, Fairfax KA, Mackay F. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine Growth Factor Rev. 2013;24(3):203-15.

12. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Böhm J, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. Proc Natl Acas Sci U S A. 2009;106(33):13945-50.

13. Matthes T, Dunand-Sauthier I, Santiago-Raber ML, Krause KH, Donze O, Passweg J, et al. Production of the plasma-cell survival factor a proliferation-inducing ligand (APRIL) peaks in myeloid precursor cells from human bone marrow. Blood. 2011;118(7):1838-44.

14. Chiu A, Xu W, He B, Dillon SR, Gross JA, Sievers E, et al. Hodgkin lymphoma cells express TACI and BCMA receptors and generate survival and proliferation signals in response to BAFF and APRIL. Blood. 2007;109(2):729-39.

15. Sakiani S, Olsen NJ, Kovacs WJ. Gonadal steroids and humoral immunity. Nat Rev Endocrinol. 2013;9(1):56-62.

16. Ahmed SA, Hissong BD, Verthelyi D, Donner K, Becker K, Karpuzoglu-Sahin E. Gender and risk of autoimmune diseases: possible role of estrogenic compounds. Environ Health Perspect. 1999;107(Suppl 5):681-6.

17. Vosters JL, Roescher N, Polling EJ, Illei GG, Tak PP. The expression of APRIL in Sjogren's syndrome: aberrant expression of APRIL in the salivary gland. Rheumatology (Oxford). 2012;51(9):1557-62.

18. Chu TV, Enghard P, Schürer S, Steinhauser G, Rudolph B, Riemekasten G, et al. Systemic activation of the immune system induces aberrant BAFF and APRIL expression in B cell in patients with systemic lupus erythematosus. Arthritis Rheum. 2009;60(7):2083-93.

19. Nile CJ, Sherrabeh S, Ramage G, Lappin DF. Comparison of circulating tumour necrosis factor superfamily cytokines in periodontitis patients undergoing supportive therapy: a case-controlled cross-sectional study comparing smokers and non-smokers in health and disease. J Clin Periodontol. 2013;40(9):875-82.

20. Lendak DF, Mihajlović DM, Novakov-Mikić AS, Mitić IM, Boban JM, Brkić SV. The role of TNF-α superfamily members in immunopathogenesis of sepsis. Cytokine. 2018;111:125-30.

21. Lendak DF, Mihajlović DM, Novakov-Mikić AS, Boban JM, Ubavić M, Brkić SV. APRIL and sTACI could be predictors of severe multiorgan dysfunction syndrome in sepsis. Virulence. 2018;9(1):946-53.

Rad je primljen 7. II 2019.
Recenziran 9. II 2019.
Prihvaćen za štampu 11. II 2019.
BIBLID.0025-8105:(2018):LXXI:11-12:377-381.

REVIEW ARTICLES
PREGLEDNI ČLANCI

University of Belgrade, School of Dental Medicine,
Clinic of Prosthetic Dentistry, Belgrade[1]
Clinic of Preventive and Pediatric Dentistry[2]
University of Belgrade, School of Medicine
Institute of Geriatric Medicine, Belgrade[3]

Review article
Pregledni članci
UDK 616.31-084-053.9-056.24
https://doi.org/10.2298/MPNS1812383P

DENTAL TREATMENT OF THE ELDERLY PEOPLE WITH DISABILITIES

STOMATOLOŠKI TRETMAN STARIH LJUDI SA HENDIKEPOM

Aleksandra POPOVAC[1], Ivica STANČIĆ[1], Ana VUKOVIĆ[2] and Nebojša DESPOTOVIĆ[3]

Summary

Introduction. The growing population of the elderly people and a proportional increase in the number of the elderly with different types of disabilities, necessitates a multidisciplinary approach to the assessment of their oral health and dental treatment. The ultimate aim is to retain a pain-free functional dentition and decrease the risk of future disease. **Material and Methods.** A PubMed search was performed and the authors contributed their experience in implementing preventive and therapeutic measures. **Oral health problems of the elderly with disabilities.** Two main factors influence the oral health: multimorbidity and polypharmacy. Oral health problems expected in this population are teeth abrasion, teeth fractures, root caries, periodontitis and problems with wearing dentures due to stomatitis caused by Candida albicans. **Oral health assessment and treatment guidelines.** This article provides guidelines for assessment and treatment planning, taking into consideration multimorbidity, polypharmacy, dementia and capacity of caregivers. **Preventive measures.** Preventive measures are crucial for long-term oral health of this population, and this paper provides guidelines for preventive treatment depending on the degree of functional dependence. **Prosthetic treatment.** Although some elderly with disabilities are suitable for conventional prosthetic treatment, often there are contraindications and specific considerations that must be taken into account. **Conclusion.** Oral health needs of the elderly people with disabilities should not be neglected and the success of treatment depends on the education of dental professionals and cooperation with other health professionals of the medical team.

Key words: Dental Care; Disabled Persons; Patient Care Planning; Dental Care for Aged; Oral Health; Dementia; Risk Assessment; Preventive Dentistry; Prosthodontics; Aged

Sažetak

Uvod. Zbog rastuće populacije starih ljudi i proporcionalnog povećanja učestalosti broja starih ljudi sa različitim tipovima hendikepa, multidisciplinarni pristup proceni njihovog oralnog zdravlja je neophodan. Krajnji cilj je postići funkcionalnu denticiju bez bolova i smanjiti rizik od budućih oboljenja. **Materijal i metode.** Pretraživanje literature u bazi *PubMed* je obavljeno, ali je takođe iskustvo autora u primeni preventivnih i terapijskih mera uzeto u obzir. **Problemi oralnog zdravlja starih ljudi sa hendikepom.** Dva glavna razloga imaju uticaj na oralno zdravlje: multimorbiditet i polifarmacija. Problemi oralnog zdravlja koji se očekuju u ovoj populaciji su abrazija zuba, frakture zuba, karijes korena, parodontopatija i problemi sa nošenjem proteza usled stomatitis uzrokovanog kandidom (*Candida albicans*). **Procena oralnog zdravlja i smernice za planiranje tipa tretmana.** Ovaj članak obezbeđuje korisne smernice za procenu i planiranje tretmana, uzimajući u obzir multimorbiditet, polifarmaciju, demenciju i kapacitet negovatelja. **Preventivne mere.** Preventivne mere su ključne za dugotrajni uspeh oralnog zdravlja ove populacije i ovde su prikazane smernice za preventivne mere u zavisnosti od stepena funkcionalne zavisnosti. **Protetski tretman.** Iako su neki stari ljudi sa hendikepom pogodni za konvencionalni protetski tretman, većina ovih pacijenata ima neke kontraindikacije i specifičnosti koje treba uzeti u obzir. **Zaključak.** Potrebe za oralnim zdravljem starih ljudi sa hendikepom ne smeju biti zanemarene, a uspeh tretmana zavisi od edukacije stomatologa kao i saradnje sa ostalim specijalistima medicinskog tima.

Ključne reči: stomatološka zaštita; osobe sa posebnim potrebama; planiranje tretmana pacijenta; stomatološka zaštita za stare osobe; oralno zdravlje; demencija; procena rizika; preventivna stomatologija; protetika; stare osobe

Introduction

The elderly population has been growing all over the world and there is a trend towards the reduction in edentulism. European countries have reported a reduction in edentulousness from 28% to 6% since the late ’70s [1]. In Serbia, the prevalence of edentulous patients is still very high, but in the last decade this has started changing [2]. Namely, the elderly population is increasingly retaining their natu-

Corresponding Author: Dr Aleksandra Popovac, Univerzitet u Beogradu, Stomatološki fakultet, Klinika za stomatološku protetiku, 11000 Beograd, Rankeova 4, E-mail: jelenkovic.aleksandra@gmail.com

ral teeth. That is a new challenge which requires knowledge and skills of dental professionals and oral health services. They mostly deal with dental pathology which occurs more frequently in the elderly (due to previous treatment throughout the years) and complex medical needs specific for this population.

The majority of the elderly patients are treated similarly to younger ones, including complex treatments such as endodontic treatment, prosthetic rehabilitation and dental implants. However, 10 – 14% of the elderly (depending on source) are defined as frail or as the elderly with disabilities [3–5]. Frailty has been defined as a dynamic state affecting an individual who experiences loss of one, or more than one domain of human functioning (physical, psychological, social), caused by the influence of a range of variables increasing the risk of adverse outcomes [6]. Disability is defined differently, but with similar outcome. People with disability experience negative aspects of interaction between their environmental and personal context as well as any functional impairments, activity limitations and participation restrictions that they may present [7]. Mostly, the elderly with disabilites experience difficulties with mobility, as a consequence of osteoporosis, osteoarthritis or stroke. Also, disabilities are also consequences of eyesight or hearing impairment and dementia. Guidelines for treating patients affected by the listed conditions provide useful overviews for dental teams. Older patients with frailty or other disabilities may hold different attitudes towards dental care [1], and therefore need to be assessed in a comprehensive and holistic manner.

Table 1. Assessment of oral care and treatment planning for the elderly with disabilities
Tabela 1. *Procena oralne nege i planiranja tretmana za starije osobe sa invaliditetom*

	Level of dependency/*Nivo zavisnosti*		
	Low/*Nizak*	Medium/*Srednji*	High/*Visok*
Assessment *Procena*	Identify cause of increasing dependency (stroke, polypharmacy, dementia) *Identifikovati uzrok rastuće zavisnosti (moždani udar, polifarmacija, demencija)*	Consult other medical specialists to assess health risks, generally *Konsultovati se sa ostalim medicinskim specijalistima u opštoj proceni zdravstvenog rizika*	Identify patients' physical, cognitive and social barriers to emergency palliative and elective oral care *Ustanoviti fizičke, kognitivne i socijalne barijere pacijenata za pružanje hitne, palijativne i elektivne oralne nege*
		Reassess oral health-related prevention *Ponovo proceniti prevenciju povezanu sa oralnim zdravljem*	Monitor the burden of oral care on the patient and health professionals *Pratiti teškoće u oralnoj nezi pacijenta i negovatelja*
Treatment *Tretman*	Identify, repair or replace strategically important teeth guided by the principle of the 'shortened dental arch', with or without implants *Idenfitikovati, popraviti ili nadoknaditi strateški značajne zube vodeći se principom skraćenog dentalnog luka, sa implantatima ili bez njih*	Repair and maintain strategically important teeth with conservative treatment (i. e., atraumatic restorative technique with fluoridated glass-ionomer materials), and design oral prostheses to simplify oral hygiene and prevent infection *Popraviti i održavati strateški značajne zube konzervativnim tretmanima (npr. atraumatska restaurativna tehnika sa glasjonomer cementom sa fluoridom) i planirati dizajn proteza na način koji olakšava oralnu higijenu i prevenira infekciju*	Palliative treatment on demand from the patient to control pain and infection and maintain social contacts and activities *Palijativni tretman na zahtev pacijenta zbog kontrole bola i infekcije kao i održavanja socijalnih kontakata i aktivnosti*
	Plan ongoing maintenance, including restorative and surgical treatment, in order to maintain function and prevent or control infection and pain *Planiranje daljeg održavanja oralnog zdravlja uključujući restorativne i hirurške tretmane radi održavanja funkcije i prevencije ili kontrole infekcije i bola*	Use prosthodontic attachments between overdentures and abutment teeth or dental implants to simplify hygiene and maintenance *Korišćenje atačmena u kompleksnom radu na zubima nosačima ili implantatima kako bi se pojednostavila higijena i održavanje*	

The purpose of this review was to discuss current literature data regarding oral health of patients with frailty or other disabilities and to list available preventive, prophylactic and dental treatment guidelines.

Material and Methods

A PubMed search, using combinations of keywords related to oral health care of the elderly with disabilities and dental treatment, was performed. All articles with available full texts were analyzed and divided in two large groups: preventive procedures and dental treatment procedures. Also, the authors contributed their experience in implementing preventive and therapeutic measures in the elderly people with disabilities.

Oral health problems of the elderly with disabilities

Two main factors influence the oral health: multimorbidity and polypharmacy [8, 9]. Multimorbidity is defined as the presence of two or more chronic conditions including mental health problems and chronic pain. The prevalence of multimorbidity is significant in the older population, affecting more than 60% of those aged 65–84 and 81% of those aged 85 and above [1, 9]. It points to the need to gather information from the relevant healthcare teams treating such patients and to cautiously consider approaches to treatment provision.

Due to the coexistence of multiple long-term conditions, an increasing number of patients experience polypharmacy; 44% of patients over 65 are taking 5 or more medications and 12% are taking 10 or more medications [2, 5, 10]. Multiple medications can influence both dental health and the oral health care delivery. Medications, such as bisphosphonates and anticoagulants, commonly impact the safety of dental treatment, including tooth extractions and small surgical treatments. Some medications, such as decongestants, antidepressants, antipsychotics and antihypertensive drugs may cause xerostomia. Further, xerostomia causes problems such as dysphagia, taste loss, and oral pain. It also increases the risk of dental caries and oral infections. Some studies reported synergistic effects of multiple xerostomia medications in the elderly patients taking multiple medications [10].

Oral health problems which are expected in this population are tooth wear, tooth fractures, root caries, periodontitis and problems with wearing removable dentures due to Candida induced stomatitis. Oral diseases are progressive and cumulative due to additional complex factors, such as difficulties with mobility, polypharmacy, chronic diseases and cognitive impairment [4, 5]. Over 90% of the elderly have periodontitis and in frail and ones with disabilities it happens in almost in 100% [11], especially in those who neglect oral hygiene at younger age. As they become frail, their oral health rapidly deteriorates. Dental problems in older people are a common cause of speech impairment, eating difficulties, and pain. Tooth loss, poorly fitting dentures and oral infections can result in poor nutrition because it can affect appetite, food enjoyment and ability to chew.

Just like poor general health and above listed conditions have an impact on the oral health, poor oral health is linked to increased risk of some potentially fatal conditions. It has been proven that oral diseases may cause cardiovascular diseases, stroke and aspiration pneumonia. Also, chronic oral infection can complicate the medical management of diabetes, chronic heart failure, and respiratory diseases [12].

Oral health assessment and treatment guidelines

The ultimate aim for the elderly patients is to retain a pain-free functional dentition, while managing the risk of future diseases. Assessment of oral health should be comprehensive and treatment planning carefully conducted having in mind general health, medications, level of disability, cognitive impairment, access to dental services and capacity of caregivers.

The Seattle Care Pathway is a very useful health care program which contains guidelines for treatment planning **(Table 1)** [5, 13]. This concept divides older patients into 5 categories based on the level of their dependency. The first two categories will not be discussed here, since they are aimed for older people without disabilities, labeled as “no dependency” and “pre-dependency”. “Low dependency” is the next category, which includes people with identified chronic conditions that affect oral health, but who currently receive oral treatment or do not require help to access dental services or to maintain oral health. These patients are not really dependent, but their disease symptoms are affecting them (Porter, 2015). “Medium dependency” includes people with an identified chronic condition that currently impacts their oral health. This category includes patients who demand to be seen at home or at a nursing home, or who need transportation to a dental office. “High dependency” includes people who have complex medical problems affecting their mobility preventing them from moving to receive dental care at a dental office. They differ from patients categorized as medium dependent because they cannot be moved and must be seen at home or the nursing home.

Generally, in those with higher dependence (or more significant comorbidities or frailty), active invasive dental treatment becomes less appropriate and it may interfere with medical and social factors. When a patient is experiencing pain or infection, an intervention is certainly necessary. For asymptomatic older individuals, the balance of risk and benefit of changes must be assessed, while full mouth reconstruction is often not feasible. As the dependency grows, the treatment should be less invasive and palliative, but that does not mean that basic dental treatment options should not be considered for the

elderly who can come to a dental office with the caregiver's help, especially those without cognitive impairment.

Specificities of dental treatment for the elderly with dementia

There are some specific considerations in the elderly with dementia. The rate of dementia progression is unpredictable and there can be benefits in providing proactive dental disease management to prevent need for intervention at a later, more advanced stage of the disease, when the risks of treatment may be greater.

At the early stages, it is important to undertake an oral health risk assessment and plan preventive strategies on mitigating the future risk. For example, assess the oral hygiene, risks of different medications and impact of xerostomia [14, 15]. At this stage, most types of dental care are still possible. The dentist should plan the treatment, keeping in mind that the person will eventually be unable to carry out hygiene procedures. Factors to consider in oral health assessment at this stage are:

– Caries risks – number of exposed root surfaces, xerostomia, diet and nutrition, oral hygiene;

– Periodontal disease risks: presence of active disease, diabetes, smoking;

– Manual dexterity;

– Dependency: family member or residential care;

– Drugs and comorbidities such as Parkinson's disease and diabetes.

The elderly with early dementia may be best treated in general dental practice and it can be highly beneficial for detection of early cognitive changes and get used to them during treatment. Patients will be more relaxed in a familiar environment and with a dentist with whom relationship of trust has been established. Rigorous preventive measures are highly recommended in order to prevent further gum disease and restorative treatment should be high quality and low maintenance. Key teeth should be identified and restored such as canines, molars and occluding pairs [15, 16]. Prosthetic treatment such as crowns, bridges and implants should be considered only if someone is prepared to carry out daily brushing for that person at a stage when they cannot do this for themselves. Shortened dental arch can be the aim, to secure adequate function and nutrition. If patients become edentulous, consideration may be given to two-implant retained overdentures [14, 16].

The middle stage dementia implies cognitive decline and sometimes alterations in behavior such as agitation and aggression. The focus of treatment is likely to be on prevention of further dental disease, from restoration to maintenance. The decision on the type of treatment should be based on the ability to cooperate, dental treatment needs, general health and social support. As dementia progresses, comprehensive clinical and radiographic examination can be difficult and pain identification can be particularly challenging [14].

At a later stage, the patients may lose the ability to clean their teeth, may stop understanding that their teeth need to be kept clean or may lose interest in doing so. Health care professionals may need to take over this task. At this stage, treatment focuses on prevention of dental disease, maintaining oral comfort and provision of emergency treatment if needed. Dental interventions should be as non-invasive as possible, for examples Carisolv gel for caries removal, atraumatic restorative techniques such as glass-ionomer cement restorations and preventive measures. In advanced dementia, the decline in cognition can lead to limited cooperation for treatment under local anesthesia, and dental team needs to consider use of anesthetist-led sedation or general anesthesia for treatment of pain or infection [17–20].

Preventive measures

Preventive treatment is crucial for oral diseases risk reduction. It is important for the elderly with minor disabilities, but also for those with high dependency and for residents of nursing homes. Preventive measures include treatment at the dental office, but also recommendations for care givers [21–23]. Recommendations for oral health of the elderly with disabilities are:

– Toothbrush every morning and every evening, the toothbrush should be soft, the tongue should be cleaned as well, and the tooth paste should be with a high concentration of fluoride (5000 ppm);

– Antibacterial product after lunch (mouth rinses etc.);

– Cut sugar intake down;

– Keep mouth moist; Lip balm should be water-based and not petroleum-based because of the risks for aspiration pneumonia and in the elderly with disability who use oxygen.

– Denture cleaning should be done using a special brush, better with a liquid soap than with toothpaste because it can be very abrasive. Patients should not wear removable dentures over night. Denture disinfection is recommended once a week with sodium-hypochlorite or chlorhexidine. Chlorhexidine is a safer option since it can be used both for acrylic dentures alone or acrylic dentures with metal frameworks. Sodium-hypochlorite can cause metal components to corrode. Denture tablets are also suitable, although instructions must be read to see recommendations for dentures with metal frameworks. It should be taken into consideration that denture tablets often contain persulfate which can cause severe allergic reaction.

It is obvious that for a certain number of older people with disabilities oral care on daily basis is conducted by caregivers, especially for those in nursing homes and those with dementia. However, caregivers mostly have insufficient knowledge about the importance of oral health and oral care. A study conducted in [22] residential homes in Serbia showed that most caregivers had no formal medical education (70.7%). Only one third (36.2%) of them

passed some training or courses related to prevention of oral diseases, and 78% considered them useful in practice. Most caregivers learned oral hygiene techniques from their colleagues (41.4%). When asked to evaluate the level of residents' oral health, 69% of them thought it was low or very low, which was proved by clinical examination by dental professionals. Lack of time of caregivers was indicated to be the main barrier of oral hygiene maintenance, since most of the caregivers (84.5%) were responsible for over 20 residents. It is obvious that comprehensive strategy for improving oral health of the elderly with disabilities is needed in Serbia, and education of caregivers in residential homes may be the first step of improvement.

