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ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

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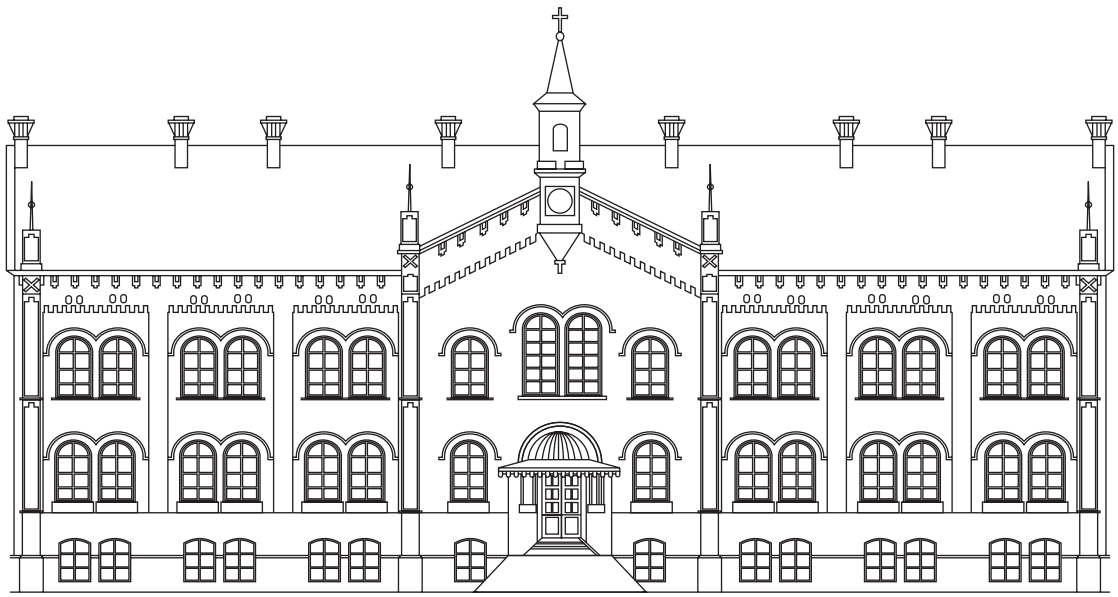

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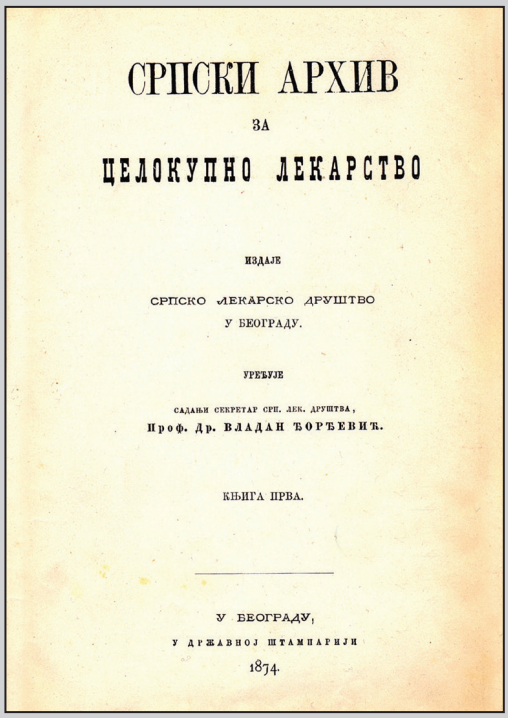


СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

Проф. Др. ВЛАДАН ЂОРЂЕВИЋ.

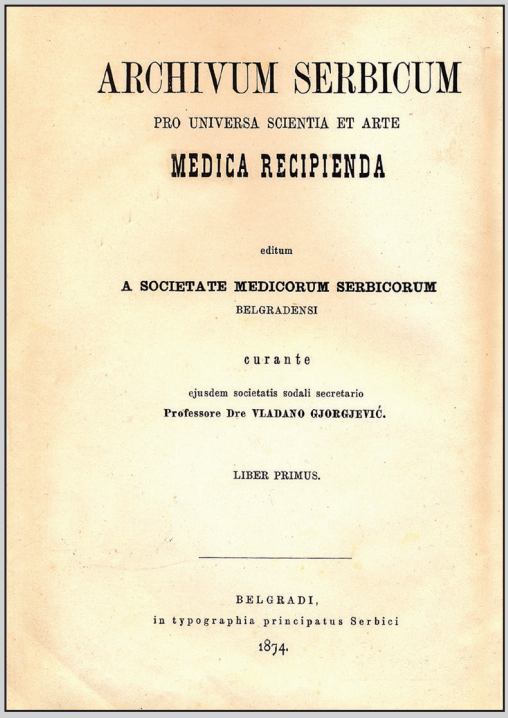
КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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MEDICA RECIPIENDA

editum

A SOCIETATE MEDICORUM SERBICORUM

BELGRADENSI

curante

ejusdem societatis sodali secretario

Professore Dre VLADANO GJORGJEVIĆ.

LIBER PRIMUS.

BELGRADI,

in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основаног 1872. године, први пут штампан 1874. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Објављују се: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике и регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози.

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Излази шест пута годишње

Српски архив за целокупно лекарство

ГОДИШТЕ 150. ЈАНУАР–ФЕБРУАР 2022. СВЕСКА 1–2

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EDITORIAL / УВОДНИК

The Serbian Archives of Medicine – the 150 Years' Long Tradition

With great pride and immense honor, we welcome the year 2022 and this issue, as the very first one of the year that marks our 150th anniversary. The Serbian Archives of Medicine, as the official journal of the Serbian Medical Society, the oldest society of its kind in modern medical Serbian history, was founded in Belgrade (Serbia), 150 years ago, more precisely on April 22, 1872 (according to the Julian calendar), i.e., May 4, 1872 (according to the Gregorian calendar) [1].

About this historical action of a group of medical doctors who were practicing in Belgrade and who were led by Dr. Vladan Đorđević, you will read in the next issue of our journal, in the written address of the current President of the Serbian Medical Society – Professor Radoje Čolović, Full Member of the Serbian Academy of Sciences and Arts.

The decision to publish the Serbian Archives of Medicine was already reached during the Second Regular Meeting of the Serbian Medical Society and during the fourth one (September 16, 1872), the structure of the journal suggested by Dr. Vladan Đorđević as the editor, was accepted. His idea was to publish the journal on a quarterly basis and that each issue should be made out of two parts. Section I, the so-called Book I, should encompass (1) independent research of national authors discussing statistical data of diseases in Serbia and other countries where Serbs were living; (2) independent discussions from different medical and allied sciences; (3) a review of current medical achievements worldwide; (4) critical analyses of existing national sanitary/epidemiological stances, national medical books and research. The second part of each journal issue, Section II (Book II) should comprise abstracts in Serbian of at least one accepted best international medical book [2].

The first issue of the Serbian Archives of Medicine was published in 1874. Its table of contents consisted of the following minutes from previous meetings (1872), of the so-called Main Meeting of the Serbian Medical Society (1872), of the ordinary and extraordinary meetings held in 1872 and 1873, original articles (Section I) and Serbian translations of foreign literature (Section II).

Over the following 21 years, i.e., up to 1895, 12 books of Section I and 27 books of Section II were published. In 1895, Dr. Milan Jovanović Batut, the new editor, introduced monthly publishing of the journal with permanent jounal sections, keeping pace with other journals of that era. Other editors afterwards gave their different personal contributions to the journal's evolution through the decades to come.

With journal's home being on the West-to-East and North-to-South crossroads, it does not come as a surprise that the two Balkan Wars, as well as two World Wars, affected Serbian Archives of Medicine and caused the discontinuity in publishing. In 1952, the Annual Assembly of the

Figure 1. Professor Radmila Janković (University of Belgrade, Faculty of Medicine, Institute of Pathology, Belgrade, Serbia)

Figure 2. Nevena Kalezić (University of Belgrade, Faculty of Medicine, University Clinical Center of Serbia, Belgrade, Serbia)

Figure 3. Aleksandar Lešić (University of Belgrade, Faculty of Medicine, University Clinical Center of Serbia, Belgrade, Serbia)

Figure 4. Professor Mladen Jovanović (University of Novi Sad, Faculty of Medicine, Department of Plastic and Reconstructive Surgery, Clinical Center of Vojvodina, Novi Sad, Serbia)

Figure 5. Professor Aleksandra Radosavljević (University of Belgrade, Faculty of Medicine, University Clinical Center of Serbia, Belgrade, Serbia)

Figure 6. Associate Professor Jasna Trbojević-Stanković (University of Belgrade, Faculty of Medicine, Dr Dragiša Mišović University Clinical Centre, Belgrade, Serbia)

Serbian Medical Society decided to count all the years since the establishment of the Serbian Medical Society, using the number of years since its foundation as a volume number in addition to the publishing year.

Despite another SARS-CoV2 pandemic year behind us, our healthcare workers' workloads did not diminish – *while the general public kept promoting the spread of COVID-19 by misinterpreting nation-wide vaccination efforts aimed to control mortality and new viral variants' formation for a "carte blanche" to embrace life with no constraints denied to so many* – our amazing reviewers stood tall, time and again (Table 1).