Prosthodontic treatment

Some elderly with disabilities are suitable to receive a full range of dental procedures, including prosthodontic treatment. This mostly refers to the elderly with movement disability, who can visit the dental office with a caregiver, who can give informed consent for treatment and who have previous positive experience with prosthodontic treatment. However, some patients may be more likely to experience diseases or poorer oral hygiene which may contraindicate specific treatments and that should be taken into consideration. Due to the risk of intervention as opposed to the complexity itself, there must be a clear justification for providing complex treatment or using complex approaches. Therefore, even in patients who can receive prosthodontic treatment, it should be minimally invasive, simple and with low maintenance demands to be sure that the caregivers can take over all oral hygiene procedures at later stages of the disease, if necessary [23].

As stated previously, maintaining shortened dental arch can provide secure mastication and nutrition, if there are enough occluding pairs of teeth (more than 8 in total). Therefore, the replacement of a full dental arch is not necessary and may outweigh the positive risk-benefit ratio [24]. In edentulous patients, two implants with overdenture can be a good solution in those without cognitive impairment and without contraindications for surgical treatment such as antiangiogenic or anticoagulant medications, osteoporosis and patients who received radiation.

In cases when the existing dentures must be replaced, it should be done with minimum visits and adaptation problems. In that respect, denture copy technique is very useful. It is done when patients have old dentures, which no longer fit well, move a lot during mastication, make ulcers on mucosa and therefore elicit both nutrition problems and psychological problems. The copy technique is a production of new dentures, as accurate as possible, but with changes to aspects which cause problems to patients. In the copy technique procedure, the existing dentures are used as an individual tray, with wax rims and for teeth set up. One of the greatest advantages is the reduction of clinical phases. The original method implies first phase as clinical examination and duplication of the existing denture. This copy denture is then modified by removing denture teeth and by placement of wax rims, and that modified denture copy is further used for functional impression and records of inter-maxillary relationship. However, this standard procedure can be even shorter in patients with disabilities; the existing dentures can be used as an individual tray and wax rims without copying, only with modifications necessary for the following phases. Denture flanges that are too long due to alveolar ridge atrophy need to be shortened, sharp edges rounded and denture teeth removed in order to make wax rims. The use of a copy technique is proved to reduce complications and to shorten the adaptation period for new dentures [2, 11].

In patients with low dependency and without cognitive impairment, removable partial dentures with double crowns may be a good solution. Older patients often have only few teeth left, but sometimes the remaining teeth are of good biological value (canines and/or first molars). In that case, a conventional removable partial denture can be less suitable as a treatment option, because clasps fractures and low level of denture retention are frequent. The remaining teeth are usually extruded which elicits additional difficulties in adjusting occlusion and well-fitting of a denture. Dentures retained by double crowns ensure reliable retention, stability, and better distribution of occlusal forces than conventional partial removable dentures. Due to a good retention, double crown-retained dentures are comfortable for patients and may be very long-standing [25]. However, in patients with disabilities, some new materials should be taken into consideration, such as zirconia for inner and outer crowns. They can be manufactured quickly and with great precision. For the outer crown and a removable partial denture, polymers such as polyoxymethylene may be suitable, because such dentures are metal-free, light in weight, with good retentive properties to metal alloys and zirconia, low cost and manufactured quickly.

In patients with old fixed restorations with evidence that the existing crowns cause the periodontal disease, worsen gum inflammation, elicit tooth sensitivity due to gum retraction, the clinician may decide to remove the restoration. The main reason for the removal of the existing fixed denture is prevention of further gum destruction and repercussions on general health. If the level of dependency is low or medium, old fixed restoration can be taken off quickly under local anesthesia, but the clinician might be worried whether a patient can withstand complex clinical procedures with a new fixed partial denture. In such cases, some materials for temporary restorations can be taken into consideration. Improvements have been made in temporary materials in terms of a resistance to occlusal forces and increased durability. Such materials are mostly a combination of acrylic resin and composite materials, and can be produced in a dental laboratory using conventional techniques or a computer-aided design/computer-

aided manufacturing technology. The main advantages of such materials are in their low cost and fast production. Their durability can be up to one year, but even more, especially when antagonistic teeth are removable partial or complete dentures.

Treatments feasible in older patients with greater dependency are those that aim to improve the existing fixed or removable dentures, such as denture relining, occlusal adjustments, teeth replacement and clasp replacement or activation [12, 15, 26]. The manufacture of a new denture is not recommended, and treatment is based on rigorous preventive measures and emergency interventions in cases of infection and pain.

Conclusion

The safety and suitability of dental treatment varies to a great extent in the elderly. Some older people with disabilities are able to entirely receive a routine dental treatment. Others, with a more complex medical background, reduced cognitive status and cooperation, may not be suitable for dental/oral rehabilitation. A decision not to perform any treatment can be entirely appropriate. In all treatment options, risks and benefits should be carefully weighed to ensure the safety of patients during any treatment.

References

1. Arnold C, Brookes V, Griffiths J, Maddock S, Theophilou S. Guidelines for oral health care for people with a physical disability [Internet]. British Society for Disability and Oral Health; 2000 [revised 2000 Jan; cited 2018 May 15]. Available from: http://www.bsdh.org/documents/physical.pdf.

2. Jelenković A, Stančić I, Tihaček Šojić Lj, Živković R, Miličić B. A preliminary evaluation of normative and realistic needs for prosthetic treatment among frail elderly patients in Serbia. J Dent Sci. 2013;8(1):15-20.

3. Stančič I, Tihaček-Šojić LJ, Jelenković A. Srpska verzija indeksa „Oral Health Impact Profile" (OHIP-14) u sklopu merenja kvaliteta života bolesnika starijeg životnog doba. Vojnosanit Pregl. 2009;66(7):511-5.

4. Erić J, Stančić I, Tihaček Šojić Lj, Kulić Lj, Popovac A, Tsakos G. Prevalence, severity and clinical determinants of oral impacts in older people in Bosnia and Herzegovina. Eur J Oral Sci. 2012;120(5):438-43.

5. Pretty IA, Ellwood RP, Lo EC, MacEntee MI, Müller F, Rooney E, et al. The Seattle Care Pathway for securing oral health in older patients. Gerodontology. 2014;31(Suppl 1):77-87.

6. Gobbens RJ, Luijkx KG, Wijnen-Sponselee MT, Schols JM. In search of an integral conceptual definition of frailty: opinions of experts. J Am Med Dir Assoc. 2010;11(5):338-43.

7. World Health Organization. World report on disability [Internet]. Geneva: World Health Organization; c2011 [cited 2018 Dec 5]. Available from: http://apps.who.int/iris/bitstream/handle/10665/70670/WHO_NMH_VIP_11.01_eng.pdf;jsessionid=ED028A6237C5830F23F845BAD27A0B9C?sequence=1.

8. Pretty IA. The life course, care pathways and elements of vulnerability. A picture of health needs in a vulnerable population. Gerodontology. 2014;31(Suppl 1):1-8.

9. Petrović M, Stančić I, Popovac A, Vasović M. Oral health-related quality of life of institutionalized elderly in Serbia. Vojnosanit Pregl. 2017;74(5):402-9.

10. Shetty SR, Bhowmick S, Castelino R, Babu S. Drug induced xerostomia in elderly individuals: an institutional study. Contemp Clin Dent. 2012;3(2):173-5.

11.Walls A. Developing pathways for oral care in elders: challenges in care for the dentate the subject? Gerodontology. 2014;31(Suppl 1):25-30.

12. Gil-Montoya JA, de Mello AL, Barrios R, Gonzalez-Moles MA, Bravo M. Oral health in the elderly patient and its impact on general well-being: a nonsystematic review. Clin Interv Aging. 2015;10:461-7.

13. Geddis-Regan A, Walton G. A guide to treatment planning in complex older adults. Br Dent J. 2018;225(5):395-9.

14. The authors journal compilation. Oral health of people with dementia. Gerodontology. 2006;23(Suppl 1):3-32.

15. Ghezzi EM, Ship JA. Dementia and oral health. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89(1):2-5.

16. Ettinger RL. Dental management of patients with Alzheimer's disease and other dementias. Gerodontology. 2000;17(1):8-16.

17. Lalić M, Melih I, Aleksić E, Gajić M, Kalevski K, Ćuković A. Oral health related quality of life and dental status of adult patients. Balkan Journal of Dental Medicine. 2017;21:93-9.

18. Chalmers JM, Carter KD, Spencer AJ. Caries incidence and increments in community-living older adults with and without dementia. Gerodontology. 2002;19(2):80-94.

19. Coleman P. Improving oral health care for the frail elderly: a review of widespread problems and best practices. Geriatr Nurs. 2002;23(4):189-99.

20. Turanjanin-Tomić G, Drašković B, Stanić D, Uram-Benka A. Specifičnosti opšte anestezije u stomatologiji kod osoba sa posebnim potrebama. Med Pregl. 2010;63(7-8):535-40.

21. Šapurić M, Tozja F. Assessment of knowledge and attitudes to preserve oral health among older people aged 60+ in FYROM. Balkan Journal of Dental Medicine. 2015;19:26-32.

22. Stančić I, Petrović M, Popovac A, Vasović M, Despotović N. Caregivers' attitudes, knowledge and practices of oral care at nursing homes in Serbia. Vojnosanit Pregl. 2016;73(7):668-73.

23. Chalmers JM. Minimal intervention dentistry: part 1. Strategies for addressing the new caries challenge in older patients. J Can Dent Assoc. 2006;72(5):427-33.

24. Tihaček-Šojić Lj, Stančić I, Jelenković A, Milić-Lemić A. Mogućnosti protetske rehabilitacije skraćenog zubnog niza u osoba starije dobi. Stomatološki glasnik Srbije. 2008;55(4):238-46.

25. Stančić I, Jelenković A. Retention of telescopic denture in elderly patients with maximum partially edentulous arch. Gerodontology. 2008;25(3):162-7.

26. Porter J, Ntouva A, Read A, Murdoch M, Ola D, Tsakos G. The impact of oral health on the quality of life of nursing home residents. Health Qual Life Outcomes. 2015;13:102.

Rad je primljen 10. XII 2018.
Recenziran 15. XII 2018.
Prihvaćen za štampu 18. XII 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:383-388.

PROFESSIONAL ARTICLES
STRUČNI ČLANCI

University of Novi Sad, Faculty of Medicine[1]
Clinical Center of Vojvodina, Novi Sad
Clinic of Vascular and Endovascular Surgery[2]

Professional article
Stručni članak
UDK 616.133-089.168:616.831-005.1
https://doi.org/10.2298/MPNS1812389M

EARLY STROKE AFTER CAROTID ENDARTERECTOMY – A CASE SERIES DURING A 5-YEAR PERIOD

RANI MOŽDANI UDAR NAKON KAROTIDNE ENDARTEREKTOMIJE – PRIKAZ SERIJE SLUČAJEVA ZA PETOGODIŠNJI PERIOD

Vladimir Č. MANOJLOVIĆ[1,2], Đorđe MILOŠEVIĆ[1], Nebojša BUDAKOV[1], Vladimir MARKOVIĆ[1] and Dragan NIKOLIĆ[1,2]

Summary

Introduction. Carotid endarterectomy is an efficient surgical procedure of primary and secondary prevention of ischemic stroke in asymptomatic and symptomatic patients with extracranial carotid artery disease. **Material and Methods.** In this paper we analyzed incidence, risk factors, potential causes and preventive measures for early stroke after carotid endarterectomy in patients (809) who underwent surgery for carotid artery stenosis at the Clinic of Vascular and Endovascular Surgery of the Clinical Center of Vojvodina in Novi Sad during a five year period (April 2013 - March 2018). **Results and Discussion.** Early operative stroke was registered in 12 patients with no significant difference between symptomatic (2.8%) and asymptomatic (1.1%) patients (odds ratio - 2,56, 95% confidence interval - 0.8063 to 8.1770 standard deviation 1,596; p = 0,11). Contralateral carotid occlusion (odds ratio - 3.1154, 95% confidence interval - 1.1620 to 8.3522, p = 0.0239) and no dual antiplatelet therapy (odds ratio - 3.1154, 95% confidence interval - 1.8537 to 526.4871; p = 0.0169) were pointed out as risk factors for operative stroke. Most of the perioperative and early postoperative strokes after carotid endarterectomy were due to arterial-arterial thromboembolism, intracerebral hemorrhage and acute carotid occlusion that developed rarely and were associated with severe neurological deficit. **Conclusion.** Even though our results are in agreement with the literature data, additional measures for surgical quality control would further decrease the incidence of operative stroke.

Key words: Stroke; Carotid Artery Diseases; Endarterectomy, Carotid; Prophylactic Surgical Procedures; Risk Factors; Carotid Stenosis; Intraoperative Complications; Postoperative Complications

Sažetak

Uvod. Karotidna endarterektomija predstavlja efikasnu meru primarne i sekundarne prevencije moždanog udara kod pacijenata sa oboljenjem karotidnih arterija, a koji nemaju simptome i pacijenata koji ih imaju. **Materijal i metode.** U ovom radu analizirani su učestalost, faktori rizika, potencijalni uzročnici i mere prevencije moždanog udara kod pacijenata (809) koji su zbog ekstrakranijalne karotidne bolesti operisani na Klinici za vaskularnu i endovaskularnu hirurgiju Kliničkog centra Vojvodine u periodu od pet godina (april 2013. - mart 2018.). **Rezultati i diskusija.** Moždani udar registrovan je kod 12 pacijenata sa učestalošću do 2,8% kod pacijenata sa simptomima i 1,1% kod pacijenata bez simptoma, bez značajne statističke razlike (odnos verovatnoće 2,56, 95% interval poverenja od 0.8063 do 8.1770 standardna devijacija 1,596 p = 0,11). Od drugih faktora rizika, značajnim su se pokazali kontralateralna okluzija arterije (odnos verovatnoće 3.1154, 95% interval poverenja 1.1620 do 8.3522, p = 0.0239) i odsustvo dvojne antiagregacione terapije (odnos verovatnoće 3.1154, 95% interval poverenja 1.8537 do 526.4871, p = 0.0169). Najveći broj perioperativnih i postoperativnih moždanih udara razvio se kao posledica arterio-arterijske embolizacije, a moždano krvarenje i akutna karotidna tromboza su bili retki uzročnici povezani sa dubljim neurološkim deficitom i lošijom prognozom. **Zaključak**. I pored toga što su dobijeni rezultati u skladu sa zvaničnim preporukama, mere operativne kontrole kvaliteta koje su predložene mogle bi dodatno da smanje rizik od moždanog udara kod operisanih pacijenata.

Ključne reči: moždani udar; oboljenje karotidnih arterija; karotidna endarterektomija; preventivne hirurške procedure; faktori rizika; karotidna stenoza; intraoperativne komplikacije; postoperativne komplikacije

Introduction

Carotid endarterectomy is an efficient surgical procedure that prevents primary and secondary ischemic stroke in asymptomatic and symptomatic patients with extracranial carotid artery disease. It has been recognized with the highest class of recommendation and the level of evidence by current guidelines [1].

Stroke after carotid endarterectomy is a major early complication of the procedure. It is an organ-specif-

Corresponding Author: Doc. dr Vladimir Č. Manojlović, Univerzitet u Novom Sadu, Medicinski fakultet, Klinički centar Vojvodine, 21000 Novi Sad, Hajduk Veljkova 1-7, E-mail: vladimirmanojlovic@mf.uns.ac.rs

Abbreviations

OR – odds ratio
CI – confidence interval
SD – standard deviation
PAD – peripheral artery disease
CT – computerized tomography
ICA – internal carotid artery
MCA – middle cerebral artery
ICU – intensive care unit
DAPT – dual antiplatelet therapy
NIHSS – National Institutes of Health Stroke Scale
HITS – high intensity transient signals

ic complication that essentially represents the opposite to the primary goal: instead of preventing it, the surgery seems to be causing a stroke. Thus, maintainance of low incidence of perioperative stroke is a condition for successful treatment of carotid artery disease. The acceptable incidence of perioperative stroke is 4% in symptomatic and 3% in asymptomatic patients. This level of risk was defined by multiple randomized controlled trials where the perioperative risk was correlated with the risk of stroke in patients who received only medical treatment without surgery [2].

Having that in mind, every early stroke after carotid endarterectomy has to be considered potentially preventable. It is necessary to analyze and professionally discuss each case. Relevant health facilities performing carotid surgery should conduct a continuous critical observation and statistical follow up of the incidence of perioperative strokes, which should be published in periodical publications and reports [3].

The aim of this research was to analyze incidence, risk factors, potential causes and preventive measures for early stroke after carotid endarterectomy in patients who underwent surgery due to asymptomatic and symptomatic carotid stenosis at the Clinic of Vascular and Endovascular Surgery of the Clinical Center of Vojvodina in Novi Sad.

Material and Methods

This observational study included 809 patients who underwent surgery due to extracranial carotid artery stenosis at the Clinic of Vascular and Endovascular Surgery of the Clinical Center of Vojvodina in Novi Sad during a 5-year period (April 2013 - March 2018). During this period, we systematically collected the following data:

– Preoperative data: age, sex, associated diseases and risk factors (hypertension, hyperlypoproteinemia, diabetes, smoking, clinically signifcant coronary artery disease and peripheral artery disease (PAD)), medications (antiplatelet and antihypertensive drugs, statins), clinical symptoms (asymptomatic and symptomatic carotid disease), degree of stenosis and contralateral carotid artery stenosis were determined by duplex ultrasound and computerized tomography (CT) angiography;

– Operative and early postoperative data: type of anesthesia, surgical technique, operative findings, use of intraluminal shunt protection, operative and early postoperative outcome: major complications in early postoperative period: stroke, myocardial infarction and/or decompensation, and death from any cause; minor complications (cranial nerve injury, wound hematoma, hemodynamic instability, stenocardia, heart rhythm disorders, transient confusion, hyperperfusion syndrome);

– Early operative stroke was defined as a newly recorded neurological deficiency corresponding to the operated carotid artery that lasted more than 24 h and occurred during the period of 30 days after the operation. A total of 12 patients with early postoperative stroke were registered;

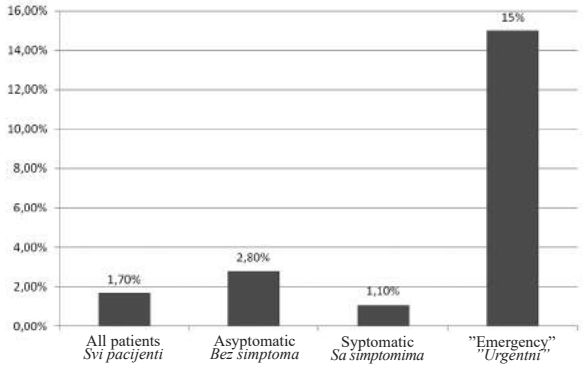
– In all patients who presented with early operative stroke, according to the current guidelines, an emergency duplex ultrasound, CT, facultative wound and carotid artery exploration were done, as well as neurological examination. The severity of neurogical defficiency was estimated by National Institutes of Health Stroke Scale (NIHSS);

– Results of extracranial carotid artery disease surgery were presented by descriptive statistic values: mean value, frequency and average. Data for postoperative stroke patients were presented as a case series (because the number of patients who had an early postopertive stroke was low), and we used univariate analysis (standard deviation (SD) and odds ratio (OR)) for analyzing risks of operative stroke.

Results

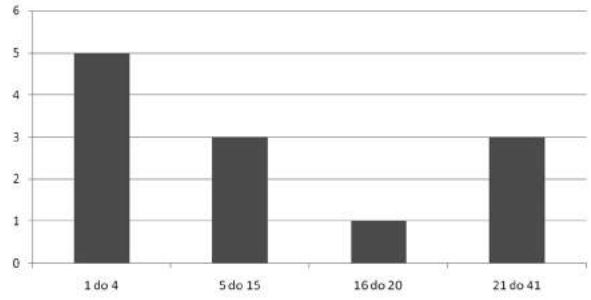
The patients who underwent surgery were 54 to 79 years of age, medain - 67 years, and 76% of patients were males.

Risk factors. Hypertension was found in 85% of patients, diabetes in 37%, hyperlipoproteinemia in 62% and 51% were smokers. Clinically significant coronary artery disease was found in 28% of pa-



Graph 1. Incidence of early stroke after carotid endarterectomy in symptomatic, asymptomatic patients and "emergency" carotid endarterectomy

Grafikon 1. *Učestalost ranog moždanog udara nakon karotidne endarterektomije kod pacijenata koji imaju simptome, pacijenata koji nemaju simptome i urgentno operisanih pacijenata*



Graph 2. Severity of stroke after carotid endarterectomy
Grafikon 2. *Težina moždanog udara nakon karotidne endarterektomije*

tients, left ventricular hypertrophy in 22%, significant PAD (class III -VI) in 12%.

Medical therapy. Most patients used a single antiplatelet therapy (acetylsalicylic acid or clopidogrel) 58%, dual antiplatelet therapy was used by 13 %, 7% used oral anticoagulation therapy and 23% of patients did not use any antiplatelet therapy. Statins were used by 70% of patients and angiotensin-converting-enzyme (ACE) inhibitors were the most common antihypertensive drugs used by 51% of patients, whereas 31% did not use any antihypertensive therapy.

Operative results. Of 809 patients who underwent surgery, the majority (447 patients, 63%) were asymptomatic, 248 patients (35%) presented with a symptomatic carotid disease and 13 (2%) were "urgent" cases, operated within the first 48 h after the neurological event. Seven patients were operated in locoregional anesthesia, in 44% of patients endoluminal protection was used, with direct or patch closure, and in 56% of patients eversion technique was used.

Five asymptomatic patients had a perioperative stroke (1.1%); 7 symptomatic patients also had a perioperative stroke (2.8%) out of which 2 patients underwent "acute" carotid endarterectomy (15% stroke incidence in "acute" carotid endarterectomy group). In total, 1.5% (12 patients) had a perioeprative stroke, 4 cases had myocardial infarction (0.7%) and 4 cases had a fatal outcome (0.7%). The difference between symptomatic and asymptomatic patients did not show a statistical significance (OR 2,56, confidence interval (CI) 95% 0.8063 to 8.1770, SD 1,596, p = 0,11). The results of operative treatment are shown in **Graph 1**.

Types of stroke. Perioperative stroke which occurred imediatly after the surgery was defined as "intraoperative stroke" and it was registred in 7 patients, while "early postoperative stroke" developed in 5 patients who did not have a neurological deficiency immediately after the surgery, but it developed more than 1 hour later. In case of intraoperative stroke, immediate carotid artery exploration was indicated. In only two cases complete carotid thrombosis and occlusion were detected as well as one case of partial thrombosis and distal intimal flap. These patients were treated by baloon catheter thrombectomy, intimal flap fixation, local removal of the thrombus, local flushing with heparin saline solutions, additional systemic heparinization and closure with patch angioplasty or direct suture. The remaining 4 patients underwent carotid artery exploration, but the cause of tromboembolic event was not found. Early postoperative stroke was registered in the rest of 5 patients, 3 cases of ischemic and 2 cases of hemorrhagic (one case of hemorrhagic transformation and one due to cerebrovacular hypeperfusion).

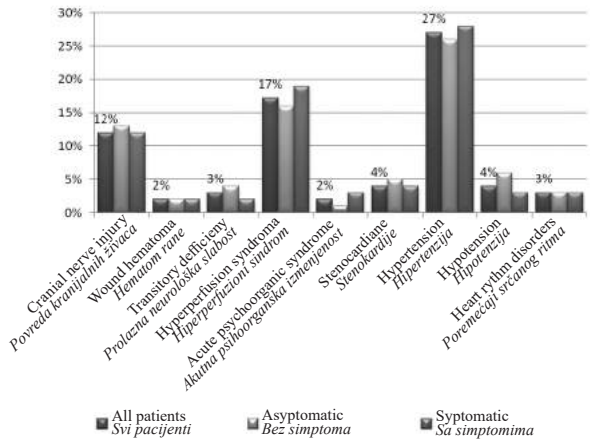
Risk factors for operative stroke. Univariate analysis showed two risk factors for operative stroke: high-grade stenosis or occlusion of the contralateral carotid artery (OR 3.1154, 95% CI 1.1620 to 8.3522, p = 0.0239) and absence of previous dual antiplatelet therapy (OR 3.1154, 95% CI 1.8537 to 526.4871, p = 0.0169).

Outcome of stroke. In all 12 cases of stroke after carotid endarterectomy, CT was done within 24 h after surgery, and ischemic stroke was found in 9 patients, and hemorrhagic stroke in 3 patients (one intracerebral hematoma and 2 hemorrhagic transformations of acute ischemic stroke).

The severity of stroke was evaluated by using NIHSS and results are shown in **Graph 2**. Most of the patients (eight) had a mild to moderate neurological deficiency. In 3 cases the stroke was lethal: in one case a severe stroke was the cause of death, and in two cases mild and moderate stroke was accompanied with acute heart failure and multiple organ failure. Along with the major complications, we analyzed the frequency of minor complications and results are shown in **Graph 3.**

Discussion

Early stroke after carotid endarterectomy is a rare complication and in our series the incidence of 1.1% in asymptomatic and 2.8% in symptomatic patients was far below the reported levels of 4% and 6%, respectively. The risk factors for operative stroke that showed to be significant in our series were contralateral carotid occlusion and absence of dual antiplatetlet



Graph 3. Incidence of "minor" early complications in patients after carotid endarterectomy
Grafikon 3. *Učestalost blažih ranih komplikacija kod pacijenata nakon karotidne endarterektomije*

therapy (DAPT), which is in agreement with literature data. Other risk factors suggested in literature, such as age, sex, symptomatic/asymptomatic, PAD, did not show a statistical significance, probably due to low incidence of perioperative strokes [4–6].

In case of intraoperative stroke, the priority is to identify carotid occlusion assuming that these patients would benefit from thrombectomy and restoration of blood flow within 1 h. Early carotid occlusion is caused by dissection of intimal flap or by thrombosis formed at endarterectomized surface of the carotid artery. Clinical findings, including a triad of hemiplegia, hemianopia and higher cortical dysfunction, are likely to be connected with internal carotid artery (ICA) or middle cerebral artery (MCA) mainstream acute occlusion [7]. Ultrasound examination in patients with acute neurological defficiency after surgery is not accurate enough, due to air and hematoma trapped in the wound. In such cases, emergency reoperation is indicated. In our seires, less than half of patients had an acute carotid thrombosis as a cause of stroke, indicating that direct ultrasound examination of carotid artery could be done after the wound exploration to avoid unnecessary carotid artery exploration prolonging the procedure.

If not acute carotid thrombosis, the most probable cause of operative stroke is movement of the thrombus during the preparation of the carotid artery, crossclamping, or movement of the thrombus adherent to intraluminal shunt after restoration of flow. In all cases of intraoperative stroke, patients should be admitted to the intensive care unit (ICU) under general anesthesia, and CT should be done as soon as possible [8].

Early postoperative stroke is caused either by the arterial-arterial embolization from the endarterectomized surface of the carotid artery (mostly by thrombus formed from the arterial wall vasa vasorum), or intracranial hemorrhage. To evaluate the cause of stroke, CT has to be done as soon as possible. In case of ischemic stroke, reoperation is indicated if there is a clear case of carotid thrombosis evaluated by duplex ultrasound or contrast enhanced CT [9].

Most efficient procedures to decrease the incidence of perioperative stroke are:

– Perioperative detection of arterial emboli by high intensity transient signals (HITS) is useful in order to abort the procedure (carotid bulb preparation and intraarterial shunt insertion) in case of increased HITS. This procedure was not performed in our series [10].

– Completion angioscopy, to exclude adherent thrombus or intimal flap larger than 2 mm, that was also not done in our series [11].

– Routine shunting is not supported by the literature, because shunt insertion may mobilize the thrombus. The only case of intracranial hemorrhage in our series was the result of prolonged brain ischemia and consecutive decrease of cerebrovascular reserve during the eversion carotid endarterectomy without shunting. That suggests that shunting may be used selectively to avoid operative stroke [12, 13].

– Routine DAPT administration at least 5 days prior to surgery, without discontinuaition of the therapy. In our series absence of DAPT was a statisticaly significant predictor of perioperative stroke. At the same time, there were no other hemorrhagic complications in patients that were using DAPT, such as wound hematoma or intracerebral hemorrhage [14].

– Postoperative strict blood pressure control should be done primarily in ICU and continued after admission to the department, at least in the following 48 to 72 hours. Blood pressure should be targeted at 140/80 mmHg to avoid the cerebral hyperperfusion syndrome. Cerebral hyperperfusion syndrome patients have 13 times greater risk for postoperative cerebral hemorrhage. In our series, it was registered in 17% [15].

– Endovascular options for perioperative stroke include emergency carotid artery stenting, thrombus aspiration from occluded MCA, while thrombolysis is rarely indicated. Evaluation of such treatment is expected in the future [16].

Conclusion

Carotid endarterectomy at the Clinic of Vascular and Endovascular Surgery is done with acceptable operating risks for major complications. Even though it is a low risk procedure, measures for surgical quality control would further decrease the incidence of operative stroke.

References

1. Eckstein HH. European Society for Vascular Surgery Guidelines on the Management of Atherosclerotic Carotid and Vertebral Artery Disease. Eur J Vasc Endovasc Surg. 2018;55 (1):1-2.

2. Clinical alert: benefit of carotid endarterectomy for patients with high-grade stenosis of the internal carotid artery. National Institute of Neurological Disorders and Stroke Stroke and Trauma Division. North American Symptomatic Carotid Endarterectomy Trial (NASCET) investigators. Stroke. 1991; 22(6):816-7.

3. Riles TS, Imparato AM, Jacobowitz GR, Lamparello PJ, Giangola G, Adelmang MA, et al. The cause of perioperative stroke after carotid endarterectomy. J Vasc Surg. 1994;19(2):206-14.

4. Spiotta AM, Vargas J, Zuckerman S, Mokin M, Ahmed A, Mocco J, et al. Acute stroke after carotid endarterectomy: time for a paradigm shift? Multicenter experience with emergent carotid artery stenting with or without intracranial tandem occlusion thrombectomy. Neurosurgery. 2015;76(4):403-10.

5. Brott TG, Halperin JL, Abbara S, Bacharach JM, Barr JD, Bush RL, et al. 2011 ASA/ACCF/AHA/AANN/AANS/ACR/ASNR/CNS/SAIP/SCAI/SIR/SNIS/SVM/SVS guideline on the management of patients with extracranial carotid and vertebral artery disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, and the American Stroke Association, American Association of Neuroscience Nurses, American Association of Neurological Surgeons, American College of Radiology, American Society of Neuroradiology, Congress of Neurological Surgeons, Society of Atherosclerosis Imaging and Prevention,

Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of NeuroInterventional Surgery, Society for Vascular Medicine, and Society for Vascular Surgery. J Am Coll Cardiol. 2011;57(8):e16-94.

6. Pasternak J, Marković V, Nikolić D, Milošević Đ, Popović V, Manojlović V. Rane postoperativne komplikacije karotidne hirurgije. Medicina danas. 2012;11(4-6):134-42.

7. Sharpe R, Sayers RD, London NJ, Bown MJ, McCarthy MJ, Nasim A, et al. Procedural risk following carotid endarterectomy in the hyperacute period after onset of symptoms. Eur J Vasc Endovasc Surg. 2013;46(5):519-23.

8. Naylor AR, Sayers RD, McCarthy MJ, Bown MJ, Nasim A, Dennis MJ, et al. Closing the loop: a 21-year audit of strategies for preventing stroke and death following carotid endarterectomy. Eur J Vasc Endovasc Surg. 2013;46(2):161-70.

9. Aleksic M, Rueger MA, Lehnhardt FG, Sobesky J, Matoussevitch V, Neveling M, et al. Primary stroke unit treatment followed by very early carotid endarterectomy for carotid artery stenosis after acute stroke. Cerebrovasc Dis. 2006;22(4):276-81.

10. Levi CR, O'Malley HM, Fell G, Roberts AK, Hoare MC, Royle JP, et al. Transcranial Doppler detected cerebral microembolism following carotid endarterectomy: high microembolic signal loads predict post-operative cerebral ischaemia. Brain. 1997;120(Pt 4):621-9.

Rad je primljen 18. XI 2018.
Recenziran 29. XI 2018.
Prihvaćen za štampu 4. XII 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:389-393.

11. Sharpe R, Sayers RD, McCarthy M, Dennis M, London NJ, Nasim A, et al. The war against error: a 15 year experience of completion angioscopy following carotid endarterectomy. Eur J Vasc Endovasc Surg. 2012;43(2):139-45.

12. Slankamenac P, Živanović Ž. The significance of collateral circulation in acute ischemic stroke. Med Pregl. 2018;71(7-8):213-6.

13. Lewis SC, Warlow CP, Bodenham AR, Colam B, Rothwell PM, Torgerson D, et al. General anaesthesia versus local anaesthesia for carotid surgery (GALA): a multicentre, randomised controlled trial. Lancet. 2008;372(9656):2132-42.

14. Payne DA, Jones CI, Hayes PD, Thompson MM, London NJ, Bell PR, et al. Beneficial effects of clopidogrel combined with aspirin in reducing cerebral emboli in patients undergoing carotid endarterectomy. Circulation. 2004;109(12):1476-81.

15. De Rango P. Cerebral hyperperfusion syndrome: the dark side of carotid endarterectomy. Eur J Vasc Endovasc Surg. 2012;43(4):337-9.

16. Perler BA, Murphy K, Sternbach Y, Gailloud P, Shake JG. Immediate postoperative thrombolytic therapy: an aggressive strategy for neurologic salvage when cerebral thromboembolysm complicates carotid endarterectomy. J Vasc Surg. 2000;31(5):1033-7.

Oncology Institute of Vojvodina, Sremska Kamenica[1]
University of Novi Sad, Faculty of Medicine[2]

Professional article
Stručni članak
UDK 618.19-006.6:615.38.035
https://doi.org/10.2298/MPNS1812394V

OPTIMAL TIME FOR ADJUVANT THERAPY INITIATION IN BREAST CANCER PATIENTS: A SINGLE CENTER EXPERIENCE

OPTIMALNO VREME ZA POČETAK ADJUVANTNE TERAPIJE KOD PACIJENATA SA KARCINOMOM DOJKE: ISKUSTVO JEDNOG CENTRA

Bojana VRANJKOVIĆ[1], Dragana PETROVIĆ[1], Jelena RADIĆ[1, 2], Maja POPOVIĆ[1, 2], Ivana KOLAROV BJELOBRK[1, 2] and Nemanja PETROVIĆ[1, 2]

Summary

Introduction. This retrospective study evaluates the association between the time of chemotherapy initiation and disease free survival in regard to breast cancer subtypes and stage at diagnosis. **Material and Methods.** The study included a total of 1075 breast cancer patients, stages I – III, treated at Oncology Institute of Vojvodina, Serbia (from 2010 to 2012; n = 617). The gathered data included prognostic factors used in everyday practice. Patients were divided into three groups according to the interval between surgery and chemotherapy (≤ 30, 31 – 60, ≥ 61 days). Disease free survival was calculated. **Results**. Among the 617 patients, the 5-year disease free survival estimate was similar: 81.5%, 81.0%, 84.6% (log-rank test, p = 0.728) regarding the time of adjuvant chemotherapy initiation: ≤ 30 days, 31 – 60, and ≥ 61 days, respectively. The study showed that 85% of our breast cancer patients started adjuvant chemotherapy within 3 months after definitive surgery. In multivariate analysis, independent prognostic factors for disease free survival were nodal status and tumor size. The 5-year disease free survival estimate was 85.8% (p = 0.001) for patients with luminal-A subtype (Estrogen +, Progesterone high, human epidermal growth factor receptor 2−, Ki-67 < 20%) with a median follow-up of 62,7 months; for patients with luminal-B (Luminal B (human epidermal growth factor receptor 2 –) Estrogen +, human epidermal growth factor receptor 2 −, either Ki-67 high or Progesterone low, Luminal B (human epidermal growth factor receptor +) Estrogen +, human epidermal growth factor receptor 2 +, any Ki-67, any Progesterone) 78.3% (p = 0.534) with a median follow-up of 55.9 months; for patients with triple negative breast cancer it was 73.4% with a median follow-up of 58,1 months, and for patients with human epidermal growth factor receptor 2+ it was 77.1% (p = 0.448) with a median follow-up of 55.5 months. **Conclusion.** Early initiation of adjuvant chemotherapy is particularly important in patients with advanced-stage breast cancer at diagnosis, and those with trastuzumab-treated triple-negative breast cancer and human epidermal growth factor receptor 2-positive tumors.

Key words: Breast Neoplasms; Chemotherapy, Adjuvant; Triple Negative Breast Neoplasms; Disease-Free Survival; Receptor, ErbB-2; Treatment Outcome

Sažetak

Uvod. Ova retrospektivna studija ispituje uticaj vremena započinjanja hemioterapije na dužinu vremena bez progresije bolesti u zavisnosti od tipa karcinoma dojke i stadijuma u momentu dijagnoze. **Materijal i metode.** U studiji je obuhvaćeno 1 075 pacijenata obolelih od karcinoma dojke, stadijum bolesti I–III, lečenih na Institutu za onkologiju Vojvodine, Srbija (od 2010. do 2012. godine, n = 617). Podaci su prikupljeni u odnosu na prognostičke faktore korišćene u svakodnevnoj praksi. Pacijenti su podeljeni u tri grupe shodno vremenu započinjanja hemioterapije nakon definitivne hirurgije (≤ 30, 31 do 60, ≥ 61 dan). Period bez bolesti je takođe izračunat. **Rezultati.** Među 617 pacijenata koji su bili uključeni, petogodišnji period bez bolesti bio je redom 81,5%, 81%, 84,6% (*log - rank test* p = 0,728), među pacijentima koji su započeli hemioterapiju ≤ 30, 31 do 60, ≥ 61 dan. Pokazano je da 85% naših pacijenata je započelo adjuvantnu hemioterapiju do tri meseca nakon završenog hirurškog lečenja. U multivarijantnoj analizi, nezavisni prognostički parametri za period bez bolesti su status limfnih čvorova i veličina tumora. Petogodišnji period bez bolesti bio je 85,5% (p = 0.001) za luminal A-tumore (estrogen +, receptor 2 humanog epidermalnog faktora rasta –, Ki - 67 < 20%, progesteron visok), medijana praćenja je bila 62,7 meseci, za luminal B-tumore (luminal B receptor 2 humanog epidermalnog faktora rasta –) estrogen +, receptor 2 humanog epidermalnog faktora rasta –, Ki - 67 visok ili progesteron nizak, luminal B (receptor 2 humanog epidermalnog faktora rasta)+, estrogen +, receptor 2 humanog epidermalnog faktora rasta +, bilo koja vrednost Ki - 67, bilo koja vrednost progesterona) 78,3% (p = 0,534), medijana praćenja bila je 55,9 meseci, za trostruko negativne tumore 73,4%, medijana praćenja bila je 58,1 mesec i za receptor 2 humanog epidermalnog faktora rasta + 77,1% (p = 0,448), medijana praćenja je bila 55,5 meseci. **Zaključak.** Rano započinjanje adjuvantne hemioterapije je naročito bitno za pacijente sa uznapredovalim karcinomom dojke, za trostruko negativne i receptor 2 humanog epidermalnog faktora rasta pozitivne karcinome lečene trastuzumabom.