Ranked as the first reviewer in 2021, is Professor Radmila Janković (University of Belgrade, Faculty of Medicine (UBFM), Belgrade, Serbia) (Figure 1), and ranked as second, with equal number of reviews, came Professors Nevena Kalezić (UBFM), University Clinical Center of Serbia (UCCS), Belgrade, Serbia (Figure 2), Aleksandar Lešić (UBFM, UCCS, Belgrade, Serbia) (Figure 3), Mladen Jovanović (University of Novi Sad, Faculty of Medicine, Department of Plastic and Reconstructive Surgery, Novi Sad, Serbia) (Figure 4), and Aleksandra Radosavljević (UBFM, UCCS, Belgrade, Serbia) (Figure 5) and Associate Professor Jasna Trbojević-Stanković (UBFM, Dr Dragiša Mišović University Clinical Center, Belgrade, Serbia) (Figure 6).

As part of the world starts to embrace living with SARS-CoV2 variants and proceeds to lifting restrictions, Serbian healthcare system still fearlessly fights rising numbers of the infected and hospitalized, while we hope that, by the end of 2022, if not sooner, our social solidarity will grow to enable equitable opportunity to both life and healthcare besides COVID-19 for all, irrelevant of age, sex, creed or any other social determinant of health.

Conflict of interest: None declared.

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Table 1. The list of the Serbian Archives of Medicine reviewers in the year 2021

1. Aksić Z Milan
2. Anejević Slađana
3. Antić Darko
4. Arsenijević Tatjana
5. Bašić Dragoslav
6. Begović Kuprešanin Vesna
7. Belojević A Goran
8. Bila Jelena
9. Bilanović Dragoljub
10. Bjeloš Rončević Mirjana
11. Blagojević Duška
12. Boscolo-Berto Rafael
13. Božić Marija
14. Božić Antić Ivana
15. Bubanja Dragana
16. Buljčik Čupić Maja
17. Bumbaširević Marko
18. Bumbaširević Uroš
19. Cekovska D Svetlana
20. Cerovac Nataša
21. Cvetković Slobodan
22. Čanović Predrag
23. Čolić Miodrag
24. Čolić Snježana
25. Čolović D Milica
26. Čolović R Nataša
27. Čupić Maja

28. Ćeranić S Miljan
29. Ćupurdija Vojislav
30. Ćurković Aleksandar
31. Daković R Dragana
32. Damjanović Tatjana
33. Davidović B Lazar
34. Dimitrijević Milovan
35. Dimković Nada
36. Dobe Madhumita
37. Dobričić Čevrljaković Nevenka
38. Dugalić Vladimir
39. Đikanović Bosiljka
40. Đorđević S Boban
41. Đorđević Vladimir
42. Đorđević Jocić Jasmina
43. Đukanović D Ljubica
44. Đukić Vojko
45. Filipović Gordana
46. Folić Miljan
47. Galun Danijel
48. Gazibara Tatjana
49. Gluhović A Aleksandar
50. Gojković Bukarica Č Ljiljana
51. Gojnić Miroslava
52. Golubović Zoran
53. Gottardo Fedra
54. Graovac Stevica
55. Gregorić Pavle
56. Gvozdenović Ljiljana
57. Ilić Dragan
58. Ilić Slobodan
59. Ille Tatjana
60. Ivanović D Mirjana
61. Ivanović Nebojša
62. Jakšić Vesna
63. Janković Radmila
64. Jeremić Branislav
65. Jeremić Jelena
66. Jokić R Radoica
67. Joković Miloš
68. Jotić Ana
69. Jovanić Tatjana
70. Jovanović Marina
71. Jovanović A Mladen
72. Jovanović Tanja
73. Jovanović Simić Jelena
74. Juloski Jovana
75. Jusufović Edin
76. Kadija Marko
77. Kalezić Nevena
78. Kalezić Tanja
79. Karamarković Aleksandar
80. Kitov Borislav
81. Knežević Miroslav
82. Knežević Srbislav
83. Kocić Gordana
84. Konstantinović Ljubica
85. Konstantinović Vitomir
86. Kovačević Ljubinka
87. Kovačević S Vojin
88. Kozomara Ružica
89. Krasnik Rastislava
90. Kravljanac Ružica
91. Krejović Trivić Sanja
92. Krivokapić Zoran
93. Krstev Cvetana
94. Krstić Zoran
95. Krstonošić Bojana
96. Kuzmanović Miloš
97. Lalić Nensi
98. Lalošević Dušan
99. Latas Milan
100. Lazić Vojkan
101. Lazović Milica
102. Lečić Toševski Dušica
103. Lešić R Aleksandar
104. Ležaić D Višnja
105. Macut Đura
106. Maksimović Nataša
107. Maliković B Aleksandar
108. Mandić Stojmenović B Gorana
109. Manojlo Kosanović Rade
110. Manojlović Slavko
111. Marjanović Ivan
112. Marković Evgenija
113. Martić Jelena
114. Matejić R Bojana
115. Matić Slavko
116. Micev Marjan
117. Michelsen Kai
118. Mihailo Nikolić Dimitrije
119. Mihailović Zoran
120. Mihalj Marija
121. Milenković Pavle
122. Milenković S Saša
123. Milisavljević M Milan
124. Milovanović Jovica
125. Milovanović Srđan
126. Milutinović Suzana
127. Miljević Čedo
128. Mitković Milan
129. Mitković Milorad
130. Mitrović M Dušan
131. Mladenović Jasmina
132. Nedeljković Milan
133. Nedeljković Nenad
134. Nenadić B Dane
135. Nešković Č Vojislava
136. Nikitović Marina
137. Nikolić Ač Eržebet
138. Nikolić M Igor
139. Nikolić Slobodan
140. Novaković R Tatjana
141. Obrenović Kirćanski Biljana
142. Odalović Marina
143. Palibrk Ivan
144. Pantić Igor
145. Parapid Biljana
146. Pašić Srđan
147. Patrinos P George
148. Pavlović Milorad
149. Pavlović Sonja
150. Peco Antić Amira
151. Pejović Milovančević Milica
152. Perić Momčilo
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157. Petrović Milan
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160. Plešinac Karapandžić Vesna
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162. Pravica Vera
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171. Radovanović Zoran
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192. Spasojević Slobodan
193. Sretenović Aleksandar
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195. Stamenković Bojana
196. Stamenković Dragoslav
197. Stamenković Miroslav
198. Stanković Goran
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206. Stepić Nenad
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208. Stojanović Dragoš
209. Stojanović Rundić Suzana
210. Stojčev Ljiljana
211. Stojičić Milan
212. Stojimirović Biljana
213. Stojković Siniša
214. Stojković Zlatanović N Sanja
215. Stojšić Milosavljević Anastazija
216. Svetel Marina
217. Šaponjski Jovica
218. Šijački Ana
219. Škodrić Trifunović Vesna
220. Teofilovski Parapid Gordana
221. Todorović Ljubomir
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225. Trbojević Stanković Jasna
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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The role of yogurt enriched with LGG culture (*Lactobacillus rhamnosus GG*) in dental caries prevention

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SUMMARY
Introduction/Objective Contemporary tendencies suggest that probiotics can significantly reduce the prevalence of caries in children, so it can be considered that they have a positive effect on general and oral health. *Lactobacillus rhamnosus GG* (LGG) is a probiotic culture of particular importance in preventive dentistry.
The aim was to assess the effects of consumption of probiotic strain *Lactobacillus rhamnosus GG* on the dental plaque accumulation in children with mixed dentition.
Methods Research included 90 children with mixed dentition (5–12 years old). The first study group consumed 200 ml of *Lactobacillus rhamnosus GG*-enriched yogurt (B-Activ LGG, Dukat) daily for a period of 14 days, while the remaining 30 formed the second study group who consumed 200 ml of yogurt (Jogurt 1.5% milk fat, Imlek) with manually added powder from probiotic capsule (Wayaforte LGG capsule, Medis) daily for 14 days. The control group consisted of 30 children who had regular diet during examination period. Silness–Löe plaque index and saliva pH (pH-Fix-0-14, Macherey-Nagel) were determined at baseline and also upon intervention completion.
Results An increase in pH values was observed in both study groups. In general sample, there is a significant decrease of mean plaque index values ($p < 0.001$). Both study groups had significant decrease of mean plaque index values on the baseline and after 14 days consumption of yogurt. In the control group the number of subjects with decrease plaque index values did not correlate and no association was found.
Conclusion Consistent consumption of LGG culture-enriched yogurt inhibits dental film accumulation and promotes saliva pH increase in children with mixed dentition.
Keywords: dental biofilm; probiotics; cariogenic bacterium

INTRODUCTION

The use of probiotics in a wide range of food products is attracting increasing interest due to their potential health benefits. The World Health Organization defines probiotics as "living microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. That microorganisms are usually part of the normal flora and this approach in therapy and prevention was first applied in the treatment of intestinal diseases. The general principle of bacteriotherapy or replacement therapy is to change the local micro-ecology, since the aim of treatment is to introduce and stimulate no pathogenic bacterial species [2]. Probiotic use is considered safe and significant, due to their positive effects, such as immunomodulation, hypocholesterolemic activity, protection against infections, and immune response normalization [3].