Ključne reči: neoplazme dojke; adjuvantna hemoterapija; tripl negativni karcinom dojke; preživljavanje bez bolesti; HER2+ receptori; ishod lečenja

Corresponding Author: Dr Bojana Vranjković, Institut za onkologiju Vojvodine,
21204 Sremska Kamenica, Put Doktora Goldmana 4, E-mail: bojanavranjkovic1985@gmail.com

Abbreviations

DFS – disease free survival
HER2 – human epidermal growth factor receptor 2
BC – breast cancer
TNBC – triple-negative breast cancer
TTC – time to chemotherapy
TNM – Tumor Node and Metastasis
ER – estrogen receptor
PgR – progesterone receptor
CIs – confidence intervals
CMF – cyclophosphamide, methotrexate, fluorouracil
OS – overall survival
ERBB2 – receptor tyrosine-protein kinase erbB-2
WT – whole time

Introduction

Meta-analysis of adjuvant chemotherapy randomized controlled trials has shown that adjuvant chemotherapy may decrease the risk of breast cancer (BC) mortality by 30 – 40% in regard to patients without chemotherapy [1]. Today, adjuvant chemotherapy is routinely recommended in 60 – 70% of BC patients after surgery. Postponing the start of adjuvant chemotherapy for more than 90 days following surgery may significantly increase the risk of death in BC patients. The optimal time for initiation of adjuvant chemotherapy after surgery is still controversial. Currently, there are no guidelines recommending the optimal time for initiation of adjuvant chemotherapy in BC patients.

Retrospective studies evaluating the role of early initiation of chemotherapy reported conflicting results [2–4]. Most patients with BC start adjuvant chemotherapy within 30 to 40 days after surgery. It is thought that chemotherapy administration delayed beyond this time can decrease the benefit provided by cytotoxic systemic therapies [5]. Possible explanations for these effects include accelerated growth of micro-metastases after primary tumor resection, increased tumor angiogenesis, or development of primary resistance [6–10].

Studies differ with respect to patient and disease characteristics including the arbitrarily selected cut-off to the definition of early versus delayed beginning of therapy [11]. On the other hand, it is known that BC is a heterogeneous disease and certain subtypes of BC, such as triple negative BC (TNBC) and human epidermal growth factor receptor 2 (HER2) positive BC are associated with worse prognosis because of increased risk of recurrence, which probably has impact on the benefit from adjuvant chemotherapy [12–14]. A most recent report from Gagliato et al. [11] indicates that the delayed adjuvant chemotherapy is particularly meaningful for patients with advanced disease, TNBC, and trastuzumab-treated HER2+ tumors.

According to the results of a retrospective study, the authors suggest that early initiation of chemotherapy is very important for the outcome of these patients [4, 15, 16].

The researchers found that factors such as socioeconomic status, health insurance coverage and ethnicity were associated with delayed treatment [17, 18].

To determine the relationship between time to chemotherapy (TTC) and survival in women with BC, we conducted a retrospective study at the Oncology Institute of Vojvodina, Serbia. It remains unclear whether TTC has a differential impact among the distinct BC subtypes. Therefore, we conducted this retrospective analysis using our single-institution data to evaluate the association between TTC and outcomes according to tumor characteristics and BC subtypes. Our country is one of the developing countries with limited financial resources for health insurance.

Material and Methods

During 2010 – 2012, there were 1075 consecutive patients who were diagnosed with stage I - III BC and underwent surgery at the Oncology Institute of Vojvodina. Patients with stage IV BC are generally treated with palliative chemotherapy and were excluded from this study. Among them, 458 were excluded for the following reasons: 256 received no adjuvant chemotherapy, 72 were having neoadjuvant chemotherapy, and 130 had inflammatory BC, unknown tumor size or surgery type, or incomplete or unknown chemotherapy or surgery data. The final study cohort included 617 patients.

Our analysis included women aged 18 to 99 years who underwent a surgical resection and adjuvant chemotherapy as initial treatment. Patients were excluded if they had not had a surgery or chemotherapy, who initiated treatment > 365 days following surgery, and those who were treated with neoadjuvant chemotherapy or radiation therapy before surgery. We examined three levels of adjuvant chemotherapy delay (≤ 30, 31 to 60, and ≥ 61 days delay). These were divided into 3 groups: less than or equal 30 days (n = 173), 31 – 60 days (n = 353) and equal or over 61 days (n = 91). The time to adjuvant chemotherapy was defined by the days from the most definitive resection of the primary site to the first administration of chemotherapy. The definitive surgical procedure at the primary site included excision biopsy, lumpectomy, and mastectomy.

We obtained information on the age at diagnosis, type of surgery, tumor pathologic stage (according to the American Joint Committee on Cancer (AJCC)/ International Union Against Cancer (IUAC) Tumor Node and Metastasis (TNM) classification, lymphovascular invasion (LVI), tumor grade, histology, and comorbidities. We also obtained data on estrogen receptor (ER), progesterone receptor (PgR), and HER2 status. BC subtype was defined as hormone receptor-positive (ER-positive and/or PgR-positive and HER2-negative), HER2-positive (HER2-positive regardless of hormone receptor status), and TNBC (HER2-negative and hormone receptor-negative). We identified the chemotherapy received and classified it as anthracycline-based, anthracycline and taxane-based, or other type. In addition, for the HER2-positive tumors, we further categorized them as trastuzumab-treated and non-trastuzumab treated, since the use of adjuvant trastuzumab was approved in our country in 2005.

Patients were categorized according to TTC categories, and this variable was calculated from the date of

definitive surgery to the date of the first dose of adjuvant chemotherapy administration. Patients' TTC categories were 30 days or less, 31 to 60 days, 61 or more days. Descriptive statistics were used to evaluate the characteristics of patients according to TTC, and the distribution was compared using $\chi 2$ test. The outcome of interest was disease free survival (DFS). DFS was calculated from the time of surgery to the first relapse (local, regional, and/or distant), last follow-up or death in the absence of relapse. All the patients were followed-up for at least 6 months. The Kaplan-Meier product limit method was used to estimate the 5-year DFS with 95% confidence intervals (CIs) in all patients according to TTC and other patients' and clinical characteristics. Groups were compared by using the log-rank statistic.

Cox proportional hazards regression models were developed to determine association between TTC and survival outcomes after adjustment for potential confounders. Variables in the model included age (as a continuous variable), pathologic tumor size according to TNM classification (T1, T2, T3), pathological nodal status according to TNM classification (N1, N2, N3), histologic grade, histologic type of BC (ductal, lobular and other), presence of lymphovascular and perineural invasion, hormone receptor (ER- and PgR positive and negative), HER2 status (positive and negative), TNBC, type of surgery and presence of comorbidities. We classified the received chemotherapy as antracycline-based, antracycline/taxane-based, cyclophosphamide, methotrexate, fluorouracil (CMF) type and hormone therapy.

Results are expressed in hazard ratios and 95% CIs. P values ≤ 0.05 were considered statistically significant. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) 18.0.

The National Code on Clinical Trials has declared that ethics approval is not necessary for retrospective studies. Before this retrospective study our institutional board was informed that this study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

The present retrospective study investigated the association between the initiation time of adjuvant chemotherapy and DFS in 617 operable BC patients. The median age at diagnosis was 55 years, and 62.4% (385) of patients were older than 50. A total of 173 (28%) patients started chemotherapy within less than 31 days; 353 (57.2%) between 31 and 60 days; and 91 (14.7%) started chemotherapy 61 or more days after surgery. Median time to initiation of adjuvant treatment was 43 days (range, 14 to 92). At the median follow-up of 54.4 months, 113 patients (18.3%) experienced distant recurrence i.e. metastases, most commonly in the bones and hepatic metastases, and 7 patients (6.2%) had experienced recurrence of BC.

Table 1. Patient and clinical characteristics by interval from surgery to adjuvant chemotherapy among patients with stage I to III breast cancer

Tabela 1. *Karakteristike pacijenata i intervali započinjanja hemioterapije nakon završenog hirurškog lečenja za pacijente stadijuma I do III sa karcinomom dojke*

Characteristics *Karakteristike*	Interval from surgery to chemotherapy initiation (days) *Interval od hirurgije do početka hemioterapije (u danima)*			
	≤ 30 days ≤ *30 dana* (n = 173)	31-60 days *31-60 dana* (n = 353)	≥ 61 days ≥*61 dan* (n = 91)	p
Age, years/*Starost, godine*				
Median/*Medijana*	52	54	57	0.001
Range/*Raspon*	26-76	25-77	36-80	
Age ≥ 50 years/≥ *50 godina starosti*	105 (60.7%)	237 (67.1%)	67 (73.6%)	0.20
Comorbidity/*Komorbiditet*				
Absent/*Odsutni*	130 (75.1%)	247 (70.0%)	50 (54.9%)	0.003
Present/*Prisutni*	43 (24.9%)	106 (30.0%)	41 (45.1%)	
Pathologic tumor size according to TNM classifikation/*Veličina tumora prema TNM klasifikaciji*				
T1	69 (39.9%)	130 (36.8%)	26 (28.6%)	0.30
T2	68 (39.3%)	157 (44.5%)	48 (52.7%)	
T3	36 (20.8%)	66 (18.7%)	17 (18.7%)	
Vascular/lymphatic invasion/*Vaskularna/limfna invazija*				0.80
Absent/*Odsutna*	67 (38.7%)	145 (41.1%)	39 (42.9%)	
Present/*Prisutna*	106 (61,3%)	208 (58.9%	52 (57.1%)	
Perineural invasion/*Perineuralna invazija*				0.83
Absent/*Odsutna*	92 (53.2%)	192 (54.4%)	52 (57.1%)	
Present/*Prisutna*	81 (46.8%)	161 (45.6%)	39 (42.9%)	

Histological grade/*Histološki gradus*				0.50
GI	9 (5.2%)	30 (8.5%)	10 (11.0%)	
GII	67 (38.7%)	138 (39.1)	32 (35.2%)	
GIII	97 (56.1%)	185 (52.4%)	49 (53.8%)	
No. of involved lymph nodes/*Broj zahvaćenih limfnih čvorova*				0.31
0	73 (42.2%)	167 (47.3%)	38 (41.8%)	
1- 3	53 (30.6%)	93 (26.3%)	34 (37.4%)	
3- 9	27 (15.6%)	51 (14.4%)	14 (15.4%)	
≥ 10	20 (11.6%)	42 (11.9%)	5 (5.5%)	
Histological type of breast cancer/*Histološki tip karcinoma dojke*				0.48
Ductal/*Duktalni*	159 (91.9%)	315 (89.2%)	77 (84.6%)	
Lobular/*Lobularni*	5 (2.9%)	15 (4.2%)	6 (6.6%)	
Other/*Ostali tipovi*	9 (5.2%)	23 (6.5%)	8 (8.8%)	
Estrogen receptor/*Estrogenski receptor*				0.86
Positive/*Pozitivan*	115 (66.5%)	227 (64.3%)	58 (63.7%)	
Negative/*Negativan*	58 (33.5%)	126 (35.7%)	33 (36.3%)	
Progesterone receptor/*Progesteronski receptor*				0.81
Positive/*Pozitivan*	107 (61.8%)	208 (58.9%)	55 (60.4%)	
Negative/*Negativan*	66 (38.2%)	145 (41.1%)	36 (39.6%)	
Breast cancer subtype/*Podtip karcinoma dojke*				
HER2-positive/*HER2 pozitivan*	29 (16.8%)	49 (13.9%)	16 (17.6%)	0.55
Triple-negative/*Trostruko negativan*	27 (15.6%)	72 (20.4%)	12 (13.2%)	0.18
Hormone receptor-positive/*Hormonski receptor pozitivan*	99 (57.2%)	195 (55.2%)	49 (53.8%)	0.85
Surgery/*Vrsta operacije*				0.92
BSC/*Poštedna operacija*	127 (73.4%)	269 (76.2%)	68 (74.7%)	
Mastectomy/*Mastektomija*	46 (26.6%)	84 (23.8%)	23 (25.3%)	
Chemotherapy/*Hemioterapija*				0.08
CMF-type/*CMF protokol*	9 (5.2%)	22 (6.2%)	9 (9.9%)	
Anthracycline-based/A*ntraciklinski protokol*	58 (33.5%)	147 (41.6%)	34 (37.4%)	
Anthracycline+Taxane/*Antraciklini+Taksani*	99 (57.2%)	166 (47.0%)	39 (42.9%)	
Hormone therapy/*Hormonska terapija*	7 (4.0%)	18 (5.1%)	9 (9.9%)	
Trastuzumab among HER2-positive patients (n= 231) *Trastuzumab kod HER2-pozitivnih pacijentkinja*				
No/*Ne*	28 (49.1%)	67 (57.7%)	26 (61.9%)	0.09
Yes/*Da*	29 (50.9%)	49 (42.3%)	16 (38.1%)	

Legend: BSC – breast conservative surgery; CMF – cyclophosphamide, methotrexate, 5-fluorouracile; P[a] value for different distribution in 3 groups tested by heterogeneous x^2
Legenda: BSC – poštedna operacija dojke, CMF – ciklofosfamid, metotreksat, 5-fluorouracil; P[a] vrednost za različitu distribuciju u okviru 3 grupe je testirana x^2 testom; HER2 – receptor 2 humanog epidermalnog faktora rasta; TNM – Tumor Nodus Metastaze

Table 2. Survival estimate for DFS according to patient characteristics among patients with stage I to III BC treated with adjuvant chemotherapy
Tabela 2. *Preživljavanje pacijenata i period bez bolesti u odnosu na karakteristike pacijenata obolelih od karcinoma dojke stadijuma I do III*

	Interval from surgery to chemotherapy initiation (days) *Interval od operacije do početka hemioterapije (u danima)*						p
	≤ 30 days/≤ *30 dana* (n = 173)		31-60 days/*31-60 dana* (n = 353)		≥ 61 days/≥ *61 dan* (n = 91)		
	No. of patients *Broj pacijenata*	5-year DFS (%) *5-godišnji DFS*	No. of patients *Broj pacijenata*	5-year DFS (%) *5-godišnji DFS*	No. of patients *Broj pacijenata*	5-year DFS (%) *5-godišnji DFS*	log-rank
Age ≥ 50 years/ ≥ *50 godina starosti*	94	76.6%	226	83.2%	65	80.0%	0.375
Age < 50 years/< *50 godina starosti*	79	87.3%	127	77.2%	26	96.2%	0.035

Comorbidity absent/*Odsutni komorbiditeti*	130	85.4%	247	81.0%	50	90.0%	0.252
Comorbidity present/*Prisutni komorbiditeti*	43	69.8%	106	81.1%	41	78.0%	0.266
Pathologic tumor size according to TNM classification/*Veličina tumora prema TNM klasifikaciji*							
T1	69	85.5%	130	90.8%	26	88.6%	0.472
T2	68	83.8%	157	75.8%	48	81.3%	0.359
T3	36	69.4%	66	74.2%	17	88.2%	0.361
Vascular/lymphatic invasion absent *Odsutna vaskularna/limfna invazija*	67	82.1%	145	86.2%	39	92.3%	0.315
Vascular/lymphatic invasion present *Prisutna vaskularna/limfna invazija*	106	81.1%	208	77.4%	52	78.8%	0.778
Perineural invasion absent *Odsutna perineuralna invazija*	92	85.9%	192	87.5%	52	86.5%	0.907
Perineural invasion present *Prisutna perineuralna invazija*	81	76.5%	161	73.3%	39	82.1%	0.497
Histological grade/*Histološki gradus*							
GI	9	100 %	30	93.3%	10	100%	0.545
GII	67	79.1%	138	83.3%	32	90.6%	0.374
GIII	97	81.4%	185	77.3%	49	77.6%	0.753
No. of involved lymph nodes/*Broj zahvaćenih limfnih čvorova*							
0	73	89.0%	167	89.2%	38	86.8%	0.879
1-3	53	92.5%	93	83.9%	34	85.3%	0.339
4-9	27	59.3%	51	72.2%	14	78.6%	0.395
≥10	20	55.0%	42	52.4%	5	80.6%	0.580
Histological type of breast cancer/*Histološki tip karcinoma dojke*							
Ductal/*Duktalni*	159	80.5%	315	81.3%	77	85.7%	0.601
Lobular/*Lobularni*	5	100%	15	86.7%	6	66.7%	0.359
Other/*Ostali tipovi*	9	88.9%	23	73.9%	8	87.5%	0.554
ER negative/*ER negativan*	58	70.7%	126	77.0%	33	81.8%	0.437
ER positive/*ER pozitivan*	115	87.0%	227	83.3%	58	86.2%	0.687
PgR negative/*PgR negativan*	66	72.7%	145	76.6%	36	83.3%	0.476
PgR positive/*PgR pozitivan*	107	86.9%	208	84.1%	55	85.5%	0.860
HER2-positive/*HER2 pozitivan*	28	75.0%	42	85.7%	14	57.7%	0.070
HER2-negative/*HER2 negativan*	145	82.8%	311	80.4%	8	89.6%	0.179
Triple-negative/*Trostruko negativan*	27	74.1%	72	73.8%	12	100 %	0.157
Hormone receptor-positive *Hormon receptor pozitivan*	99	87.9%	195	83.6%	49	83.7%	0.658
Hormone receptor-negative *Hormon receptor negativan*	50	70.0%	113	75.2%	27	77.8%	0.702
Surgery/*Operacija*							
BSC/*Poštedna*	127	85.6%	269	83.6%	68	88.6%	0.908
Mastectomy/*Mastektomija*	46	71.7%	84	73.8%	23	87.0%	0.384
Chemotherapy/*Hemioterapija*							
CMF-type/*CMF protokol*	9	44.4%	22	90.9%	9	77.8%	0.020
Anthracycline-based/*Antraciklinski protokol*	58	84.5%	147	79.6%	34	91.2%	0.253
Anthracycline+Taxane *Antraciklini+Taksani*	99	81.9%	166	80.1%	39	79.5%	0.963
Hormone therapy/*Hormonska terapija*	7	100%	18	88.9%	9	88.9%	0.767
Trastuzumab among HER2-positive patients/*Trastuzumab kod HER2 pozitivnih pacijentkinja*	28	75.0%	42	85.7%	14	57.1%	0.070

Legenda: DFS - period bez bolesti; ER - receptori za estrogen, PgR - receptori za progesteron, HER2 – receptor 2 humanog epidermalnog faktora rasta; CMF - ciklofosfamaid, metotreksat, 5-fluorouracil, TNM – Tumor Nodus Metastaze

Table 1 lists the patients' characteristics according to timing of adjuvant chemotherapy. Significant differences among groups were found regarding the age (p = 0.001) and comorbidity (p = 0.003). However, women with delayed chemotherapy were likely to to be older and have associated diseases. Globally, 69.2% of patients presented with hormone receptor positive tumors, versus 30.8% of patients that pre-

Table 3. Univariate analysis hazard ratio: DFS according to timing of adjuvant chemotherapy
Tabela 3. *Hazard racio univarijantna analiza: periodi bez bolesti u odnosnu na započinjanje adjuvantne terapije*

Variable *Varijable*		B	HR	CI	p
Age/*Starost*	Continuous/*Kontinuirana*	-0.002	0.998	0.980-1.015	0.784
Comorbidity/*Komorbiditeti*	absent vs. present/*Prisutan vs odsutan*	0.363	1.438	0.978-2.114	0.065
Pathologic tumor size according to TNM calssification/*Tumorska veličina prema TNM klasifikaciji*					
T1	Reference/*Referentna vrednost*				
T2		0.737	2.091	1.306-3.347	0.002
T3		0.930	2.535	1.490-4.313	0.001
Vascular/lymphatic invasion *Vaskularna/limfna invazija*	Absent vs. present/*Prisutan vs odsutan*	0.472	1.603	1.075-2.389	0.021
Perineural invasion *Perineuralna invazija*	Absent vs. present/*Prisutan vs odsutan*	0.684	1.981	1.357-2.892	0.001
Histological grade/*Histološki gradus*					
GI	Reference/*Referentna vrednost*				
GII		1.473	4.364	1.054-18.072	0.042
GIII		1744	5.722	1.402-23.350	0.015
No. of involved lymph nodes/*Broj zahvaćenih limfnih čvorova*					
0	Reference/*Referentna vrednost*				
1-3		0.214	1.239	0.726-2.114	0.432
4-9		1.140	3.127	1.874-5.219	0.001
≥10		1.597	4.940	2.985-8.175	0.001
Histological type of breast cancer/*Histološki tip karcinoma dojke*					
Ductal/*Duktalni*		-0.083	0.921	0.448-1.893	0.822
Lobular/*Lobularni*		-0.261	0.770	0.232-2.559	0.670
Other/*Ostali tipovi*	Reference/*Referentna vrednost*				
Estrogen receptor/*Estrogenski receptor*	Positive vs. negative/*Pozitivan vs negativan*	0.492	1.635	1.129-2.368	0.009
Progesteron receptor *Progesteronski receptor*	Positive vs. negative/*Pozitivan vs negativan*	0.504	1.656	1.145-2.396	0.007
HER2 receptor/*HER2 receptor*	Negative vs. positive/*Pozitivan vs negativan*	0.244	1.276	0.794-2-051	0.314
Triple-negative/*Trostruko-negativni*	Positive vs. negative/*Pozitivan vs negativan*	0.595	1.813	1.226-2.681	0.003
Surgery modality/*Tip operacije*	BCS vs. Mastectomy/*Poštedna vs mastektomija*	-0.510	0.600	0.406-0.887	0.010
Scheme of chemotherapy used/*Hemioterapijski protokol*					
CMF-type/*CMF protokol*		0.950	2.586	0.699-9.560	0.154
Anthracycline-based *Antraciklinski protokol*		0.696	2.006	0.621-6.480	0.245
Anthracycline+Taxane *Antraciklini+ Taksani*		0.797	2.220	0.694-7.098	0.179
Hormone therapy/*Hormonska terapija*	Reference/*Referentna vrednost*				
Hormone receptor/*Hormonski receptor*	Positive vs. negative/*Pozitivan vs negativan*	0.599	1.820	1.254-2.642	0.002
TTC, days/*Vreme do započinjanja hemioterapije*					
≤ 30	Reference/*Referentna vrednost*				
31-60		0.008	1.008	0.662-1.537	0.969
≥ 61		-0.221	0.802	0.428-1502	0.490

Legenda: *HR – odnos rizika, CI - indeks poverenja,* BCS – breast conservative surgery; *TNM - Tumor Nodus Metastaze*; *HER2 – receptor 2 humanog epidermalnog faktora rasta*; *CMF - ciklofosfamaid, metotreksat, 5-fluorouracil, B- beta*

sented with hormone receptor negative tumors. However, 15.2% of patients had HER-2 positive and 23.2% TNBC. A total number of 239 (38.7%) patients received adjuvant anthracycline-based chemotherapy; 304 (49.3%) received anthracycline and taxane based chemotherapy; 40 (6.5%) received CMF-type chemotherapy; 94 (15.2%) received trastuzumab, and 343 (69%) patients received adjuvant hormone therapy.

Table 2 summarizes the 5-year DFS for all the investigated patients according to TTC, patients' and tumor characteristics. Median follow-up was 54.4 months. Survival analysis, using the Kaplan-Meier method for DFS according to TTC, demonstrated that there were no differences in DFS among the groups that received adjuvant treatment at different timings **(Graph 1)**. The 5-year DFS estimate was 81.5%, 81.0%, 84.6% (log-rank p = 0.728) among patients who initiated chemotherapy < 30 days, 31–60, and > 61 days, respectively, after surgery.

The Cox proportional hazards model was used to adjust the analysis for known prognostic factors such as age, comorbidity, pathologic tumor size, number of positive lymph nodes, vascular and perineural invasion, hormonal receptors status, tumor grade, HER-2 status, histological type of BC, surgery modality and chemotherapy regimen. In the univariate

Table 4. Multivariate analysis of prognostic factors for disease-free survival (DFS)
Tabela 4. *Prognostički faktori koji utiču na period bez bolesti u multivarijantnoj analizi*

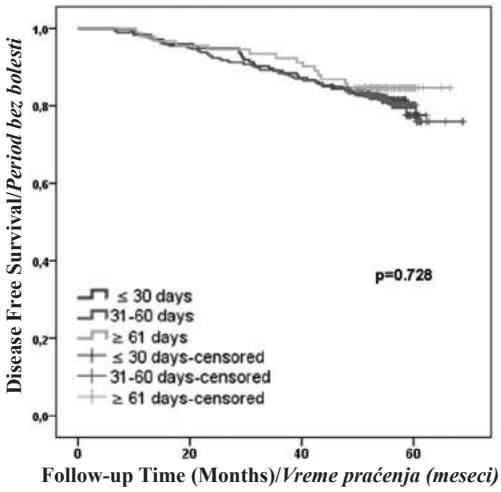
Variable/*Varijabla*		B	HR	CI 95%	p
Pathologic tumor size according to TNM classification/*Tumorska veličina prema TNM klasifikaciji*					
T1	Reference/*Referentna vrednost*				
T2		0.412	1.509	0.925-2.462	0.099
T3		0.618	1.856	1.070-3.549	0.050
Vascular/lymphatic invasion *Vaskularna/limfna invazija*	Absent vs. present/*odsutan vs prisutan*	0.151	1.163	0.730-1.853	0.525
Perineural invasion *Perineuralna invazija*	Absent vs. present/*odsutan vs prisutan*	0.285	1.330	0.854-2.072	0.207
Histological grade/*Histološki gradus*					
GI	Reference/*Referentna vrednost*				
GII		1.138	3.120	0.742-13.128	0.121
GIII		1.185	3.272	0.782-13.690	0.105
No. of involved lymph nodes/*Broj zahvaćenih limfnih čvorova*					
0	Reference/*Referentna vrednost*				
1-3		0.126	1.135	0.648-1.985	0.658
4-9		0.926	2.525	1.460-4.365	0.001
≥10		1.381	3.981	2.320-6.830	0.0001
Estrogen receptor *Estrogenski receptor*	Positive vs. negative/*pozitivan vs negativan*	-0.077	0.926	0.280-3.008	0.898
Progesteron receptor *Progesteronski receptor*	Positive vs. negative/*pozitivan vs negativan*	0.181	1.198	0.587-2.443	0.620
Triple-negative/*Trostruko-negativan*	Positive vs. negative/*pozitivan vs negativan*	0.408	1.503	0.756-2.988	0.245
Surgery modality/*Tip operacije*	BSC vs. Mastectomy/*Poštedna vs mastektomija*	-0.142	0.868	0.537-1.402	0.562
Hormone receptor *Hormonski receptori*	Positive vs. negative/*pozitivan vs negativan*	0.228	1.256	0.286-5.505	0.763

Legenda: TNM - *Tumor Nodus Metastaze,* CI - *interval poverenja,* HR - *odnos rizika,* B - *beta*

Table 5. Survival estimate for DFS according to breast cancer subtype among patients with stage I to III BC treated with adjuvant chemotherapy
Tabela 5. *Procena preživljavanja i period bez bolesti u odnosu na podtip karcinoma dojke za pacijente stadijuma I do III*

Breast cancer subtype *Podtip karcinoma dojke*	No. of patients *Broj pacijenata*	No. of events *Broj događaja*	5-Year DFS *5-godišnji DFS*	p
Luminal-A/*Luminal-A*	380	54	85.8%	0.001
Luminal-B/*Luminal-B*	46	10	78.3%	0.534
HER2-positive/*HER2 pozitivan*	48	11	77.1%	0.448
Triple-negative/*Trostruko negativan*	143	38	73.4%	0.003

Legenda: BSC – breast conservative surgery, *HER2 – receptor 2 humanog epidermalnog faktora rasta; DFS - period bez bolesti*



Graph 1. Kaplan-Meier plot for disease-free survival according to interval between surgery and initiation of adjuvant chemotherapy

Grafikon 1. *Kaplan Majerova kriva za period bez bolesti u odnosu na interval koji prođe od definitivne hirurgije do početka adjuvantne terapije*

analysis, DFS was significantly higher in patients with T1 tumor, without vascular and perineural invasion, histological grade 2, up to three lymph nodes involved, estrogen and progesterone positive tumor, and breast conservative surgery **(Table 3)**.

Multivariate analysis of independent prognostic factors for DFS identified nodal status and tumor size **(Table 4)**.

Subsequently, we investigated 5-year DFS for all the patients according to the BC subtype. The 5-year DFS estimate was 85.8% (p = 0.001) in patients with luminal-A subtype (ER positive, HER2 negative, Ki-67 < 20%, Progesterone high) at a median follow-up of 62.7 months; in patients with luminal-B subtype (Luminal B (HER2 negative) ER positive, HER negative and either Ki-67 > 20% or Progesterone low, Luminal B (HER2 positive) ER positive, HER2 positive, any Ki-67, any Progesterone) 78.3% (p = 0.534) at a median follow-up of 55.9 months; in patients with TNBC 73.4% at a median follow-up of 58,1 months, and in patients with HER2+ 77.1% (p = 0.448) at a median follow-up of 55.5 months **(Table 5)**.

Discussion

Adjuvant chemotherapy is one of the most important therapies for BC patients. The optimal time to initiate chemotherapy after surgery is still unknown. Due to the potential ethical problems it is unlikely that a prospective clinical trial will be undertaken to explore the association between delayed chemotherapy initiation and survival in BC patients. The lack of change in the attitude towards timing of adjuvant chemotherapy might be related not only to the reported controversial results, but also to the increased requests for additional testing or procedures used to decide whether or not to offer adjuvant chemotherapy [19].

All the published findings related to the treatment outcome associated with the timing of adjuvant chemotherapy initiation are retrospective. The categorization of the time of initiation as proper, early or delayed has no clinical basis, and it is based on the prejudices related to habits in the clinical practice. In clinical trials, a routine criterion for the adjuvant chemotherapy of BC is the initiation of adjuvant therapy within 6 to 8 weeks after surgical treatment, and the initiation out of this timeframe seems to be unusual and potentially harmful. The published randomized controlled clinical trials do not directly suggest the time to initiate chemotherapy after surgery. The time to therapy initiation ranges from 2 to 12 weeks in different trials [20–23]. In clinical practice, many factors may affect the time interval between surgery and adjuvant chemotherapy. Some of the frequently involved factors are related to patients' clinical condition and comorbidities. Delays in treatment initiation are more likely to occur in Medicare patients and in low-income populations [24]. In a large, multi-institutional cohort of women with BC, time from diagnosis to initiation of adjuvant chemotherapy was approximately 12 weeks. This interval increased steadily from 10.8 to 13.3 weeks between 2003 and 2009. The greatest effects were associated with diagnostic and therapeutic interventions, including immediate post-mastectomy reconstruction, re-excision, and use of the 21-gene reverse transcriptase polymerase chain reaction (RT-PCR) assay [25].

Our study showed that 85% of BC patients started adjuvant chemotherapy within 3 months of definitive surgery. Within these 3 months, we found no association between the initiation of chemotherapy and DFS, meaning that the prognosis was similar for patients starting chemotherapy within 3 weeks after surgery and those starting chemotherapy up to 13 weeks after surgery. This is in agreement with results of other studies [26, 27]. A large population-based study did not demonstrate any benefit in overall survival (OS) from an early start of adjuvant chemotherapy among Danish BC patients treated within 3 months of definitive surgery, or for any subgroups with potentially fast growing tumors according to increasing number of involved axillary lymph nodes, increasing malignancy grade or negative hormone receptor status [3]. If chemotherapy is delayed for more than 5 months, then the concept of being adjuvant no longer holds.

Adjuvant chemotherapy decreases the risk of BC mortality through eradication of micro-metastatic tumor deposits. Some clinical studies suggest that an adjuvant chemotherapy delay for up to 12 weeks will significantly reduce the effectiveness of systemic therapy. Results of meta-analyses show that OS decreases by 13% and DFS by 14% every four weeks that adjuvant chemotherapy is delayed [28].

In regard to BC subtype, Gagliato et al. [11] and Chavez-MacGregor et al. [5] categorized patients in to hormone receptor-positive, receptor tyrosine-protein kinase erbB-2 (ERBB2) positive, and TNBC subgroups. Results showed that a WT (whole time) of 31–60 days had no significant impact on patients

with ERBB2+ tumors or hormone receptor-positive tumors, while with TNBC, a WT 31–60 days resulted in a 26% increased risk of death. We did not find a statistically significant adverse effect on DFS among patients with ERBB2+ tumors or hormone receptor-positive tumors who had a WT longer than 60 days. In our study, all patients who were ERBB+ received trastuzumab. The HER-2 overexpression or amplification in BC is associated with worse prognosis in untreated patients and may also be associated with poor prognosis [29–31]. Also, our patients who were TNBC had statistically significantly shorter DFS. TNBC is known to have a more aggressive behavior when compared with other BC subtypes [32]. Gagliato et al. have shown that the TNBC subgroup experienced a detrimental effect in delaying initiation of adjuvant chemotherapy in terms of OS, with a 75% and 54% increased risk of death for those women who received chemotherapy 31 to 60 days and ≥ 61 days after definitive surgery [11]. Despite the differences in OS, no differences in relapse free survival (RFS) or DFS were seen in TNBC patients [11]. It is important to mention that there is a lack of targeted therapies for this population and that chemotherapy is the only effective known treatment.

As expected, in a multivariate analysis, independent predictive factors for shorter DFS were more than 3 lymph nodes involvement and tumor size T3. In our study, there was no difference in DFS between hormone receptor positive and negative BC patients. Early initiation of chemotherapy had an impact on hormone receptor-negative patients in comparison to hormone receptor-positive patients [27]. Many trials have demonstrated that the magnitude of benefit of adjuvant chemotherapy is less pronounced among hormone receptor-positive patients [33]. Tamoxifen and aromatase inhibitors are important and effective agents in the treatment of BC and their use in adjuvant treatment reduces the risk of death and recurrence [34–36].

There is also a possibility that the detrimental effect associated with delayed TTC among hormone receptor-positive patients is related to a delay in the initiation of endocrine therapy [3].

The main limitation of this study is that it is not randomized. Despite our median follow-up of 54.4 months, it is possible that longer follow-up is needed, particularly to evaluate the effect of delay in adjuvant chemotherapy initiation among patients with hormone receptor-positive BC. Two thirds of the patients had hormone receptor–positive disease, and there was no indication that TTC for these patients made any difference at all. Endocrine manipulation may act a more positive role than earlier initiation of chemotherapy in ER positive patients.

A larger number of studies, especially those with subgroup analyses are needed. Smaller subpopulations of patients were identified in whom delays (beyond 60 days) to chemotherapy initiation might have been avoided. The magnitude of the benefit of adjuvant chemotherapy might indeed be greater in locally advanced BC, with higher probability of micro-metastatic disease, and initiation within 60 days might be an appropriate guideline for these patients. The impact also varies in regard to subtypes and an early initiation of adjuvant chemotherapy is particularly important for patients with luminal-B, TNBC and HER2+ tumors.

The recognition of the heterogeneity of BC has recently led to the concept that investigations of tailored adjuvant treatment in specific subpopulations, through international collaboration, are the key to improve the outcome in patients with early BC.

Conclusion

In conclusion, our retrospective study did not show that the timing of adjuvant chemotherapy initiation affected the outcome in patients with early breast cancer. The current report suggests that unnecessary delay in initiation of chemotherapy may be unwise in patients in whom the effect of adjuvant chemotherapy is expected to be significant. Early initiation of adjuvant chemotherapy is particularly relevant in patients with advanced-stage breast cancer at diagnosis, those with triple-negative breast cancer, and patients with trastuzumab-treated human epidermal growth factor receptor 2-positive tumors. Since the realization of a prospective trial that should definitely provide reliable data seems to be unlikely, only retrospective trials with sufficient statistical power and valuable data, to detect differences among groups, can help to explain this interesting issue.

References

1. Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012;379(9814):432-44.

2. Shannon C, Ashley S, Smith IE. Does timing of adjuvant chemotherapy for early breast cancer influence survival? J Clin Oncol. 2003;21(20):3792-7.

3. Cold S, Düring M, Ewertz M, Knoop A, Møller S. Does timing of adjuvant chemotherapy influence the prognosis after early breast cancer? Results of the Danish Breast Cancer Cooperative Group (DBCG). Br J Cancer. 2005;93(6):627-32.

4. Lohrisch C, Paltiel C, Gelmon K, Speers C, Taylor S, Barnett J, et al. Impact on survival of time from definitive surgery to initiation of adjuvant chemotherapy for early-breast cancer. J Clin Oncol. 2006;24(30):4888-94.

5. Chavez-MacGregor M, Clarke CA, Lichtensztajn DY, Giordano SH. Delayed initiation of adjuvant chemotherapy among patients with breast cancer. JAMA Oncol. 2016;2(3):322-9.

6. Fisher B, Gunduz N, Coyle J, Rudock C, Saffer E. Presence of a growth-stimulating factor in serum following primary tumor removal in mice. Cancer Res.1989;49(8):1996-2001.

7. Folkman J. Endothelial cells and angiogenic growth factors in cancer growth and metastasis. Introduction. Cancer Metastasis Rev. 1990;9(3):171-4.

8. Goldie JH, Coldman AJ. A mathematic model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep. 1979;63(11-12):1727-33.

9. Gunduz N, Fisher B, Saffer EA. Effect of surgical removal on the growth and kinetics of residual tumor. Cancer Res.1979;39(10):3861-5.

10. Simpson JF, Page DL. Status of breast cancer prognostication based on histopathologic data. Am J ClinPathol. 1994;102(4 Suppl 1):S3-8.

11. Gagliato Dde M, Gonzalez-Angulo AM, Lei X, Theriault RL, Giordano SH, Valero V, et al. Clinical impact of delaying initiation of adjuvant chemotherapy in patients with breast cancer. J Clin Oncol. 2014;32(8):735-44.

12. Yamamoto Y, Iwase H. Clinicopathological features and treatment strategy for triple-negative breast cancer. Int J Clin Oncol. 2010;15(4):341-51.

13. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363(20):1938-48.

14. Pritchard KI, Shepherd LE, O'Malley FP, Andrulis IL, Tu D, Bramwell VH, et al. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med. 2006;354 (20):2103-11.

15. Alkis N, Durnali AG, Arslan UY, Kocer M, Onder FO, Tokluoglu S, et al. Optimal timing of adjuvant treatment in patients with early breast cancer. Med Oncol. 2011;28(4):1255-9.

16. Jara Sánchez C, Ruiz A, Martín M, Antón A, Munárriz B, Plazaola A, et al. Influence of timing of initiation of adjuvant chemotherapy over survival in breast cancer: a negative outcome study by the Spanish Breast Cancer Res Group (GEICAM). Breast Cancer Res Treat. 2007;101(2):215-23.

17. Nurgalieva ZZ, Franzini L, Morgan RO, Vernon SW, Liu CC, Du XL. Impact of timing of adjuvant chemotherapy initiation and completion after surgery on racial disparities in survival among women with breast cancer. Med Oncol. 2013;30(1):419.

18. Fedewa SA, Ward EM, Stewart AK, Edge SB. Delays in adjuvant chemotherapy treatment among patients with breast cancer are more likely in African American and Hispanic populations: a national cohort study 2004-2006. J Clin Oncol. 2010;28(27):4135-41.

19. Colleoni M, Gelber RD. Time to initiation of adjuvant chemotherapy for early breast cancer and outcome: the earlier, the better? J Clin Oncol. 2014;32(8):717-9.

20. Bonadonna G, Valagussa P, Moliterni A, Zambetti M, Brambilla C. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: the results of 20 years of follow-up. N Engl J Med. 1995;332(14):901-6.

21. Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S, et al. Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol. 2003;21(6):976-83.

22. Levine MN, Bramwell VH, Pritchard KI, Norris BD, Shepherd LE, Abu-Zahra H, et al. Randomized trial of intensive cyclophosphamide, epirubicin, and fluorouracil chemotherapy compared with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer. national cancer institute of canada clinical trials group. J Clin Oncol. 1998;16(8):2651-8.

23. French Adjuvant Study Group. Benefit of a high-dose epirubicin regimen in adjuvant chemotherapy for node-positive breast cancer patients with poor prognostic factors: 5-year follow-up results of French Adjuvant Study group 05 randomized trial. J Clin Oncol. 2001;19(3):602-11.

24. Bleicher RJ, Ruth K, Sigurdson ER, Ross E, Wong YN, Patel SA, et al. Preoperative delays in the US Medicare population with breast cancer. J Clin Oncol. 2012;30(36):4485-92.