Most probiotics are Gram-positive bacteria that belong to the genera *Lactobacillus* or *Bifidobacterium* [4]. Studies based on the use of the intestinal probiotics *Lactobacillus rhamnosus* GG [5], *Lactobacillus reuteri*, and *Bifidobacterium* [6] have each reported achieving reduced levels of *Streptococcus mutans (S. mutans)*.

Probiotics can effectively prevent and treat some infectious diseases in the oral cavity, such as halitosis and periodontitis, and can reduce the development of dental caries and the concentration of harmful bacteria, according to clinical studies [7].

For decades, it has been known that the main cariogenic bacterium *S. mutans* is one of the dental biofilm constituents. It is the most important microorganism for the development of caries, both because of its rapid metabolism of sucrose, glucose and fructose, which lowers the pH, and due to the fact that it alters microbial homeostasis towards the caries-causing flora.

Increased, *S. mutans* count is associated with a higher risk of caries and its more rapid progression. Given the essential role of *S. mutans* in the development of caries, efforts have been made to influence its prevalence and cariogenic ability in the oral cavity.

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After probiotics' use, there have been shown the suppressed growth of *S. mutans* and other oral streptococci with cariogenic potential [8, 9] has been demonstrated *in vitro*.

Clinical trials investigated the effect of probiotics on caries prevalence as a final goal in preschool and school children [8, 10, 11, 12].

In most of these cases, probiotics contained in milk, ice cream, yogurt, and other dairy products have been examined. Empirical evidence also shows that probiotic technology is a revolutionary approach to maintaining optimal oral cavity health [13].

Lactobacillus rhamnosus GG (LGG) is a probiotic with a very important role in preventive dentistry, as it is believed to reduce caries prevalence in children and was shown to confer the same benefits in adults when combined with fluoride [14, 15, 16].

This microorganism is capable of colonizing the oral cavity and thus replacing cariogenic streptococci bound to the tooth surface because the adherent ability of *Lactobacillus rhamnosus* for oral tissues is greater than the adherent ability of streptococci [15]. Short and long-term intake of probiotics could reduce the caries risk among children, decrease gum bleeding and reduce gingivitis, reduce the pocket depth and positively affect the gain of clinical attachment, as well as reduce the counts of *Candida albicans* in elderly [17].

Consequently, the aim of this study was to investigate the impact of consuming strain of *Lactobacillus rhamnosus GG* on dental plaque accumulation in preschool- and school-aged children in period of mixed dentition.

METHODS

The research presented here was conducted at the Dentistry Clinic of Vojvodina in Novi Sad, at the Department of Pediatric and Preventive Dentistry. This randomized double-blind study recruited 90 healthy children with the age range of 5–12 years old of both sexes (mean age = 7.86 ± 1.7 years). Participants had not received any products containing probiotic, xylitol, corticosteroids, systemic antibiotics, and local fluoride therapy at least four weeks before taking part in the study. The study had three parallel groups: 30 children in the control group and two study groups (each group consisting of 30 children). Children from the first study group consumed 200 ml of *Lactobacillus rhamnosus GG* enriched yogurt (B-Activ LGG, Dukat) daily for 14 days, this widely available yogurt includes *L. rhamnosus ATCC53103*. Other study group consumed 200 ml of yogurt without probiotic culture (Jogurt 1.5% milk fat, Imlek) with manually added powder from probiotic capsule (Waya forte LGG capsule, Medis) daily for 14 days, this product also contains the same strain *L. rhamnosus ATCC53103* with at least 10×10^9 colony forming units. Tooth brushing was not allowed at least an hour after eating probiotic yogurt. The control group consisted of children who had normal diet during examination period. Oral hygiene habits were not a factor for inclusion or exclusion from the study. The parents were asked not to change the children`s oral hygiene habits during the two-week period. To ensure the use of the probiotic yogurt participants were asked to fill out a table for two weeks each time they used the yogurt.

Prior to commencing the study, parents of the participating children were informed of all research procedures (Appendix 1: Informing parents / guardians about the study) and provided signed consent for the child's participation (Appendix 2: Parent's / guardian's consent to for their child's participation in the study).

Oral examinations were conducted by an experienced pediatric dentist, using mirrors and periodontal probes under focused flashlights in a conventional dental chair.

Silness–Löe plaque index and saliva pH (pH-Fix-0-14, Macherey-Nagel) were determined for all subjects on the first and last day of the study.

The Silness–Löe plaque index was measured by examining four tooth surfaces (vestibular, vestibulo-mesial, vestibulo-distal, and lingual) with a periodontal probe, and each of the surfaces was rated on a 0–3 scale (0 = no dental biofilm, 1 = small amount of biofilm not visible to the naked eye, 2 = greater amount of biofilm visible to the naked eye, 3 = abundance of dental biofilm). The plaque index was calculated by summing the scores pertaining to all four surfaces of all teeth and dividing by four (number of surfaces examined) and the number of teeth examined in both upper and lower jaw.

The saliva pH was determined using pH-Fix indicators, which measure pH in the range from 0 to 14.

The research was conducted in accordance with the Helsinki declaration and was approved by the Committee on Ethics of the Dentistry Clinic of Vojvodina in Novi Sad.

The data obtained were analyzed using the statistical software *The jamovi project,* Jamovi (2020) (Version 1.6). The Shapiro–Wilk test was used to test normality, prior to selection of appropriate parametric/nonparametric test. The overall data were analyzed using Friedman nonparametric test and Durbin–Conover pairwise comparisons. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

The first study group that consumed yogurt enriched with LGG included 30 children, 14 (46.6%) of whom were boys, and 16 (53.4%) were girls. The second study group that consumed yogurt with manually added probiotic powder incorporated 30 children, 12 (40%) of whom were boys and 18 (60%) were girls. The control group comprised 30 children, 15 (50%) of whom were boys and 15 (50%) were girls.

The distribution of the age of children was analyzed using the Friedman test, which indicates that the sample is uniformed since there is no statistically significant difference in all three examined groups ($p = 0.114$).

The mean pH value does not increase significantly in general sample ($p = 0.155$). Mean pH value did not increase significantly in the first study group which consumed

Table 1. Impact of probiotic treatment on mean pH value at baseline and after 14 days of consuming LGG probiotic culture

Parameters	Control group pH 1 (n = 30)	Control group pH 2 (n = 30)	Study group yogurt pH 1 (n = 30)	Study group yogurt pH 2 (n = 30)	Study group capsule pH 1 (n = 30)	Study group capsule pH 2 (n = 30)
Mean	6.43	6.43	6.70	6.90	6.63	6.60
Median	6.50	6.50	7.00	7.00	7.00	7.00
Standard deviation	0.626	0.626	0.702	0.662	0.490	0.498
Shapiro–Wilk W	0.742	0.742	0.781	0.794	0.612	0.624
Shapiro–Wilk p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

*pH 1 – pH value at the baseline
*pH 2 – pH value after 14 days

Table 2. Comparison of mean pH values of all three studied groups using Pairwise Comparison (Durbin–Conover) test

Groups		Statistic	p
Control g. pH 1	Control g. pH 2	0.0000	1.000
Control g. pH 1	Study g. yogurt pH 1	1.1122	0.268
Control g. pH 1	Study g. capsule pH 1	1.0232	0.308
Control g. pH 2	Study g. yogurt pH 2	2.4468	0.016
Control g. pH 2	Study g. capsule pH 2	0.7563	0.451
Study g. yogurt pH 1	Study g. yogurt pH 2	1.3346	0.184
Study g. yogurt pH 1	Study g. capsule pH 1	0.0890	0.929
Study g. yogurt pH 2	Study g. capsule pH 2	1.6905	0.093
Study g. capsule pH 1	Study g. capsule pH 2	0.2669	0.790

*pH 1 – mean pH value at baseline
*pH 2 – mean pH value after 14 days

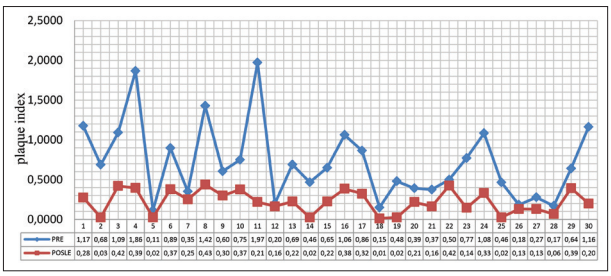

Figure 1. Plaque index values at the baseline and 14 days after intake of commercial yogurt

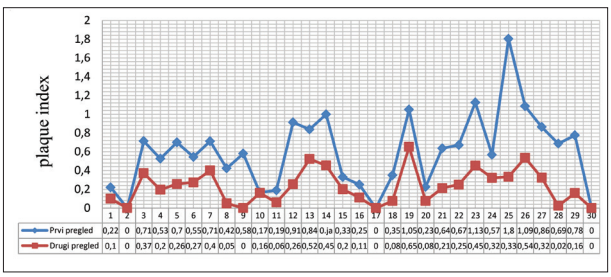

Figure 2. Plaque index values at the baseline and 14 days after intake of yogurt with manually added probiotic powder

commercial yogurt, at the baseline and after 14 days of consuming. The second study group which consumed yogurt with manually added probiotic powder also did not show significantly increase of mean pH value before and after consuming probiotic. The findings are reported in Tables 1 and 2.