25. Vandergrift JL, Niland JC, Theriault RL, Edge SB, Wong YN, Loftus LS, et al. Time to adjuvant chemotherapy for breast cancer in National Comprehensive Cancer Network institutions. J Natl Cancer Inst. 2013;105(2):104-12.

26. Buzdar AU, Smith TL, Powell KC, Blumenschein GR, Gehan EA. Effect of timing of initiation of adjuvant chemotherapy on disease-free survival in breast cancer. Breast Cancer Res Treat. 1982;2(2):163-9.

27. Colleoni M, Bonetti M, Coates AS, Castiglione-Gertsch M, Gelber RD, Price K, et al. Early start of adjuvant chemotherapy may improve treatment outcome for premenopausal breast cancer patients with tumors not expressing estrogen receptors. The International Breast Cancer Study Group. J Clin Oncol. 2000;18(3):584-90.

28. Zhan QH, Fu JQ, Fu FM, Zhang J, Wang C. Survival and time to initiation of adjuvant chemotherapy among breast cancer patients: a systematic review and meta-analysis. Oncotarget. 2018;9(2):2739-51.

29. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987;235(4785):177-82.

30. Paik S, Hazan R, Fisher ER, Sass RE, Fisher B, Redmond C, et al. Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in primary breast cancer. J Clin Oncol. 1990;8(1):103-12.

31. Gilcrease MZ, Woodward WA, Nicolas MM, Corley LJ, Fuller GN, Esteva FJ, et al. Even low-level HER2 expression may be associated with worse outcome in node-positive breast cancer. Am J Surg Pathol. 2009;33(5):759-67.

32. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011;365(14):1273-83.

33. Joensuu H, Kellokumpu-Lehtinen PL, Bono P, Alanko T, Kataja V, Asola R, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. N Engl J Med. 2006;354(8):809-20.

34. Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. Lancet. 2002;359(9324):2131-9.

35. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med. 2003;349(19):1793-802.

36. Coombes RC, Hall E, Gibson LJ, Paridaens R, Jassem J, Delozier T, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. N Engl J Med. 2004;350(11):1081-92.

Rad je primljen 5. IX 2018.
Recenziran 1. X 2018.
Prihvaćen za štampu 15. X 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:394-403.

CASE REPORTS
PRIKAZI SLUČAJEVA

University of East Sarajevo, Faculty of Medicine Foča, Bosnia and Herzegovina
Department of Pathology and Cytodynamics, Foča[1]
University Hospital Foča, Center of Laboratory Diagnosis[2]
University of Banja Luka, Faculty of Medicine, Banja Luka, Bosnia and Herzegovina
University Clinical Center Banja Luka, Department of Pathology[3]
Mother and Child Health Care Institute "Dr Vukan Čupić", Belgrade[4]
University of East Sarajevo, Faculty of Medicine Foča, Foča, Bosnia and Herzegovina
Department of Surgery[5]
University Hospital Foča, Foča, Bosnia and Herzegovina[6]

Case report
Prikaz slučaja
UDK 616.346.2-006.6-07/-08
https://doi.org/10.2298/MPNS1812405C

ADENOCARCINOMA OF THE APPENDIX MIMICKING COMPLICATED APPENDICITIS IN THE ELDERLY – A REPORT OF TWO CASES

ADENOKARCINOM APENDIKSA KOJI IMITIRA KOMPLIKOVANI APENDICITIS KOD STARIJIH PACIJENATA – PRIKAZ DVA SLUČAJA

Mirjana ĆUK[1,2], Radoslav GAJANIN[3], Slaviša ĐURIČIĆ[3,4], Veljko MARIĆ[5], Radmil MARIĆ[5] and Miodrag KOVAČEVIĆ[6]

Summary

Introduction. Primary appendiceal adenocarcinoma is a very rare malignancy which accounts for 0.1% of all appendectomy specimens. In both patients presented in this paper, appendectomy was performed due to suspected acute complicated appendicitis. **Case Reports.** The first patient, a 77-year-old man, presented with a low grade colonic-type pT3 adenocarcinoma of the appendix, diagnosed by histopathological examination of the resected appendix delivered in a fixative. A month after appendectomy, the patient underwent right hemicolectomy of a tumor at the edge of the resection. Due to a cardiovascular disease, adjuvant chemotherapy was not indicated. The second patient, a 74-year-old female, presented with a low grade mucinous adenocarcinoma of the appendix with subserous infiltration, diagnosed by histopathological analysis of the resected appendix. Eight months after appendectomy, the patient developed a recurrent tumor in the cecal area. After radical surgical excision of the recurrent tumor, the patient received adjuvant chemotherapy. Both patients had a 5-year survival without relapse. **Conclusion.** Preoperative diagnosis of appendiceal adenocarcinoma is a challenge due to overlapping symptoms of complicated acute appendicitis. Our results suggest that in elderly patients with symptoms of complicated acute appendicitis, appendectomy should be done with intraoperative histopathological frozen section consultation. In advanced stages of adenocarcinoma, right hemicolectomy is a better choice than appendectomy.

Key words: Adenocarcinoma; Appendiceal Neoplasms; Appendectomy; Diagnosis; Acute Disease; Appendicitis; Morphological and Microscopic Findings

Sažetak

Uvod. Primarni adenokarcinom apendiksa je veoma redak maligni tumor sa učestalošću 0,1% u ukupnom broju hirurški odstranjenih apendiksa. Kod oba pacijenta prikazana u našem radu apendektomija je urađena zbog sumnje na akutni komplikovani apendicitis. **Prikaz dva slučaja.** Prvi pacijent je muškarac starosti 77 godina sa adenokarcinomom apendiksa, kolonični tip, niskog gradusa, u stadijumu pT3, dijagnostikovan patohistološkim pregledom reseciranog apendiksa dostavljenog u fiksativu. Tumor je bio prisutan na rubu resekcije. Desna hemikolektomija je urađena mesec dana nakon apendektomije. Zbog bolesti kardiovaskularnog sistema, pacijent nije lečen adjuvantnom hemioterapijom. Drugi pacijent je 74-godišnja žena kod koje je patohistološkom analizom reseciranog apendiksa postavljena dijagnoza mucinoznog adenokarcinoma niskog gradusa s infiltracijom supseroze. Prvobitno je urađena samo apendektomija. Osam meseci nakon prve operacije pacijentkinja je imala rekurentni tumor u predelu cekuma. Nakon radikalne hirurške operacije rekurentnog tumora, pacijentkinji je ordinirana adjuvantna terapija. Oba pacijenta su preživela pet godina bez relapsa bolesti. **Zaključak.** Preoperativna dijagnoza adenokarcinoma apendiksa je izazov zbog preklapanja simptoma sa komplikovanim akutnim apendicitisom. Naši rezultati ukazuju na to da apendektomiju kod starijih pacijenata sa slikom komplikovanog akutnog apendicitisa treba raditi sa intraoperativnom patohistološkom konsultacijom na zaleđenim rezovima. Desna hemikolektomija je bolja metoda od apendektomije kod pacijenata sa adenokarcinomom apendiksa u uznapredovalom stadijumu tumorske bolesti.

Ključne reči: adenokarcinom; neoplazme apendiksa; apendektomija; dijagnoza; akutna bolest; apendicitis; morfološki i mikroskopski nalazi

Corresponding Author: Dr Mirjana Ćuk, Univerzitet u Istočnom Sarajevu, Medicinski fakultet u Foči, Univerzitetska bolnica u Foči, Foča, Studentska 5, Bosna i Hercegovina E-mail: akicuk@yahoo.com, mirjana.cuk@ues.rs.ba

Abbreviations
AA – appendiceal adenocarcinoma
WBC – white blood cell
LA – laparoscopic appendectomy

Introduction

Primary appendiceal adenocarcinoma (AA) is a rare malignancy with an incidence of 0,12 cases per 1,000,000 persons per year [1]. Primary neoplasms of the appendix are histologically diverse. They are broadly classified as colonic-type adenocarcinoma, mucinous adenocarcinoma, goblet cell adenocarcinoma, or neuroendocrine carcinoma [2]. Carcinoids are the most common tumors of the appendix (66%), followed by mucinous adenocarcinoma (20%), and colonic type adenocarcinoma (10%) [3]. Most primary tumors of the appendix occur in the elderly, between the fifth and seventh decades of life, with the exception of carcinoids, that are usually diagnosed before the age of 40 [3]. The clinical symptoms of the tumors of the appendix are nonspecific and resemble the symptoms of acute appendicitis, rarely to other gastrointestinal or urogenital diseases [4]. The five-year survival rate for AA is 49 - 60%, and the prognosis is favorable when the tumor is diagnosed at an early stage of the disease [4]. We present two cases of AA which were clinically mimicking complicated appendicitis and manifested with symptoms of acute abdomen.

Case 1

A 77-year-old male was admitted to our hospital with a right lower quadrant abdominal pain and peritoneal tenderness. The patient complained about melena, and constipation lasting a few days. The patient's medical history showed age-related cardiovascular disorders. Routine laboratory test results were within normal limits. Ultrasound examination showed a dilated and thickened appendix, hypoechoic with loss of normal mural stratification, and appendicular lump with free fluid in the peritoneal cavity. Laparotomy and appendectomy were performed. On gross section, the proximal part of the resected appendix was firm, dilated and ruptured with purulent exudate on the surface. Histologically, an invasive colonic type, low grade adenocarcinoma of the appendix **(Figure 1),** infiltrating up to subserosa was noted. There was no lymphatic or vascular invasion. The right hemicolectomy was done a month after appendectomy, and the histologic examination revealed that the rest of the tumor invaded the cecal wall up to the serosal surface. Metastases were not found in any of the 15 ileocecal lymph nodes. The postoperative course was uneventful. Adjuvant chemotherapy was not applied due to cardiovascular comorbidity. The patient was disease free 62 months after the surgery, which was determined by imaging techniques.

Case 2

A 74-year-old female was admitted to our hospital due to abdominal pain and peritoneal tenderness. The patient complained about constipation, bloating, and loss of appetite in the last few months. She was subfebrile (37.7° C) during the last month, especially in the evening. A complete blood count revealed only an elevated white blood cell (WBC) count: 10,5 x 10^9/l. Tumor markers were within the normal range. Ultrasound examination showed an appendicular lump with a cystic space and free fluid in the peritoneal cavity. A laparotomy with appendectomy was performed. On gross section, the resected appendix was dilated and ruptured. It was softened with mucous content on the surface and impacted mucus in the wall. The histopathological diagnosis was low grade mucinous adenocarcinoma of the appendix without lymphovascular invasion **(Figure 2)**. The postoperative course was uneventful and the patient did not respond to the calls for consideration of the Oncology Advisory Board. Eight months after appendectomy, the patient was readmitted due to abdominal pain, constipation, and poor appetite. She lost 10 kilos in the last seven months. Colonoscopy revealed a tumor in the cecal region and right hemicolectomy was done. The tumor was diagnosed as a recurrent cecal invasive mucinous adenocarcinoma staged as r T3 N0 Mx. The patient underwent adjuvant therapy with Capecitabine, 1,250 mg/m^2/day for 14 days every 21-days, for a total of 6 cycles. The patient was disease-free at follow up, 65 months after the second surgery.

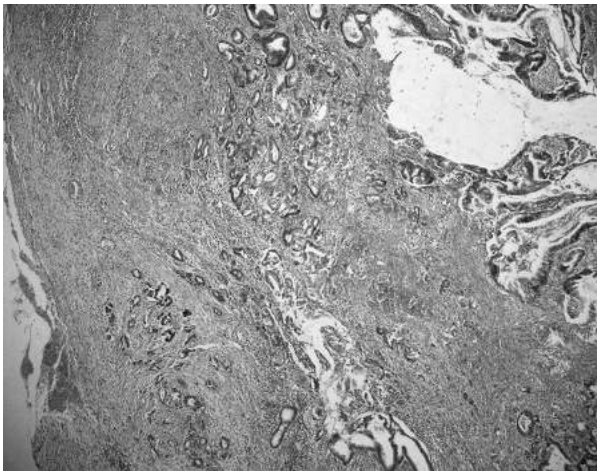


Figure 1. Adenocarcinoma of the appendix, colonic type, low grade, HE x 100
Slika 1. *Adenokarcinom apendiksa, kolonični tip, niskog gradusa, HE x 100*

Discussion

During the 21st century, the pooled incidence of appendicitis was (in per 100,000 person-years): 100 for Northern America; 113 for Northern Europe; 112 for Southern Europe; 105 for Eastern Europe; and 151 for Western Europe [5]. Appendicitis is a common disease in the elderly, accounting for 5% of all abdominal surgical emergencies in this population [6]. Classical presentation of appendicitis with

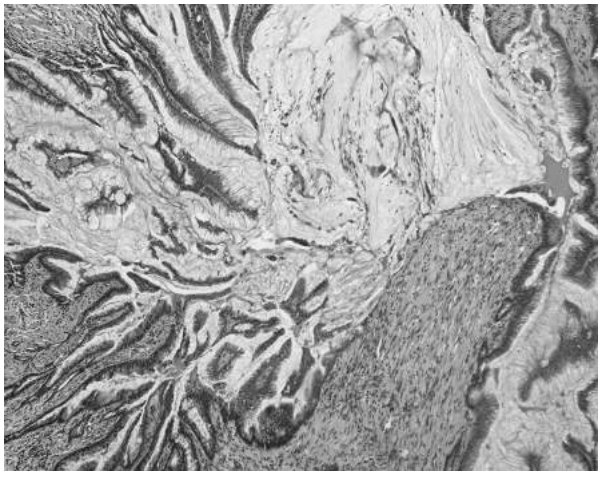


Figure 2. Mucinous adenocarcinoma of the appendix, low grade, HE x 200
Slika 2. *Mucinozni adenokarcinom apendiksa, niskog gradusa, HE x 200*

abdominal pain showing typical migration from epigastrium to the right lower abdominal quadrant and mild WBC elevation may not be representative in this subgroup of patients. Appendicitis in the elderly needs special attention, because atypical presentations and complications are common [7]. The laparoscopic appendectomy (LA) is the most dominant method of current operative therapy and the gold standard technique in the elderly. Population based studies have shown a lower rate of complications and death in the elderly (2.4 vs. 0.5%) for open vs. LA in patients over the age of 65 years [8]. The high incidence of complicated appendicitis in elderly patients can lead to an increased rate of the open procedure. In the elderly, perforation can occur very quickly, supposedly because of degenerative blood vessel changes [9].

Adenocarcinoma of the appendix is the most common perforating cancer of the gastrointestinal tract. Anatomically, there are several reasons for this, namely the extremely thin subserosal layer, gentle submucosal and thin muscular layer of the appendix. Interestingly, in a large number of patients perforation does not have a significant impact on the outcome [4]. Generally, the clinical manifestations of appendiceal tumors are similar to those of acute appendicitis, or a tumor may present as a palpable mass in the abdomen [4]. Both our patients presented with a perforation of the appendix with a classical clinical manifestation of acute abdomen, leading to wrong intraoperative approach. No recurrence in these patients shows that perforation of AA does not imply a worse prognosis.

Malignancies of the appendix are rarely diagnosed preoperatively or intraoperatively. Most tumors are identified only after histopathological examination of the resected specimen following appendectomy [1]. Advances in imaging techniques in the past 20 years have changed the way suspected acute appendicitis is evaluated. The presentation of acute appendicitis may be atypical, complicating the diagnosis. Plain abdominal radiographs are not routinely recommended for the evaluation of suspected acute appendicitis, or AA. Advances in ultrasound technology and the graded compression technique have improved the ability to visualize the appendix. The graded compression technique involves applying steady, gradual pressure to the right lower quadrant in an effort to collapse the normal bowel and eliminate normal bowel gas to visualize the appendix. In case of acute appendicitis, the appendix wall is immobile, noncompressible, and thickened with a diameter greater than 6 to 7 mm [10]. In elderly patients presenting with an appendicular lump, the differential diagnosis of malignancy should be kept in mind [9]. The urgency of the operation in our patients restricted the range of diagnostic procedures. Preoperative abdominal computerized tomography is mandatory in the elderly patients to provide an early diagnosis and to decrease unnecessary surgical exploration if acute appendicitis is suspected [11]. There are some controversies regarding the surgical therapy of adenocarcinoma of the appendix. Murphy et al. suggested that appendectomy was appropriate for accidentally intraoperatively identified tumors if the tumor was confined to the appendix, less than 2 cm in diameter, without obvious infiltration of the mesoappendix and the base of the appendix [12]. Arellano et al. conducted a retrospective study including 53.019 appendectomies; there were 44 (0.01%) cases with primary adenocarcinoma of the appendix, and a 12-year survival in 59% of these patients. Right hemicolectomy was suggested as elective treatment in this study. These authors concluded that preoperative diagnosis of appendiceal malignancies was very rare because of similarities of clinical presentations with appendicitis [13].

Conclusion

Our results suggest that in patients with appendiceal adenocarcinoma and an advanced stage of tumor, right hemicolectomy is a better choice than appendectomy alone. In elderly patients with clinical symptoms of complicated acute appendicitis, appendectomy should be done with intraoperative histopathological frozen section examination. Preoperative diagnosis of appendiceal adenocarcinoma is a challenge due to overlapping of symptoms with complicated acute appendicitis. Ultrasound is not a method of choice in the diagnosis in the elderly patients.

References

1. McCusker ME, Coté TR, Clegg LX, Sobin LH. Primary malignant neoplasms of the appendix: a population-based study from the surveillance, epidemiology and end-results program, 1973-1998. Cancer. 2002;94(12):3307-12.

2. Kelly KJ. Management of appendix cancer. Clin Colon Rectal Surg. 2015;28(4):247-55.

3. Carr NJ, Sobin LH. Adenocarcinoma of the appendix. In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system. Lyon: IARC; 2010. p. 122-5.

4. Ko YH, Park SH, Jung CK, Won HS, Hong SH, Park JC, et al. Clinical characteristics and prognostic factors for primary appendiceal carcinoma. Asia Pac J Clin Oncol. 2010;6(1):19-27.

5. Ferris M, Quan S, Kaplan BS, Molodecky N, Ball CG, Chernoff GW, et al. The global incidence of appendicitis: a systematic review of population-based studies. Ann Surg. 2017;266(2):237-41.

6. Lu SJ, Osmany S, Khoo JB, Ng DC. Synchronous adenocarcinoma of the appendix demonstrated on F-18 FDG PET/CT. Clin Nucl Med. 2010;35(4):256-7.

7. Kirshtein B, Perry ZH, Mizrahi S, Lantsberg L. Value of laparoscopic appendectomy in the elderly patient. World J Surg. 2009;33(5):918-22.

8. Guller U, Jain N, Peterson ED, Muhlbaier LH, Eubanks S, Pietrobou R. Laparoscopic appendectomy in the elderly. Surgery. 2004;135(5):479-88.

9. Kalpande S, Pandya J, Sharma T. Adenocarcinoma mimicking appendicular lump: a diagnostic dilemma - a case report. World J Surg Oncol. 2016;14(1):283.

10. Parks NA, Schroeppel TJ. Update on imaging for acute appendicitis. Surg Clin North Am. 2011;91(1):141-54.

11. Trivedi AN, Levine EA, Mishra G. Adenocarcinoma of the appendix is rarely detected by colonoscopy. J Gastrointest Surg. 2009;13(4):668-75.

12. Murphy EM, Farquharson SM, Moran BJ. Management of an unexpected appendiceal neoplasm. Br J Surg. 2006; 93(7):783-92.

13. Arellano ML, González-Domínguez Y, Molina-Ortiz F, Heras Garceau MA, Cantero R, Rodríguez-Montes JA. Primary adenocarcinoma of the appendix: experience at La Paz University Hospital of Madrid (1967–2014). International Journal of Surgery Open. 2016;4:23-6.

Rad je primljen 24. VII 2018.
Recenziran 17. XI 2018.
Prihvaćen za štampu 26. XI 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:405-408.

University of Belgrade, School of Medicine, Belgrade[1]
Clinical Center of Serbia, Belgrade
Clinic of Otorhinolaryngology and Maxillofacial Surgery[2]

Case report
Prikaz slučaja
UDK 616.311-006.2-07/-08
https://doi.org/10.2298/MPNS1812409D

DERMOID CYST OF THE ORAL CAVITY – A CASE REPORT

DERMOIDNA CISTA USNE DUPLJE – PRIKAZ SLUČAJA

Milovan V. DIMITRIJEVIĆ[1,2], Ana M. DIMITRIJEVIĆ[1], Ivan V. BORIČIĆ[1] and Petar M. ĐURKOVIĆ[1]

Summary

Introduction. Dermoid cysts are benign developmental skin growths that can occur in any part of the body. Dermoid cysts of the head and neck account for 7% of all cysts, and are most frequently located near the lateral aspect of the eyebrow. They are rarely found in the oral cavity, accounting for 0.01% of all oral cavity cysts. **Case Report.** A 15-years-old patient was referred to our Clinic due to a growth in the mouth. Clinical examination and magnetic resonance imaging showed a clearly demarcated, oval, cystic growth in the midline sublingual region. Intraoral incision, typical for frenectomy, with cyst excision was performed. Histopathological findings suggested a dermoid cyst. **Conclusion.** Dermoid cysts of the oral cavity are very rare; they grow slowly and when they reach certain dimensions, they interfere with chewing, swallowing, and lead to progressive breathing difficulty. Dermoid cysts should be included in the differential diagnosis of sublingual mass. Magnetic resonance imaging provides complete information about the localization, size, and content of the growth and contributes significantly to the decision on the surgical approach.

Key words: Dermoid Cyst; Mouth; Morphological and Microscopic Findings; Magnetic Resonance Imaging; Epidemiology; Adolescent

Sažetak

Uvod. Dermoidne ciste su razvojni benigni izraštaji, koji se mogu naći na svim delovima tela i u predelu glave i vrata su zastupljene sa 7%, sa najčešćom lokalizacijom u predelu lateralnog dela obrve. Lokalizacija u usnoj duplji je veoma retka i predstavlja 0,01% svih cista usne duplje. **Prikaz slučaja.** Pacijent star 15 godina upućen je zbog izraštaja u ustima. Kliničkim i magnetnorezonantnim pregledom utvrđen je cistični izraštaj ovalnog oblika u usnoj duplji, u srednjoj liniji, jasnih granica. Intraoralnom incizijom, kao za frenulektomiju, učinjena je ekstirpacija. Histopatološki nalaz je potvrdio dermoidnu cistu. **Zaključak.** Dermoidne ciste u regionu usne duplje su veoma retke, rastu sporo, kada dostignu značajnu dimenziju mogu dovesti do otežanog žvakanja, gutanja i disanja. Magnetna rezonacija obezbeđuje kompletnu informaciju o lokalizaciji, veličini i značajno doprinosi odluci o hirurškom pristupu.

Ključne reči: dermoidna cista; usna duplja; morfološki i mikroskopski nalazi; magnetna rezonanca; epidemiologija; adolescent

Introduction

Dermoid cysts are benign developmental growths that can be found in any part of the body. Cysts of the head and neck account for 7% of all cysts, and are most commonly located near the lateral aspect of the eyebrow. The second most common localization in the head and neck region is the floor of the mouth. Of all dermoid cysts, only 1.6% are located in the mouth, i. e. 23% of all cysts in the head and neck region. The floor of the mouth is the most common localization in the mouth, but rarely dermoid cysts are found in the tongue, lips, buccal mucosa, maxilla and mandible [1–3]. The origin of dermoid cysts still remains incompletely understood; they are considered to be congenital developmental abnormalities, with entrapment of pluripotent stem cells, implantation of the epithelial tissue, anomaly of thyroglossal duct or canal, or a consequence of a trauma. The most widely accepted theory is that cysts occur due to entrapment of ectodermal tissue in midline in third or fourth week of embryonic development, from the first or second branchial arch, therefore the term congenital cyst was accepted. They are commonly located in the midline. In 1955, Meyer classified the cysts of the oral cavity by histological features into three groups: epidermoid, dermoid and teratoid [1]. Dermoid cysts are layered by stratified squamous epithelium and contain skin and skin adnexa (glands and hair follicles). Epidermoid cysts are lined with stratified squamous epithelium and teratoid also contain skin adnexa, along with mesodermal and endodermal elements (muscles, bones, respiratory and gastrointestinal tissues) [1, 2, 4]. Dermoid and teratoid cysts may have a malignant potential [5].

Corresponding Author: Prof. dr Milovan V. Dimitrijević, Univerzitet u Beogradu, Medicinski fakultet, 11000 Beograd, Pasterova 2, Email: milovan.v.dimitrijevic@gmail.com

Abbreviations
MRI – magnetic resonance imaging

These cysts are characterized by slow growth. They are most frequently found in children, in the second or third decade of life, equally in both genders, and are located in the midline [2]. These cysts may displace the tongue upwards and cause disturbances in speech, swallowing, and big cysts may cause airway obstruction. Sublingual localization is considered for cysts between the oral mucosa and geniohyoid muscles, submental most frequently between geniohyoid and mylohyoid muscles and outer between mylohyoid muscle and skin [4, 5]. The symptoms depend on the size and localization, but they frequently cause disturbances in speech and swallowing, or airway obstruction [1, 2, 5].

To confirm the clinical diagnosis and select a surgical strategy, it is necessary to perform imaging studies, ultrasound, computerized tomography or magnetic resonance imaging (MRI) [3, 6, 7].

The main therapeutical procedure is surgery, with intraoral approach for sublingual and submental cysts and external approach for cysts localized between mylohyoid muscles and skin [1, 2, 8].

Case report

A 15-year-old girl was referred to our Clinic due to a growth in the mouth, which was observed one year before. She complained of foreign body sensation and minimal disturbances in speech and swallowing. Her personal history showed a mitral valve prolapse.

Clinical examination: external inspection and palpation showed no pathological findings. During intraoral examination, intact mucosa was observed, with sublingual and submucosal growth in the midline. The body of the tongue was in the midline, motile, moderately displaced towards the palate. The mouth opening was within physiological limits. On palpation, a tumor in the body of the tongue was observed, with a diameter of 30 mm, with a soft, painless, elastic consistency. Laboratory findings were within the normal range.

The MRI of the floor of the mouth: in the midline, a clearly demarcated, oval, cystic lesion was observed,

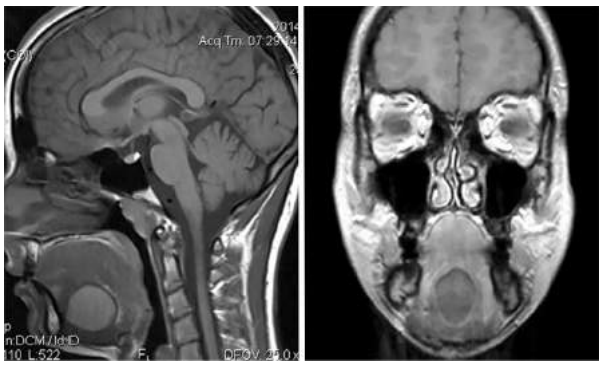


Figure 1. MRI of the sagittal and coronal sections
Slika 1. *Magnetna rezonancija sagitalnog i koronalnog preseka*

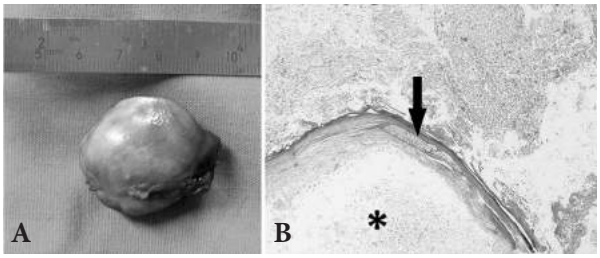


Figure 2. Macroscopic view of the cyst (A) and histopathological findings (B): The cyst wall is lined by a stratified squamous epithelium and contains mature sebaceous glands (arrow). Cystic lumen contains keratin (asterisk) (HE, x 10)
Slika 2. *Makroskopski prikaz ciste (A) i histopatološkog nalaza (B): zid ciste obložen je slojevitim skvamoznim epitelom i sadrži zrele lojne žlezde (strelica); cistični lumen sadrži keratin (zvezdica) (HE, x 10)*

with dimensions of 31 x 29,5 x 19 mm, divided with a capsule from the surrounding muscles of the tongue, beyond the mylohyoid muscle, filled with liquid content, with homogenous hyperintense signal in T1W and T2W images. The airway was without pathological findings. On both sides, the jugular lymph node chains showed normal findings **(Figure 1)**. After preoperative preparation, in general endotracheal anesthesia, using intraoral approach, typical for frenectomy, tumor excision was performed, with careful preparation of the lingual nerve and Wharton's duct, saving all blood vessels of the tongue, with blunt and sharp dissection. The tumor, adherent to the surrounding muscle tissues, was entirely dissected and extirpated. Macroscopically, it was a clearly demarcated tumor, with dimensions of 30 x 30 mm, of soft, elastic consistency. Preoperatively, the patient received antibiotic therapy (Ampicillin 2,0 i.v.). The postoperative course was uneventful, and on the fourth postoperative day the patient was discharged from hospital.

The cyst wall was lined with a stratified squamous epithelium and contained mature sebaceous glands. Keratin was also seen within the cyst cavity (HE, x 10). The histopathological findings suggested a dermoid cyst **(Figure 2)**.

The patient was seen on regular follow-up visits, two years after surgery, having no subjective complaints and no clinical signs of recurrence.

Discussion

Dermoid cysts of the head and neck region account for 7% of all dermoid cysts. They are most frequently localized in the periorbital region [1, 3]. Other localizations are the floor of the mouth, submental or lateral submandibular region, forehead, neck and nose. The floor of the mouth is the most common localization for dermoid cysts of the mouth [1–3, 6]. Dermoid and epidermoid cysts of the mouth are rare lesions, i. e. from all dermoid cysts of the head and neck they account for 1–2%. They account for less than 0.01% of all cysts of the mouth.

Dermoid cysts of the body of the tongue are very rare and in 90% of cases are found in childhood [3, 6]. Epidermoid cysts are layered with epithelium, while dermoid cysts contain skin adnexa and hair [2, 4, 5]. The origin of dermoid cysts of the mouth is dual. They are the consequence of the embryonic developmental disorder and entrapment of epithelial cells in the time of fusion of the first and second brachial arches, in the midline in the third and fourth embryonic weeks. Furthermore, dermoid cysts may occur due to iatrogenic damage or due to trauma, with epithelial and skin adnexa inclusion [4]. Dermoid cysts are most frequently diagnosed in the second and third decades of life, with no gender predominance, in midline, encapsulated [2]. Symptoms associated with dermoid cysts are dysphagia, dysphonia and speech problems, depending on their location and size. In our case, symptoms were moderate. A broad spectrum of lesions can be considered in differential diagnosis: infections, ranula, obstruction of the submandibular duct, tumors of the sublingual and other minor salivary glands, lymphadenopathy, thyroglossal duct cysts, lymphatic malformations, epidermoid cysts, neurofibromas, hemangiomas, heterotypic gastrointestinal cysts, foregut duplication cysts [1, 4, 5, 9]. The clinical findings are not sufficient. Valuable information can be gathered by ultrasound, but MRI is most important in the identification of the cysts, establishing the relationship with muscles of the floor of the mouth, and determining the surgical approach. The MRI findings of dermoid cysts have signals of variable intensity. Cysts can be hyperintense or isointense on T1-weighted images and usually the signal is of high intensity - hyperintense on T2-weighted images [3, 5, 6]. In our case report, like in other studies, dermoid cyst had shown a high intensity signal. Fine-needle aspiration biopsy is useful in making the diagnosis, but is not necessary [5]. The therapeutic surgical approach depends on the location of the cyst, anatomic localization and relationship with muscles of the floor of the mouth. In 1925, Colp classified dermoid cysts according to their relationship with the surrounding muscles as sublingual beyond the geniohyoid muscle, geniohyoid between geniohyoid and mylohyoid muscles and lateral beneath the mylohyoid muscle [8, 9]. Surgical excision is the only effective therapy. Most of the cysts beyond the mylohyoid muscle are appropriate for intraoral surgical approach, but with big cysts and those that are beneath the mylohyoid muscle – submandibular cysts, external approach is more appropriate [1]. In our case, the cyst was removed through intraoral incision approach, typical for frenectomy with precise dissection, which enabled thorough removal of the cyst. This approach allowed successful excision without or with minimal postoperative morbidity. A combined intra– and extraoral approach is reserved for cysts large in size [5]. Postoperatively, the attention should be paid to pain, edema, bleeding, troubles with breathing and swallowing, sensibility and function of the tongue, and on the course of healing [4]. It can be found in recent literature that endoscopic approach is suitable for growths in the medial upper region of the neck [8]. Provided that meticulous, thorough extirpation of the cyst is performed, no recurrences are found [3]. Most cysts already described in literature are of the same dimensions as in our case report [2, 5, 6, 8]. Rarely, they are larger [1, 3]. Definitive diagnosis is made by histological examination of the specimen. Dermoid cysts contain keratin, caseous contents, sweat and sebaceous glands, and hair follicles [4]. Malignant transformation of these cysts in squamocellular carcinoma is extremely rare, up to 5%, and was reported in case of sublingual dermoid cyst and in case of teratoid cyst of the floor of the mouth, after a long evolution process [3, 4, 10]. Radiation therapy is advised postoperatively.

Conclusion

Dermoid cysts in the region of the oral cavity are very rare; they are painless and grow slowly, but when they reach certain dimensions, they may disturb chewing, swallowing, and cause troubles with breathing. These cysts are benign lesions layered with epithelium which contains skin adnexa in the cyst wall. Dermoid cysts have a malignant potential. Magnetic resonance imaging provides complete information about the location, size, and content of the growth and contributes significantly to the decision about the surgical approach. Surgery is the only effective mode of therapy.

References

1. Aydin S, Demir MG, Demir N, Sahin S, Kayipmaz SS. A giant plunging sublingual dermoid cyst excised by intraoral approach. J Maxillofac Oral Surg. 2016;15(2):277-80.

2. Vieira EM, Borges AH, Volpato LE, Porto AN, Carvalhosa AA, Botelho Gde A, et al. Unusual dermoid cyst in oral cavity. Case Rep Pathol. 2014;2014:389752.

3. Shah SP, Quiroz F, Shah CJ. Dermoid cyst. Ultrasound Q. 2012;28(3):215-7.

4. Pirgousis P, Fernandes R. Giant submental dermoid cysts with near total obstruction of the oral cavity: report of 2 cases. J Oral Maxillofac Surg. 2011;69(2):532-5.

5. Papadogeorgakis N, Kalfarentzos EF, Vourlakou C, Alexandridis C. Surgical management of a large median dermoid cyst of the neck causing airway obstruction. A case report. Oral Maxillofac Surg. 2009;13(3):181-4.

6. Edwards PC, Lustrin L, Valderrama E. Dermoid cysts of the tongue: report of five cases and review of the literature. Pediatr Dev Pathol. 2003;6(6):531-5.

7. Kolomvos N, Theologie-Lygidakis N, Tzerbos F, Pavli M, Leventis M, Iatrou I. Surgical treatment of oral and facial soft tissue cystic lesions in children. A retrospective analysis of 60 consecutive cases with literature review. J Craniomaxillofac Surg. 2014;42(5):392-6.

8. Woo SH, Jeong HS, Kim JP, Park JJ, Baek CH. Endoscope-assisted frenotomy approach to median upper neck masses: clinical outcomes and safety (from a phase II clinical trial). Head Neck. 2014;36(7):985-91.

9. King RC, Smith BR, Burk JL. Dermoid cyst in the floor of the mouth. Review of the literature and case reports. Oral Surg Oral Med Oral Pathol. 1994;78(5):567-76.

10. Devine JC, Jones DC. Carcinomatous transformation of a sublingual dermoid cyst. A case report. Int J Oral Maxillofac Surg. 2000;29(2):126-7.

Rad je primljen 13. XI 2018.
Recenziran 17. XII 2018.
Prihvaćen za štampu 10. I 2019.
BIBLID.0025-8105:(2018):LXXI:11-12:409-412.

SEMINAR FOR PHYSICIANS
SEMINAR ZA LEKARE U PRAKSI

Health Center Novi Sad, Novi Sad[1]
University of Novi Sad, Faculty of Medicine
Department of Pharmacy[2]
Department of General Education Subjects[3]

Seminar for physicians
Seminar za lekare u praksi
UDK 615.35.035.3
https://doi.org/10.2298/MPNS1812413N

THE USE OF ANABOLIC ANDROGENIC STEROIDS: A FOCUS ON POLYPHARMACY

UPOTREBA ANABOLIČKIH ANDROGENIH STEROIDA SA FOKUSOM NA POLIFARMACIJU

Božana NIKOLIĆ[1,2] and Dušica RAKIĆ[1,3]

Summary

Introduction. Anabolic androgenic steroids, such as testosterone and its synthetic analogue, nandrolone, have clear clinical indications. However, their abuse is practiced to enhance physical performance in professional, recreational and non-professional athletes; outside of sports, their nonmedical use is associated with different social groups (criminal activities, substance abuse). **Polypharmacy.** Testosterone and its synthetic analogues are also used for nonmedical purposes, mainly administered in supraphysiological doses in cycles lasting a few weeks. In order to potentiate the anabolic properties and control the adverse effects, the users also administer other pharmacological agents. Thus, growth hormone and insulin are complement to anabolic steroids; clenbuterol, amphetamine and thyroid hormones stimulate body fat loss; diuretics reduce the body weight and improve muscle definition; and erythropoietin increases the training capacity and accelerates the recovery after hard competitions. To control adverse effects, cardiovascular drugs, central nervous system depressants, central nervous system stimulants, human chorionic gonadotropin, sexual enhancement drugs, estrogen antagonists, analgesics/opioids, nonsteroidal anti-inflammatory drugs and others, are administered. Probenecid, finasteride and diuretics mask the administration of other doping agents. Additionally, during the last two decades, attention has increasingly been focused on the relationship between the use of anabolic androgenic steroids and psychoactive substances (alcohol, cannabis, amphetamines, cocaine, hallucinogens). **Conclusion.** Supraphysiological doses and polypharmacy additionally increase the risk of adverse effects, including withdrawal syndrome; therefore, prevention of nonmedical use of anabolic androgenic steroids should be a public health priority.

Key words: Anabolic Agents; Androgens; Drug Users; Polypharmacy; Substance-Related Disorders

Sažetak

Uvod. Anabolički androgeni steroidi kao što su testosteron i njegov sintetski analog nandrolon imaju jasne kliničke indikacije. Međutim, zloupotreba u svrhu poboljšanja fizičkih performansi u vrhunskom, ali i u rekreativnom i neprofesionalnom sportu se praktikuje; izvan sporta, nemedicinska upotreba steroida se dovodi u vezu sa različitim socijalnim grupama (kriminalne aktivnosti, zavisnost od lekova i drugih supstancija). **Polifarmacija.** U nemedicinske svrhe testosteron i njegovi sintetski analozi se uglavnom primenjuju u suprafiziološkim dozama u ciklusima koji traju nekoliko nedelja, a kako bi potencirali anaboličke efekte i kontrolisali neželjene reakcije, korisnici primenjuju i druge farmakološke agense. Tako se u kombinacijama sa anaboličkim steroidima nalaze hormon rasta i insulin; klenbuterol, amfetamin i tiroidni hormoni koji podstiču sagorevanje telesnih masti; diuretici koji redukuju telesnu težinu i doprinose boljoj definiciji mišića; eritropoetin koji povećava mogućnosti treninga i ubrzava oporavak nakon napornih takmičenja. Za kontrolu neželjenih reakcija koriste se kardiovaskularni lekovi, depresori centralnog nervnog sistema, stimulansi centralnog nervnog sistema, humani horionski gonadotropin, lekovi u terapiji seksualne disfunkcije, antagonisti estrogena, analgetici/opioidi, nesteroidni antiinflamatorni lekovi i drugi. Probenecid, finasterid i diuretici maskiraju pozitivne rezultate u doping kontroli. Dodatno, tokom poslednje dve decenije pažnja se sve više usmerava na vezu između upotrebe anaboličkih steroida i psihoaktivnih supstancija (alkohol, kanabis, amfetamin, kokain, halucinogeni). **Zaključak.** Suprafiziološke doze i polifarmacija dodatno uvećavaju rizik od neželjenih reakcija, uključujući i apstinencijalni sindrom; stoga, prevencija nemedicinske upotrebe anaboličkih steroida treba da bude javnozdravstveni prioritet.