In the general sample, there is significant decrease in mean plaque index values ($p < 0.001$). In the first study group, there is statistically significant decrease of mean plaque index value on the baseline and after 14 days consumption of commercially available yogurt ($p < 0.01$). The baseline and after 14 days Silness–Löe plaque index values ranged from 0.113 to 1.972, and 0.013 to 0.437, respectively, as shown in Figure 1. It is evident from the graph that LGG yogurt consumption has led to marked plaque index reduction. The results of mean plaque index values in the second study group who consumed yogurt with probiotic powder from capsules, also showed a significant reduction ($p < 0.01$). In the second study group, the baseline and after 14 days Silness–Löe plaque index values ranged from 0. to 1.803, and 0 to 0.523, respectively, as shown in Figure 2. In the control group, the number of subjects with decrease plaque index values did not correlate and no association was found. Statistically significant difference is noticed between mean plaque index values (PI2 – after 14 days) of control group and both study groups mean values of plaque index after consumption of LGG culture ($p < 0.01$).

There is no significant difference in mean plaque index values between two study groups at the end of the observation period ($p > 0.05$). The findings are reported in Tables 3 and 4.

DISCUSSION

The aim of the present study was to demonstrate the effect of the use of *L. rhamnosus* enriched commercial yogurt and yogurt with manually added probiotic powder on the degree of dental plaque accumulation after two weeks of consumption. The results obtained by analyzing the saliva pH and plaque index in children measured before and after consuming yogurt indicated an increase in pH and a significant decrease in dental biofilm in all participants, from these facts we could indirectly infer a decreased presence of *S. mutans*. The mean saliva pH value at baseline in first study group was 6.70, increasing to 6.90 after two weeks of commercial yogurt intake. As expected [2, 4, 12], the amount of dental biofilm declined, as indicated by the mean plaque index of 0.717 and 0.224, before and after intervention, respectively. Although the second study group which consumed yogurt with manually added probiotic powder, did not show increase of mean pH value, the amount of dental biofilm declined, as indicated by the mean plaque index of 0.598 at the baseline, and 0.227 after 14 days.

In the majority of studies examining the effect of probiotics on the oral microflora, probiotics were consumed for up to 15 days, which is in line with the methodology adopted in the present investigation [6, 18–25]. The most

Table 3. Impact of probiotic treatment on plaque index value at baseline and after 14 days of consuming LGG probiotic culture

Parameters	Control group Plaque index 1	Control group Plaque index 2	Study group yogurt Plaque index 1	Study group yogurt Plaque index 2	Study group capsule Plaque index 1	Study group capsule Plaque index 2
Mean	0.668	0.622	0.717	0.224	0.598	0.227
Median	0.633	0.705	0.645	0.222	0.609	0.206
Standard deviation	0.428	0.361	0.477	0.142	0.400	0.180
Shapiro–Wilk W	0.938	0.954	0.915	0.921	0.948	0.943

*Plaque index 1 – mean value of Plaque index at baseline
*Plaque index 2 – mean value of Plaque index after 14 days

Table 4. Comparison of mean plaque index values of all three examined groups using Pairwise Comparison (Durbin–Conover) test

Group comparison	Statistic	p
Control group PI 1 – Control group PI 2	0.843	0.401
Control group PI 1 – Study group yogurt PI 1	0.295	0.768
Control group PI 1 – Study group capsule PI 1	0.674	0.501
Control group PI 2 – Study group yogurt PI 2	4.762	< 0.001
Control group PI 2 – Study group capsule PI 2	5.142	< 0.001
Study group yogurt PI 1 – Study group yogurt PI 2	5.310	< 0.001
Study group yogurt PI 1 – Study group capsule PI 1	0.379	0.705
Study group yogurt PI 2 – Study group capsule PI 2	0.379	0.705
Study group capsule PI 1 – Study group capsule PI 2	5.310	< 0.001

*PI 1 – mean value of Plaque index at baseline
*PI 2 – mean value of Plaque index after 14 days

commonly studied probiotics are those contained in fortified milk, ice cream, yogurt, and other dairy products. For example, Chinnappa et al. [24] observed a decrease in *S. mutans* count after a week-long daily consumption of ice cream and whey containing a probiotic. Similar results were obtained by Caglar et al. [6] with *Bifidobacterium lactis*, Jiang et al. [17] with the probiotic *L. rhamnosus*, Hedayati-Hajikand [11] with ProBiora3® blend of three strains of probiotic bacteria (*S. uberis* KJ2™, *S. oralis* KJ3™, *S. rattus* JH145™), and Burton et al. [26] using *S. salivarius M18*.

Very important factor is the use of different means of delivery such as dairy products, chewing gums and drops to transfer probiotics. The probiotic channels of supply are suitable for all ages, especially for young children [27]. In the presented study, yogurt is selected because it is safe, available and used routinely in Serbian children's diet. Unfortunately, the commercial yogurt, which is used in the study is not available in our country, although it is produced in Serbia. In conclusion, it appears that yogurt with manually added probiotic powder has shown same effect on the formation of dental plaque, even though commercial yogurt is easier to use.

The effects of *Lactobacillus acidophilus ATCC 4356* and *Bifidobacteriumbifidum ATCC 29521* changing *S. mutans* counts have been evaluated in study conducted by Ghasemi et al. [27], in both groups, S. mutans counts on the first day, second week, and fourth weeks after the intervention were significantly lower than baseline values.

L. rhamnosus is capable of colonizing the oral cavity and thus replacing cariogenic streptococci bound to the tooth surface because the adherent ability of *Lactobacillus rhamnosus* for oral tissues is greater than the adherent ability of streptococci. Hukioja et al. [28] noted that *L. rhamnosus GG* adheres well to hydroxyapatite, although there is a difference in the quality of adhesion between different individuals.

Despite the issue having been addressed in numerous studies and their findings indicated that the *Lactobacillus* probiotic plays a beneficial role in caries prevention, the exact mechanism of probiotic action has not been established [15, 16, 17, 19, 20, 22, 25, 26, 27].

Based on a review of the available literature, it can be presumed that the microflora in children is less stable and more susceptible to change compared to the microbial communities in adults. Consequently, probiotics may have a more lasting effect on the resident microbial population in children [16]. Owing to this disparity, the work presented here focused specifically on preschool- and school-aged children with mixed dentition.

Numerous strains of lactobacilli have been identified, but only a small subset of these strains promotes caries development. Available evidence indicates that *L. salivarius w24*, owing to its sucrose metabolism and pH-reducing capability, could act cariogenically. On the other hand, *L. rhamnosus*, *L. paracasei*, and *L. reuteri* can have a safe and positive effect on caries inhibition. Nonetheless, *in vitro* results preclude specific conclusions and recommendations. It can generally be stated that the effect of lactobacilli may be desirable in the case of carefully selected probiotic candidates [25].

In more recent literature, the role of prebiotics and symbiotics is increasingly being emphasized, in terms of promoting the growth of probiotic bacteria. Bijle at al. [29] demonstrated the usefulness and benign effects of a novel symbiotic with synergistic inhibitory effect on cariogenic bacterium *S. mutans*. Their results demonstrated that the use of L-arginine as a prebiotic enhanced the growth of the probiotic – *L. rhamnosus* GG, whose increase prevents adhesion of *S. mutans*. This observation and application of symbiotics and probiotics opens up opportunities for our new research.

It is important to emphasize that even strains of the same species have different characteristics and each should be individually investigated. It is possible that the same species is not optimal for all oral conditions, hence bacteriotherapy should be tailored to the oral health status of each individual.

CONCLUSION

Regular consumption of LGG enriched yogurt (*Lactobacillus rhamnosus GG*) has an inhibitory effect on the accumulation of dental biofilm and promotes saliva pH increase in children with mixed dentition. By using probiotic products, it is possible to modify dental biofilm composition and metabolism. Since microflora in children is more susceptible to change, it would be advantageous to include products such as LGG enriched yogurt in the regular diet of children of preschool and school age as a means of caries prevention.

Conflict of interest: None declared.

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Улога јогурта обогаћеног пробиотском културум *Lactobacillus rhamnosus GG* у превенцији каријеса

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САЖЕТАК

Увод/Циљ Савремени трендови говоре у прилог томе да пробитици могу значајано да утичу на смањење преваленце каријеса у дечјем узрасту, те се сматра да позитивно утичу на опште и орално здравље. Пробиотска култура која је у области превентивне стоматологије показала значајне резултате је *Lactobacillus rhamnosus GG* (ЛГГ).
Циљ рада је испитати утицај употребе соја ЛГГ на акумулацију денталног плака код деце са мешовитом дентицијом.