Ključne reči: anabolici; androgeni; narkomani; polifarmacija; bolesti zavisnosti

Introduction

Anabolic androgenic steroids (AAS) are testosterone and its synthetic analogues **(Figure 1)**, and most of these compounds have few clinical indications. For example, testosterone is a replacement therapy for male hypogonadism; nandrolone is an adjunct treatment in catabolic conditions after severe trauma or chronic diseases followed by cachexia, and osteoporosis with specific parameters [1, 2]. Illicit steroids and those ille-

Corresponding Author: Doc. dr Božana Nikolić, Univerzitet u Novom Sadu, Medicinski fakultet, Zavod za farmaciju, 21000 Novi Sad, Hajduk Veljkova 3, E-mail: bozana.nikolic@mf.uns.ac.rs

Abbreviations
AAS – anabolic androgenic steroids
hCG – human chorionic gonadotropin
T/E – testosterone epitestosterone ratio

gally diverted from legitimate sources are used to improve physical performance in elite athletes (e. g., sprint, shot, discus, hammer, javelin) as well as in recreational and non-professional sport (e. g., fitness, body-building); outside the world of sports, the nonmedical use is practiced in different social groups (criminal activities, abuse of drugs and other substances).

The estimated prevalence of nonmedical use is between 1.27 and 4% in young adults, and there are significant differences at the level of some subpopulations [3, 4]. For example, approximately 20% of body-builders and 11% of prisoners use anabolic steroids [5, 6].

Polypharmacy

For nonmedical purposes, testosterone and its synthetic analogues are ingested orally, injected intramuscularly, or applied to the skin. The doses are 5 – 29 times higher than doses for conventional hormone replacement [7, 8]. Users typically combine different AAS ("steroid stacking"), injectable and oral ones. Oral formulations have a short half-life and are used daily;intramuscular formulations are generally administered weekly or biweekly. Nandrolone, testosterone, trenbolone, boldenone, methandrostenolone and stanozolol are some of the most popular **(Table 1)** [3]. After administration in cyclic manner, generally lasting 6 – 18 weeks, a period of abstinence follows [7]. It should enable the recovery of the hypothalamic-pituitary-gonadal axis and return the endogenous testosterone to the normal level.

To potentiate anabolic properties, control adverse health effects, or avoid a positive result in doping-control, other pharmacological agents are also administered [3]. Indeed, polypharmacy is widespread and between 50 and 95% users practice it [6, 9, 10]. The most commonly reported adjunct substances are growth hormone, insulin, thyroxine, clenbuterol, human chorionic gonadotropin (hCG), analgesics/opioids, dietary supplements, diuretics, alcohol and some illicit pharmacological agents [11].

Enhancement of anabolic effects

There are multiple mechanisms by which anabolic steroids can increase physical performance

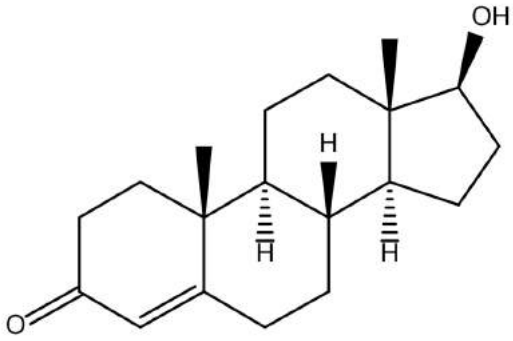


Figure 1. The chemical structure of testosterone
Slika 1. *Hemijska struktura testosterona*

(Table 2); undoubtedly, they increase muscle mass and strength in dose-dependent manner; in case of action on the blood vessels, erythropoiesis and central nervous system, the evidence is less well established [12].

In order to complement anabolic effects, approximately one-fourth of AAS users administer human growth hormone (hGH), insulin-like growth factor-1 (IGF-1) or insulin [10, 13]; however, these extensive pharmacological regimens increase the risk of adverse effects [3]. Furthermore, several other performance enhancing agents are combined with steroids; clenbuterol, amphetamine, and some hormones as thyroxine, to stimulate body fat burning; diuretics (e. g., furosemide, thiazides) to improve muscle definition or to reduce body weight, erythropoietins to increase the ability of training and accelerate the recovery after hard competitions. Finally, a relationship between the use of AAS and dietary supplements has been documented [5, 14]; besides performance enhancing properties, simultaneous administration for long periods and/or in high doses increases the risk of synergistic adverse effects as acute renal insufficiency and cardiovascular events [9].

Adverse effects control

A wide range and a high prevalence of AAS adverse effects have been reported (nearly 100%) by illicit AAS users [3, 10]. Cardiovascular (dyslipidemia, atherosclerosis, cardiomyopathy, hypertension), neuropsychiatric (mood disorders, addiction, aggression, cognitive deficits), and neuroendocrine (infertility, gynecomastia) adverse effects are of particular interest . There are also well-recognized, but either less serious or less common, effects on other

Table 1. Commonly used anabolic androgenic steroids
Tabela 1. *Često korišteni anabolički androgeni steroidi*

PO Formulations/*per os formulacije*	IM Formulations/*i. m. formulacije*
Methandrostenolone/*Metandrostenolon*	Testosterone enanthate/*Testosteron enantat*
Stanozolol/*Stanazolol*	Nandrolone decanoat/*Nandrolon dekanoat*
Oxandrolone/*Oksandrolon*	Boldenone/*Boldenon*
Oxymetholone/*Oksimetolon*	Trenbolone/*Trenbolon*

Legend: PO - per os; IM - intramuscular/*Legenda: i. m. – intramuskularno*

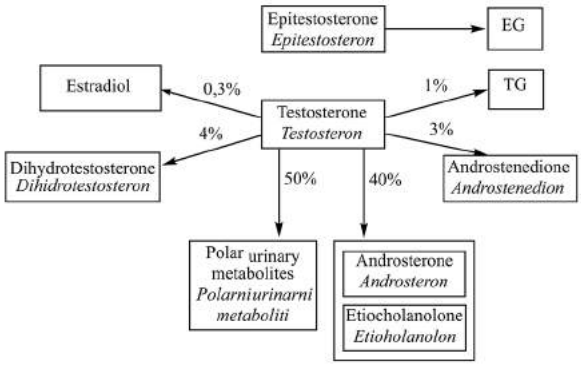


Figure 2. Testosterone metabolism
Slika 2. *Metabolizam testosterona*
Legend: EG – epitestosterone glucuronide, TG – testosterone glucuronide
Legenda: EG – epitestosteron glukuronid, TG – testosteron glukuronid

organ systems **(Table 3)** [3]. Hence, in control of undesirable clinical outcomes, the use of medications such as captopril, carvedilol, digoxin, central nervous system depressants, central nervous system stimulants, gonadotropins, sexual enhancement agents, estrogen antagonists, aromatase inhibitors, is being practiced [11]. In addition, analgesics and non-steroidal anti-inflammatory drugs (NSAID) are frequently used in the treatment as well as in pain prophylaxis [15]; however, the latter mask the pain and additionally increase the risk of muscle injury.

Masking agents

The use of AAS in sports has been recognized as a common practice [16]. Maintaining safety and fairness in sports, the International Olympic Committee tests athletes for these prohibited substances, but athletes and their teams use different strategies to avoid doping violations. Among others, manipulations are based on pharmacological, biochemical, and genetic characteristics of testosterone and its synthetic analogues' metabolism [16].

Testosterone undergoes hepatic and extrahepatic metabolism. The inactivation pathway occurs mainly in the liver via conjugation into testosterone conjugates (glucuronides and to a lesser extent sulfates) and in equal proportions into the 17-ketosteroids (androsterone and etiocholanolone). The conjugates of testosterone and its hepatic metabolites are excreted in the urine and bile. A small proportion of steroid is converted to biologically active metabolites, estradiol and dihydrotestosterone. In addition to the metabolites, approximately 1% of daily synthesized testosterone is excreted into the urine unchanged **(Figure 2)** [1, 17].

Epitestosterone is a 17α epimer of testosterone with unknown biological activity. Biosynthesis of these steroid molecules in human beings is constant, and in the urine the normal testosterone glucuronide and epitestosterone glucuronide ratio (T/E) ranges between 0.4 and 2 (bimodal variation is in line with genetic polymorphism). T/E > 4 is considered to be a doping violation. In case of a simultaneous administration of testosterone and epitestosterone in a ratio of 30 to 1, the T/E is not changed and the doping result is false-negative [18]. Therefore, epitestosterone has been added to the list of banned substances as a masking agent for illicit testosterone use.

The World Anti-Doping Agency list also includes probenecid, 5α reductase inhibitors, diuretics and ketoconazole [19]. These drugs have different mechanisms of action and masking effects (AAS is mainly detected in a urine sample). Probenecid, uricosuric drug in the treatment of gout, interferes with renal excretion of steroid glucuronides. Inhibitors of 5α reductase, the class of pharmacological agents in the treatment of benign prostatic hyperplasia, alter metabolism of anabolic steroids reducing excretion of dihydrotestosterone and androsterone. Diuretics increase the volume of urine and subsequently decrease the concentration of AAS and their metabolites to undetectable levels. Ketoconazole, antifungal drug, inhibits synthesis of endogenous testosterone [16, 18].

Table 2. Ergogenic effects of anabolic androgenic steroids
Tabela 2. *Ergogeni efekti anaboličkih androgenih steroida*

Organ system/*Organski sistem*	Effects/*Efekti*
Muscles/*Mišići*	Increased muscle mass/*Uvećana mišićna masa*
	Increased muscle strength/*Uvećana mišićna snaga*
	GH, IGF-1 secretion stimulation/*Stimulacija sekrecije HR, IGF-1*
	Decreased recovery time/*Redukovano vreme oporavka*
Blood vessels/*Krvni sudovi*	Vasodilatation/*Vazodilatacija*
Erythropoiesis/*Eritropoeza*	Erythropoiesis stimulation/*Stimulacija eritropoeze*
CNS/*CNS*	Increased motivation/*Uvećana motivacija*
	Increased aggression/*Uvećana agresivnost*
	Increased competitiveness/*Uvećana kompetitivnost*

Legend**:** GH, growth hormone; IGF-1, insulin-like growth factor 1; CNS, central nervous system
Legenda: HR, hormon rasta; IGF-1, insulinu sličan faktor rasta 1; CNS, centralni nervni sistem

Table 3. Adverse effects of anabolic androgenic steroids
Tabela 3. *Neželjene reakcije anaboličkih androgenih steroida*

Organ System/*Organski sistem*	Effects/*Efekti*
Cardiovascular/*Kardiovaskularni*	Dyslipidemia /*Dislipidemija,* Atherosclerosis/*Ateroskleroza,* Cardiomyopathy/*Kardiomiopatija*, Hypertension/*Hipertenzija,* Coagulopathy/*Koagulopatije*, Polycythemia/*Policitemija*
Neuroendocrine/*Neuroendokrini*	Infertility/*Infertilitet*, Gynecomastia/*Ginekomastija*
Neuropsychiatric/*Neuropsihijatrijski*	Mood disorders/*Poremećaj raspoloženja*, Addiction/*Adikcija*, Aggression/*Agresivnost*, Cognitive deficits/*Kognitivni deficiti*
Liver/*Jetra*	Inflammation and cholestasis/*Inflamacija i holestaza*, Neoplasms (rare)/*Neoplazme (retko)*
Musculature/*Muskulatura*	Tendon rupture/*Rupture tetiva*
Kidney/*Bubrezi*	Rhabdomyolysis and renal failure/*Rabdomioliza i renalna insuficijencija* Neoplasms (rare)/*Neoplazme (retko)/*
Immunity/*Imunitet*	Immunosuppression/*Imunosupresija*
Skin/*Koža*	Acne/*Akne*, Striae/*Strije*

AAS, psychoactive substances and addiction

Over the last two decade, attention has been increasingly focused on the relationship between the use of AAS and other psychoactive substances (e.g., alcohol, heroin, amphetamine, cocaine, hallucinogens) [14, 20]. At first sight, testosterone and the above mentioned substances are an extremely heterogeneous pharmacological group; however, there is a link at molecular and cellular level. All of these substances, with certain quantitative differences, activate the mesolimbic dopaminergic pathway (reward pathway) and precipitate hedonic effects [21]; an inherent characteristic of the activation of pathway is the desire to repeat activity that leads to feeling of pleasure. Whether and when the loss of control over substance intake is experienced, it depends on the substance being used, the dose and route of administration as well as genetic makeup of the user.

Furthermore, AAS may accelerate addiction via anabolic and androgenic mechanisms [21]. Anabolic effect is mainly the motivation to begin using illicit AAS; in some cases, when the use is discontinued, due to a little reduction in muscularity, users become anxious and continuously administer steroids despite harmful consequences. In line with androgenic mechanisms, breaking the cycle (particularly a long cycle) often results in suppression of hypothalamic-pituitary-gonadal axis and consequently in hypogonadism related symptoms (fatigue, loss of libido, depression) that may prompt some users to quickly resume using AAS.

Using the criteria of the Diagnostic and Statistical Manual of Mental Disorders, it was estimated that 30% of AAS users develop an addictive disorder [21]. However, in case of its manifestation, therapeutic interventions have been insufficiently studied [20, 21]. Clinical experiences suggest that serotonergic antidepressants as well as cognitive-behavioral therapy may be useful in the treatment of muscle dysmorphia and depressive symptoms [21]. In association with hypogonadism, hCG can accelerate the synthesis of testosterone in the testes; clomiphene can stimulate pituitary function; phosphodiesterase inhibitors (e. g., sildenafil) may be used in the treatment of sexual dysfunction; and collective administration of hCG, clomiphene, and tamoxifen in varying time courses is also possible [21]. Finally, in animal studies, naltrexone, an opioid antagonist, blocked self-administration of testosterone (Hedonic effects of AAS are likely modulated by opiodergic mechanisms) [22]. Therefore, it is speculated that it could be used to treat AAS addiction [20, 21]; precipitated withdrawal syndrome may be treated by clonidine, benzodiazepines, non-opioid analgesics and antiemetic agents, if needed [21]. In addition, it seems that psychosocial therapy may be beneficial, especially in situations where AAS addiction is comorbid with other illicit substance use disorders [21].

Conclusion

Nonmedical use of anabolic androgenic steroids is practiced among professional and non-professional athletes as well as non-athletes. Steroids are administered in supraphysiological doses and usually in combination with other licit and illicit substances. These patterns of use additionally increase the risk of adverse effects, including withdrawal syndrome. Therefore, prevention of nonmedical anabolic androgenic steroids use should be a public health priority.

References

1. Testosteron depo®, rastvor za injekciju: sažetak karakteristika leka [Internet]. Beograd: Galenika a.d; 2016 [updated 2016 Dec; cited 2018 Jun 21]. Available from: https://www.alims.gov.rs/ciril/files/lekovi/smpc/515-01-01895-16-001.pdf.

2. Deca-Durobolin®, rastvor za injekciju: sažetak karakteristika leka [Internet]. Beograd: Agencija za lekove i medicinska sredstva Srbije; 2013 [updated 2013 Jul; cited 2018 Jun 21]. Avail-

able from: https://www.alims.gov.rs/ciril/files/lekovi/smpc/515-01-01547-13-001.pdf.

3. Pope HG Jr, Wood RI, Rogol A, Nyberg F, Bowers L, Bhasin S. Adverse health consequences of performance-enhancing drugs: an Endocrine Society scientific statement. Endocr Rev. 2014;35(3):341-75.

4. Sandvik MR, Bakken A, Loland S. Anabolic-androgenic steroid use and correlates in Norwegian adolescents. Eur J Sport Sci. 2018;18(6):903-10.

5. Nogueira FR, Brito Ade F, Oliveira CV, Vieira TI, Gouveia RL. Anabolic-androgenic steroid use among Brazilian bodybuilders. Subst Use Misuse. 2014;49(9):1138-45.

6. Lood Y, Eklund A, Garle M, Ahlner J. Anabolic androgenic steroids in police cases in Sweden 1999-2009. Forensic Sci Int. 2012;219(1-3):199-204.

7. Goldman A, Basaria S. Adverse health effects of androgen use. Mol Cell Endocrinol. 2018;464:46-55.

8. Perry PJ, Lund BC, Deninger MJ, Kutscher EC, Schneider J. Anabolic steroid use in weightlifters and bodybuilders: an internet survey of drug utilization. Clin J Sport Med. 2005;15(5):326-30.

9. Bordin DM, Bettim BB, Perdona GC, de Campos EG, De Martinis BS. Understanding alterations on blood and biochemical parameters in athletes that use dietary supplements, steroids and illicit drugs. Toxicology. 2017;376:75-82.

10. Parkinson AB, Evans NA. Anabolic androgenic steroids: a survey of 500 users. Med Sci Sports Exerc. 2006;38(4):644-51.

11. Sagoe D, McVeigh J, Bjørnebekk A, Essilfie MS, Andreassen CS, Pallesen S. Polypharmacy among anabolic-androgenic steroid users: a descriptive metasynthesis. Subst Abuse Treat Prev Policy. 2015;10:12.

12. Cheung AS, Grossmann M. Physiological basis behind ergogenic effects of anabolic androgens. Mol Cell Endocrinol. 2018;464:14-20.

Rad je primljen 6. VIII 2018.
Recenziran 27. XI 2018.
Prihvaćen za štampu 3. XII 2018.
BIBLID.0025-8105:(2018):LXXI:11-12:413-417.

13. Brennan BP, Kanayama G, Hudson JI, Pope Jr HG. Human growth hormone abuse in male weightlifters. Am J Addict. 2011;20(1):9-13.

14. Dodge T, Hoagland MF. The use of anabolic androgenic steroids and polypharmacy: a review of the literature. Drug Alcohol Depend. 2011;114(2-3):100-9.

15. Fernando ADA, Bandara LMH, Bandara HMST, Pilapitiya S, de Silva A. A descriptive study of self-medication practices among Sri Lankan national level athletes. BMC Res Notes. 2017;10(1):257.

16. Alquraini H, Auchus RJ. Strategies that athletes use to avoid detection of androgenic-anabolic steroid doping and sanctions. Mol Cell Endocrinol. 2018;464:28-33.

17. von Deutsch DA, Abukhalaf IK, Socci RR. Anabolic doping agents. In: Mozayani A, Raymon LP, editors. Handbook of drug interactions: a clinical and forensic guide. New York: Humana Press; 2004. p. 493-596.

18. van de Kerkhof DH, de Boer D, Thijssen JH, Maes RA. Evaluation of testosterone/epitestosterone ratio influential factors as determined in doping analysis. J Anal Toxicol. 2000;24(2):102-15.

19. World Anti-Doping Agency. The world anti-doping code: prohibited list January 2018 [Internet]. 2018 [cited 2018 Mar 10]. Available from: https://www.wada-ama.org/sites/default/files/prohibited_list_2018_en.pdf.

20. Grönbladh A, Nylander E, Hallberg M. The neurobiology and addiction potential of anabolic androgenic steroids and the effects of growth hormone. Brain Res Bull. 2016;126(Pt 1):127-37.

21. Kanayama G, Brower KJ, Wood RI, Hudson JI, Pope HG Jr. Treatment of anabolic-androgenic steroid dependence: emerging evidence and its implications. Drug Alcohol Depend. 2010;109(1-3):6-13.

22. Peters KD, Wood RI. Androgen dependence in hamsters: overdose, tolerance, and potential opioidergic mechanisms. Neuroscience. 2005;130(4):971-81.

REGISTAR ZA 2018. GODINU

INDEKS AUTORA

INDEX KEY WORDS

INDEKS KLJUČNIH REČI

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

Korisnici časopisa treba da se registruju na adresi:
http://aseestant.ceon.rs/index.php/medpreg/user/register

Prijava rada treba da se učini na adresi:
http://aseestant.ceon.rs/index.php/medpreg/

U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

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

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.