Методе У истраживању је учествовало укупно 90 деце у периоду мешовите дентиције (узраста 5–12 година). Прву екперименталну групу чинило је 30 испитаника који су током 14 дана конзумирали једном дневно по 200 *ml* јогурта са додатком ЛГГ (*B-Activ LGG*, Дукат), другу групу чинило је 30 деце који су користили једном дневно прах из пробиотске капсуле (*Waya forte* ЛГГ капсуле, Медис) растворен у 200 *ml* јогурта који раније није садржао пробиотске културе (јогурт 1,5% млечне масти, Имлек) током 14 дана. Контролну групу чинило је 30 испитаника који су се уобичајено хранили. Плак индекс по Силнесу и Лоу и *pH* вредност пљувачке (*pH-Fix-0-14*, *Macherey-Nagel*) одређивани су првог и последњег дана истраживања.

Резултати У обе експерименталне групе уочен је пораст средњих *pH* вредности. У целокупном узорку дошло је до смањења средњих вредности плак индекса ($p < 0,01$). У обе експерименталне групе дошло је до значајног смањења средњих вредности плак индекса на почетку истраживања и након 14-дневног конзумирања јогурта обогаћеног ЛГГ културом. У контролној групи није забележена промена средњих вредности плак индекса.

Закључак Редовна употреба јогурта обогаћеног ЛГГ културом делује инхибиторно на акумулацију денталног биофилма и подстиче пораст *pH* вредности пљувачке код деце са мешовитом дентицијом.

Кључне речи: дентални биофилм; пробиотици; кариогене бактерије

APPENDIX 1

Informing parents / guardians about the study titled:

The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention

Dear parents,

This scientific study aims to demonstrate that the consumption of LGG culture (*Lactobacillus rhamnosus*) reduces the microbial flora of the oral cavity and plays a significant role in the prevention of caries.

The study involves a two-week consumption of LGG yogurt (b-Activ LGG, Dukat) or LGG probiotic capsules, before and after which a dental examination will be performed, including assessment of the plaque deposits on teeth and measuring the acidity of saliva.

The study poses no risk to the physical or mental health of your child. It is conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Dentistry Clinic of Vojvodina.

Your child's participation in this research is voluntary and yields no material gain to you or your child. You can withdraw your child from the investigation at any time without any consequences. The data obtained will be treated as confidential and will be used only for the purposes of this research.

If you agree with your child's participation in this scientific research, please sign the form "Parent's / guardian's consent for their child's participation in the study."

Thank you for your cooperation.

APPENDIX 2

Parent's / guardian's consent for their child's participation in the study titled:

The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention

I am fully informed of the details of the scientific research entitled "The role of probiotic culture *Lactobacillus rhamnosus GG* in caries prevention". I have read the information related to the planned investigation, and was given the opportunity to ask further questions about this study, whereby all my queries have been addressed to my satisfaction.

I consent to my child's participation in the aforementioned research study and am aware that I can withdraw my consent at any time without any consequences.

Child's first name and surname: _____

Child's date of birth: _____

Parent's/guardian's first name and surname: _____

Date: _____

Parent's/guardian's signature: _____

Researcher's signature: _____

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Mesiodistal dimensions of teeth in Serbian orthodontic patients with hypodontia

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SUMMARY

Introduction/Objective Hypodontia is a common dental anomaly that occurs either in a non-syndromic form or as a part of various syndromes. It is considered a multifactorial condition with genetic, epigenetic, and environmental influences, the interplay of which can lead to various anomalies in tooth size and number.

The aim of this study was to assess mesiodistal tooth dimensions in Serbian hypodontia orthodontic patients and compare them to healthy controls using digital study models.

Methods Fifty subjects (30 females, 20 males) divided into two groups – 25 with hypodontia (15 females, 10 males) and 25 sex-matched controls (15 females, 10 males) – were included in the study. Alginate impressions were taken and plaster models poured, digitized, and imported into software where mesiodistal dimensions were obtained.

Results Intra-operator reliability was high. All teeth in the hypodontia group had smaller mesiodistal dimensions compared to controls. Statistical significance was noted for all teeth except for upper canines. No statistically significant differences were found between males and females in neither the hypodontia nor the control group, except for lower canines, which were significantly smaller in both hypodontia and control females. The most commonly missing teeth were upper lateral incisors, and lower and upper premolars.

Conclusion Hypodontia group presented with smaller mesiodistal dimensions compared to controls. The greatest difference in mesiodistal dimensions was found in upper lateral incisors and lower first molars. Lower canines were significantly larger in males compared to females in both groups.

Keywords: hypodontia; tooth agenesis; mesiodistal dimensions; tooth size

INTRODUCTION

Tooth agenesis is a common dental anomaly that occurs either in a non-syndromic form or as a part of various syndromes. Non-syndromic hypodontia of permanent teeth is one of the most common developmental dental anomalies in humans. Different terms, such as hypodontia, oligodontia, and anodontia are used to describe it. Hypodontia is used when one to six teeth (excluding third molars) are congenitally missing. Oligodontia means that more than six teeth (excluding third molars) are missing, whereas anodontia denotes extreme cases of complete absence of teeth. Hypodontia is more common in permanent than in primary dentition. According to the literature, prevalence of tooth agenesis in permanent dentition varies 1.6–36.5% depending on the population [1]. Results of a recent systematic review on the prevalence of hypodontia, which included 93 studies from 2002 to 2012, concluded the prevalence of hypodontia was 6.4%. The same study found statistically significant differences in the geographic prevalence of hypodontia. It was highest in Africa (13.4%), followed by Europe (7%), Asia (6.3%), and Australia (6.3%), with the lowest prevalence in North America (5%) and Latin America and the Caribbean (4.4%). However, the authors did not find statistically significant differences in prevalence depending on the examined population, i.e., school children, dental patients, and orthodontic patients. This study also showed that most commonly, one or two teeth were congenitally missing (about 81%), three to five teeth were missing in 14% of cases, while six or more teeth were missing in only 3% of cases [2]. Janošević et al. [3] have reported the prevalence of hypodontia in Serbian children to be 6.28%, which is similar to hypodontia prevalence amongst other south-Slavic nations, i.e., in the Croatian (5.52%), Slovenian (6.9%), and Macedonian (7.52%) populations [4, 5, 6]. Several studies reported hypodontia to be more prevalent in females than in males [2, 7].

The etiology of tooth agenesis is still unclear. Hypodontia has been regarded as a multifactorial condition with genetic, epigenetic and environmental influences, the interplay of which can lead to various anomalies in tooth size and number [8]. Hundreds of genes have been connected with the patterning, morphogenesis, and cell differentiation in teeth so far [9]. Numerous studies have reported on the connection between tooth number and tooth size anomalies, and most of them have concluded that tooth dimensions were smaller in

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patients with hypodontia compared to controls [10, 11, 12]. Furthermore, a reduction in tooth size has also been observed in unaffected relatives of hypodontia patients [13], indicating a genetic influence on the link between the number and size of teeth.

Tooth size discrepancies affect buccal interdigitation, overjet, overbite, and midline position. Moreover, several authors have concluded that hypodontia impacts functional and psychosocial aspects of the patient's well-being, therefore affecting their quality of life [14–17]. Thus, the evaluation of tooth size and tooth number anomalies plays an important part in orthodontic diagnosis and treatment planning [13]. Both researchers and clinicians have used different techniques to evaluate and quantify tooth size and shape. The most common tool used for more than a century has been a caliper, which has been modernized into a digital caliper. Three-dimensional (3D) imaging and scanning has been introduced to orthodontics at the beginning of the 21st century. Laser scanners, cone-beam computed tomography scanners, stereophotogrammetry, amongst others, have been used for obtaining 3D images of teeth, jaws, and soft-tissues for over a decade now [18, 19]. Apart from being used to store patients' models and information electronically, 3D imaging has also found its place in virtual 3D diagnostics and tooth movement analyses by superimposition of pretreatment and posttreatment models [20, 21].

The aim of our study was to assess mesiodistal tooth dimensions in hypodontia patients and compare them to those of healthy controls using digital study models.

METHODS

Study sample

The study involved 50 subjects (30 females, 20 males) treated at the Department of Orthodontics, School of Dental Medicine, University of Belgrade. The sample was divided into two groups – one consisted of 25 subjects (15 females, 10 males) with hypodontia and the other consisted of 25 sex-matched controls (15 females, 10 males) without hypodontia.

Inclusion criteria

Inclusion criteria for the hypodontia group were as follows:

- one or more congenitally missing teeth (excluding third molars);
- no evidence, reported by the patient or noted upon clinical examination, of any syndrome known to be associated with hypodontia.

Inclusion criteria for the control group were as follows:

- no sign of hypodontia (excluding third molars);
- sex-matched to the hypodontia group.

Diagnosis of tooth agenesis was based on clinical examination, panoramic radiographs and anamnestic data. Deciduous teeth, erupting teeth, impacted teeth, teeth with large lesions or dental restorations, and teeth with defects on the dental casts were excluded from the study. Sixty-seven teeth were excluded from the hypodontia group, while 82 teeth were congenitally missing. Thirty-eight teeth were excluded from the control group. Upper and lower incisors, canines, premolars and first molars were measured. A total number of 451 teeth were measured in the hypodontia group and 562 in the control group. In the hypodontia group, 56% of patients had one or two teeth congenitally missing, 28% had three to six teeth missing, and 16% had more than six teeth missing.

This research was approved by the Human Research Ethics Committee of the School of Dental Medicine, University of Belgrade (resolution number 36/31 from December 4, 2014).

Data collection

Alginate impressions were taken for patients in both groups. Plaster models were poured on the same day and study models were trimmed. Each study model was positioned on a stand and scanned by a single operator (MZ) using the NextEngine 3D scanner HD (Next Engine Inc., Santa Monica, CA, USA; Figure 1). Digitized study models were saved as stereolithography (.stl) files and imported into the Geomagic Control software (Raindrop

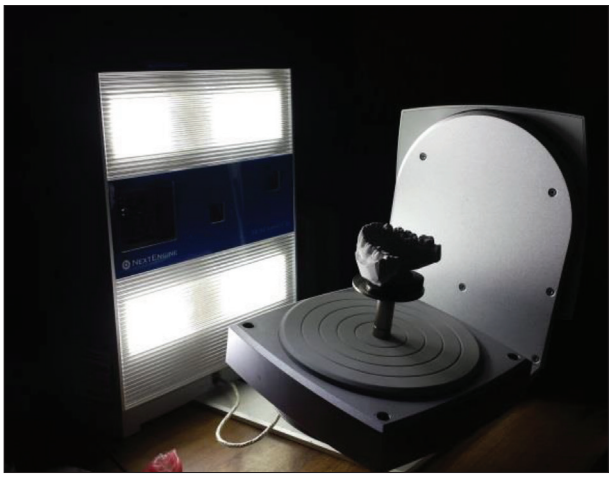

Figure 1. Scanning of a study model positioned on a stand with NextEngine 3D scanner HD (Next Engine Inc., Santa Monica, CA, USA)

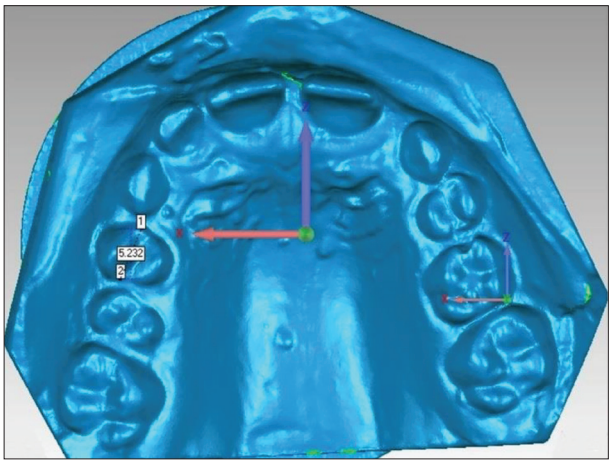

Figure 2. Measuring the mesiodistal dimension of upper right first premolar in the Geomagic software

Geomagic Inc, Cary, NC, USA), where they were converted to the .wrp format, a file format proprietary to Geomagic. Mesiodistal crown width was measured as the greatest distance between the contact points on the interproximal surfaces of tooth crowns (Figure 2). Upper and lower incisors, canines, premolars and first molars were measured on both the left and the right side of each dental arch and the dimensions were averaged. All measuring was done by the same operator (MZ). All teeth in the hypodontia group were measured twice, approximately one week apart. A total number of 451 teeth from the hypodontia group were included in the intra-operator error study. The values for left and right teeth were averaged and compared between two measurements.

Statistical analysis

All recorded data was analyzed using IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov–Smirnov test revealed the sample was normally distributed – therefore, parametric tests were used. The independent samples t-test was used to evaluate whether the diagnosis (presence/absence of hypodontia) and sex had an effect on the measurements. Paired samples t-test was used to evaluate the differences between measurements. Level of significance was set at $p < 0.05$.

RESULTS

Intra-operator reliability levels were high, with no statistically significant differences between the two sets of measurements (Table 1).

All the teeth in the hypodontia group had smaller mesiodistal dimensions compared to the controls (Tables 2 and 3). Statistically significant differences in mesiodistal dimensions between the groups were noted for all teeth, except for upper canines (Table 2). Upper lateral incisors (Table 2) and lower first molars (Table 3) showed the greatest differences in mesiodistal dimensions between the hypodontia and the control group.

No statistically significant differences were found between males and females in neither the hypodontia (Tables 4 and 5) nor the control group (Tables 5 and 6), except for lower canines, which were significantly smaller in females in both the hypodontia (Table 5) and the control group (Table 7).

Table 1. Intra-operator error assessment between two measurements in the hypodontia group

Tooth	Maxilla			Mandible		
	n	Error (mm)	SD	n	Error (mm)	SD
I1	25	0	0.24	24	-0.08	0.23
I2	13	0.07	0.21	25	0.02	0.22
C	15	0.08	0.31	20	-0.01	0.29
PM1	21	0.02	0.19	19	0.07	0.19
PM2	13	0.02	0.24	12	-0.01	0.15
M1	24	0.02	0.17	24	0.10	0.33

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar

Table 2. Mean values and standard deviations for the hypodontia and the control group – maxilla

Tooth	Group	n	Mean	SD	p
I1	Hypodontia	25	8.04	0.45	< 0.001***
	Control	25	8.59	0.48	
I2	Hypodontia	13	5.47	0.74	< 0.001***
	Control	25	6.74	0.48	
C	Hypodontia	15	7.44	0.54	0.114
	Control	25	7.67	0.38	
PM1	Hypodontia	21	6.43	0.46	0.001**
	Control	14	7.03	0.44	
PM2	Hypodontia	13	6.16	0.43	0.003**
	Control	25	6.61	0.42	
M1	Hypodontia	24	9.25	0.59	< 0.001***
	Control	25	10	0.53	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 3. Mean values and standard deviations for the hypodontia and the control group – mandible

Tooth	Group	n	Mean	SD	p
I1	Hypodontia	24	5	0.4	< 0.001***
	Control	25	5.4	0.27	
I2	Hypodontia	25	5.49	0.38	< 0.001***
	Control	25	6.01	0.32	
C	Hypodontia	20	6.59	0.51	0.029*
	Control	25	6.89	0.38	
PM1	Hypodontia	19	6.51	0.57	0.006**
	Control	21	6.95	0.39	
PM2	Hypodontia	12	6.33	0.42	< 0.001***
	Control	25	6.96	0.48	
M1	Hypodontia	24	9.38	0.72	< 0.001***
	Control	24	10.44	0.58	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 4. Mesiodistal dimensions of teeth by sex in the maxilla – hypodontia group

Tooth	Sex	n	Mean	SD	p
I1	Male	10	7.86	0.45	0.104
	Female	15	8.16	0.43	
I2	Male	5	5.56	0.61	0.753
	Female	8	5.41	0.85	
C	Male	5	7.61	0.69	0.397
	Female	10	7.35	0.47	
PM1	Male	7	6.34	0.25	0.541
	Female	14	6.48	0.53	
PM2	Male	5	5.82	0.3	0.018*
	Female	8	6.37	0.37	
M1	Male	9	9.28	0.68	0.841
	Female	15	9.23	0.55	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 5. Mesiodistal dimensions of teeth by sex in the mandible – hypodontia group

Tooth	Sex	n	Mean	SD	p
I1	Male	9	5.12	0.48	0.304
	Female	15	4.94	0.35	
I2	Male	10	5.58	0.4	0.312
	Female	15	5.43	0.36	
C	Male	7	6.90	0.48	0.040*
	Female	13	6.42	0.46	
PM1	Male	7	6.70	0.7	0.265
	Female	12	6.39	0.48	
PM2	Male	5	6.2	0.21	0.395
	Female	7	6.42	0.52	
M1	Male	9	9.23	0.96	0.434
	Female	15	9.47	0.55	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 6. Mesiodistal dimensions of teeth by sex in the maxilla – control group

Tooth	Sex	n	Mean	SD	p
I1	Male	10	8.68	0.54	0.414
	Female	15	8.52	0.45	
I2	Male	10	6.94	0.34	0.098
	Female	15	6.61	0.53	
C	Male	10	7.75	0.37	0.454
	Female	15	7.63	0.4	
PM1	Male	9	7.13	0.46	0.264
	Female	5	6.85	0.38	
PM2	Male	10	6.61	0.41	0.977
	Female	15	6.61	0.44	
M1	Male	10	9.97	0.42	0.810
	Female	15	10.02	0.61	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

Table 7. Mesiodistal dimensions of teeth by sex in the mandible – control group

Tooth	Sex	n	Mean	SD	p
I1	Male	10	5.40	0.31	0.982
	Female	15	5.4	0.26	
I2	Male	10	6.01	0.31	0.947
	Female	15	6.01	0.33	
C	Male	10	7.12	0.42	0.012*
	Female	15	6.74	0.28	
PM1	Male	10	6.98	0.45	0.742
	Female	11	6.92	0.34	
PM2	Male	10	6.92	0.44	0.762
	Female	15	6.98	0.52	
M1	Male	9	10.66	0.43	0.140
	Female	15	10.3	0.63	

I1 – central incisor; I2 – lateral incisor; C – canine; PM1 – first premolar; PM2 – second premolar; M1 – first molar;
*p < 0.05;
**p < 0.01;
***p < 0.001

The most commonly missing teeth in our study sample were upper lateral incisors (35% of all congenitally missing teeth), followed by lower (24%) and upper (16%) second premolars.

DISCUSSION

The purpose of this study was to evaluate and compare mesiodistal dimensions of teeth in patients with and without hypodontia using 3D scans of dental casts and the Geomagic software. Results of our study showed that patients with hypodontia had significantly smaller mesiodistal crown dimensions compared to controls, except for upper canines, where no statistical significance was found. This is in accordance with the results published by Brook et al. [10], Gungor and Turkkahraman [11], Al Shahrani et al. [22], Fekonja [23], and Kerekes-Mathe et al. [24]. Brook et al. [10] have also found no statistically significant differences for upper canines, and upper left, and lower right first premolars in females. However, in their hypodontia male group, all teeth were significantly smaller compared to controls, except for lower right central incisors and upper right first and second premolars, which even showed an increase. They have found the difference in size to be greater in patients with more severe hypodontia [11]. Fekonja [23] has found teeth in the hypodontia group to be significantly smaller compared to controls as well, and so have Kerekes-Mathe et al. [24]. Al-Shahrani et al. [22] have similarly reported a decrease in tooth dimension in the hypodontia group compared to controls, with statistical significance present in the severe hypodontia group only, while Gungor and Turkkahraman [11] reported statistical significance for both the mild and the severe hypodontia group, the latter showing greater differences.

According to the results of our study, upper lateral incisors and lower first molars showed the greatest differences in mesiodistal dimensions between the hypodontia group and the controls. The fact that upper lateral incisors showed that the greatest difference is in line with the theory of morphogenetic fields (incisor, canine, premolar, molar), according to which "key teeth" (maxillary central incisor, mandibular lateral incisor, canine, first premolar, first molar) display the highest heritability, whereas those positioned more distally within the field show lower heritability, and therefore are more prone to morphological variability [25]. Several studies, including a recent one of the Croatian population, by Vidaković et al. [26], have confirmed this theory, while other authors failed to find proof for these trends [27]. The reasons stated in the research of authors who failed to find proof for the morphogenetic fields theory might explain the fact that in our research lower first molars, which are considered "key" teeth, showed greater variability and were significantly smaller in the hypodontia group compared to the control group. Gungor and Turkkahraman [11] found upper first premolars (mild hypodontia group) and upper lateral incisors (severe hypodontia group) to be the teeth with the greatest differences in mesiodistal dimensions. According to the results published by Brook et al [10], upper lateral

incisors were again the teeth with the smallest mesiodistal dimensions, followed by lower central incisors. These authors have also found upper first molars and lower canines to be markedly smaller in the female hypodontia group of their sample. On the other hand, Kerekes-Mathe et al. [24] reported that in female subjects, upper first premolars had the smallest dimensions, followed by upper canines, upper central incisors, lower central incisors, and lower second premolars. In male subjects of the same study, teeth with the smallest dimensions were upper central incisors, upper lateral incisors, upper canines, lower second premolars and lower central incisors, respectively. According to a recent study by Khalaf et al. [2], upper lateral incisors were, again, the most affected in terms of tooth size reduction, whereas the least affected were lower first molars, which is opposite to our findings.

Comparing tooth sizes between sexes in our sample has revealed that lower canines had significantly greater mesiodistal dimensions in males than in females in both groups. However, in the hypodontia group upper second premolars were significantly larger in females. This might not have been the case if larger samples had been available, since second premolars are often congenitally missing, and only five second premolars were available in the male hypodontia group, and eight in the female hypodontia group of our sample. Insignificantly larger mesiodistal dimension in females compared to males were found for upper first premolars and central incisors, and lower second premolars and first molars in the hypodontia group, and for upper first molars and lower second premolars in the control group. Gungor and Turkkahraman [11] also found insignificantly larger upper central incisors in the female hypodontia group, while Fekonja [23] found insignificantly larger upper left first premolars and upper right first molars in the female hypodontia group. Kerekes-Mathe et al. [24] reported significantly smaller tooth crown dimensions in females compared to males of the hypodontia group.

The most commonly missing teeth in our study sample were upper lateral incisors, followed by lower and upper second premolars. These results are in line with those published in the meta-analyses by Khalaf et al. [2] and Polder et al. [7], which reported the most commonly congenitally missing teeth were lower second premolars, upper lateral incisors, and upper second premolars. Same results were published by Janošević et al. [3], where lower second premolars and upper lateral incisors were the most commonly missing teeth in the Serbian population sample.

Even though we did not evaluate the reliability or accuracy of digital versus plaster study model measurements, we thought we should mention that most studies published so far have found excellent reproducibility, reliability, and accuracy of measurements made on scanned digital models and the differences between measurements made on digital and plaster models were clinically acceptable and reproducible [28, 29]. The software used in our research, Geomagic, was also used by Zhou et al. [30], who found the mean difference between the plaster and virtual model measurements were approximately 0.05 mm. That is both clinically and statistically insignificant and speaks in favor of the reliability of the measurements obtained in our study. High intra-operator reliability levels and no significant differences between measurements in our study also confirm the reliability of Geomagic software for obtaining mesiodistal tooth dimensions.

CONCLUSION

Hypodontia group presented with smaller mesiodistal dimensions compared to healthy controls. The greatest difference in mesiodistal dimensions was found in upper lateral incisors and lower first molars. Lower canines were significantly larger in males compared to females in both groups.

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Мезиодисталне димензије зуба ортодонтских пацијената са хиподонцијом у српској популацији

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САЖЕТАК

Увод/Циљ Хиподонција је честа стоматолошка аномалија која се јавља одвојено или у оквиру различитих синдрома. Сматра се мултифакторијалним обољењем на које утичу генетика, епигенетика и спољашња средина, чија интеракција доводи до различитих неправилности у величини и броју зуба.
Циљ ове студије био је да се одреде мезиодисталне димензије зуба пацијената са хиподонцијом и да се упореде са промерима зуба здравих пацијената помоћу дигиталних студијских модела.

Методе Педесет пацијената (30 особа женског пола, 20 особа мушког пола) подељено у две групе – 25 са хиподонцијом (15 особа женског пола, 10 особа мушког пола) и 25 полно усклађених здравих особа (15 особа женског пола, 10 особа мушког пола) – укључено је у истраживање. На основу алгинатних отисака изливени су гипсани модели, који су дигитализовани и унети у рачунарски програм у коме су одређени мезиодистални промери зуба.

Резултати Интраоператорска поузданост је била висока. Сви зуби пацијената са хиподонцијом су имали мање мезиодисталне промере у поређењу са контролном групом. Статистичка значајност је уочена за све зубе, осим за горње очњаке. Статистички значајне разлике између полова нису уочене ни у оквиру групе са хиподонцијом ни у оквиру контролне групе, осим за доње очњаке, који су били значајно мањи код особа женског пола у обе групе. Најчешће су недостајали латерални секутићи, затим доњи, па горњи премолари.

Закључак Пацијенти са хиподонцијом су имали мање мезиодисталне промере зуба у односу на контролну групу. Највећа разлика је уочена код горњих латералних секутића и доњих првих молара. Доњи очњаци су били значајно већи код особа мушког пола у обе групе.

Кључне речи: хиподонција; агенеза зуба; мезиодисталне димензије; величина зуба

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Lithium disilicate and PEEK implant-retained single crowns – a randomized, prospective clinical study

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SUMMARY

Introduction/Objective Comparing two materials under the same conditions is the best way to define differences between them. Ceramic-reinforced polyether-etherketone (PEEK) is a polymer that has many possible uses in dentistry as already well-known lithium disilicate ceramics.
The aim of this study was to compare peri-implant soft tissue healing and evaluate patient satisfaction with esthetics in different observation periods, as well as the success and survival rate of both types of crowns.
Methods The study was conducted as a clinical, prospective, randomized split-mouth study on 17 patients with bilaterally missing upper teeth of the same type, replaced with dental implants. Study outcomes have been analyzed with subjective (visual analogue scale – VAS scale) and objective parameters (modified bleeding index – MBI, modified plaque index – MPI and peri-implant probing depth – PPD) baseline, six and twelve months after fixing crowns onto the implants.
Results Comparison of the results between PEEK and lithium disilicate crowns showed no statistical differences in terms of MPI, MBI, and PPD in the observed periods. Analyzing MPI during observation periods in the PEEK group of crowns, statistical significance was registered between baseline values and after six months. Also, statistical significance was noticed in terms of PPD during the observation time both in the study and control group of crowns. Results for VAS for the esthetics showed no statistically significant difference between the groups, while VAS for restoration satisfaction showed a statistically significant difference.
Conclusion This study showed that scores of the applied subjective and objective parameters can be a reliable tool to rate the clinical outcome of implant-retained single crowns over time.
Keywords: lithium disilicate; PEEK; single crowns; implants

INTRODUCTION

Nowadays, all-ceramic materials are frequently used for implant-retained single crowns to improve the esthetic result. As the esthetic demands in implant treatment have increased, the abutments started to be fabricated of ceramic materials, which are designed as one component and Titanium base (Ti-base) abutments. The most often used materials for this purpose are zirconia and lithium disilicate ceramic [1]. These materials are also used for implant-retained fixed restorations. Apart from ceramic materials, there are some new polymer materials on the market that are used for prosthetic restorations in conventional and implant prosthodontics. Lithium disilicate is a well-known material, which can be used for single crowns and all-ceramic fixed partial dentures framework veneered with ceramic [2, 3]. Crystals of lithium disilicate of 0.5–0.6 μm diameter are added to the glass matrix, depending on the technological method of fabrication. Lithium disilicate ceramic can be fabricated with a "press" technique in the laboratory, or CAD-CAM technique by the milling process, for chairside and laboratory settings. Both materials can be fabricated in full contour, stained and glazed in the cut back-body form layered with ceramics [4].

Also, there are some polymer materials on the market used for prosthetic restorations in conventional and implant prosthetics, and one of these materials is ceramic-reinforced polyether-etherketone (PEEK) with 30% of ceramic particles [5]. This material has constant homogeneity due to reinforced ceramic particles of 0.3 to 0.5 μm diameters [6, 7]. As crystalline thermoplastic resin is reinforced with ceramic particles, it withstands extreme forces [5, 6, 7]. It is biologically stable, so there are no reactions with other materials and ion exchanges. Also, there is no galvanic cell in the oral cavity and it does not cause pigmentation [5, 6, 7]. This material shows good biological properties in terms of biocompatibility; moreover, its elasticity is similar to human biomechanics [5, 6, 7]. As a base for prosthetic restoration, it satisfies the high esthetic criteria of contemporary implant dentistry. Due to its white color, it is an ideal base for veneering with conventional composite materials. It can be highly polished, so it does not cause abrasion of antagonist teeth [5, 6, 7].

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The aim of this study was to compare lithium disilicate and PEEK implant-retained single crowns, in terms of peri-implant soft tissue healing, esthetics and restoration satisfaction, as well as the success rate.

METHODS

The study was conducted as a clinical, prospective, randomized split-mouth study of implant retained lithium-disilicate and PEEK screw-retained crowns, and consisted on two groups - study and control group. Both of the groups consisted of the same 17 patients (70% females and 30% males, aged 24 to 62 years, mean age 44.33 ± 15.4) with bilaterally extracted teeth in the same region of the upper jaw. The study was conducted at the Clinic of Oral Surgery and Clinic for Prosthodontics, the School of Dental Medicine, University of Belgrade; it was approved by the Ethical Committee of the School of Dental Medicine, University of Belgrade.

The patients were recruited consecutively from the mentioned clinics. The inclusion criteria were: patients with already osseointegrated implants in the same region of the left and right upper dental arches, older than 18 years old, with maintained natural antagonists and vertical dimension of occlusion, and canine guided or group function occlusion. Exclusion criteria were the existence of bruxism and temporomandibular disorders, missing of the opposing teeth, and unmotivated patients for maintenance adequate oral care. The patients were fully informed about the protocol of the study, and all gave their written consent.

All the patients received Blue Sky implants (Bredent®, Senden, Germany), 4 mm diameter, and 10 mm length, on both sides. Implants were placed after planning with a cone-beam computed tomography (CBCT) scan, in a one-stage surgical procedure. Crowns were made of two different materials, put on osseointegrated implants with delayed loading protocol. Considering the split - mouth study design, each group of crowns was randomly assigned to either left or right halves of the upper jaw. The study group of crowns, PEEK based crowns (BioHpp®), were made on Sky Elegance abutment®, which consisted of a titan base coated with ceramic, reinforced with PEEK polymer. These crowns have been directly pressed on Sky Elegance abutment®, using For2press® system, made in a cut-back body form, and furtherly prepared for veneering with CreaLign® veneering material. The control group consisted of litihium disilicate crowns (IPS e.max) made on Sky Uni. fit® abutment, which consisted of a titan base and "burn out" cap as the modeling base. Core for the control group crowns was first modeled in wax on the Sky Uni. fit® abutment, shaped in a cut-back body form. After pressing in „Emax press system" (IPS e.max Press®), veneering was performed with Emax® veneering ceramics. After Emax crowns finishing, DTK® bonding material was used for connection with Sky Elegance® abutment (Figure 1). Both groups of crowns were fabricated on Ti-base, screw-retained abutment.

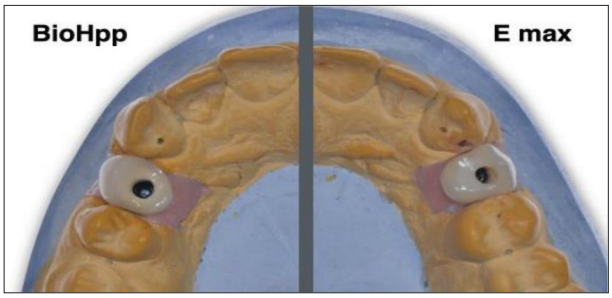

Figure 1. BioHpp® and Emax® crowns

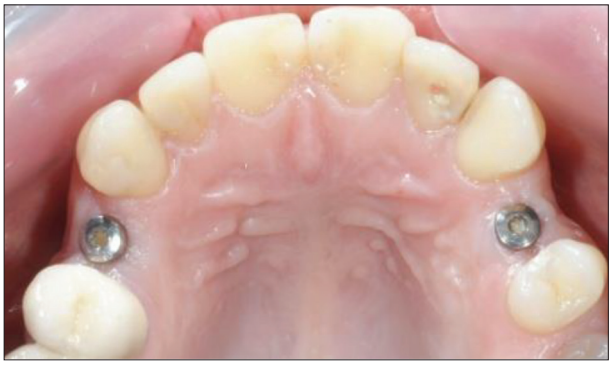

Figure 2. Osseointegrated implants and healing abutments

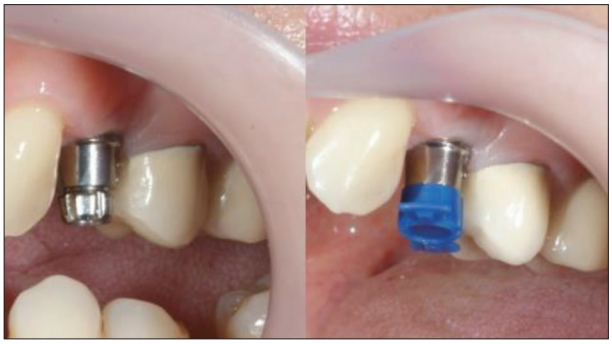

Figure 3. Transfers for closed tray impression technique

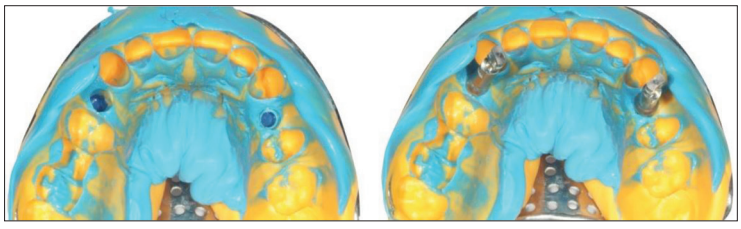

Figure 4. a) Closed tray technique impression; b) analogue reposition

After implant placement, healing abutments were placed on the implants in order to create a profile and to protect the implants (Figure 2). Full arch impression on implant level was obtained using the closed tray method with esthetic transfer (Esthetic transfer closed tray) - Figure 3. Impressions were taken with a silicone material (Elite HD+ Putty Soft Normal Set, Zhermack®, Italy), by use of a standard tray, in a single-step technique (Figure 4). Impressions of the opposite jaw were made with alginate (Hydrogum 5, Zhermack®, Italy) in standard steel trays. Finally, inter-occlusal registration in centric relation was made in Silicon A material. After defining vertical

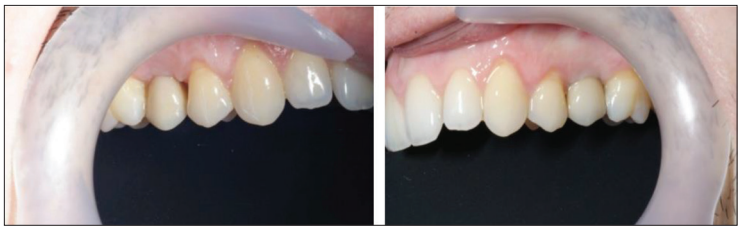

Figure 5. a) BioHpp®crown; b) IPS e.max® crown

dimension, models were transferred into an articulator (Artex CR, Amman® Girrbach, Austria) with a face bow.

The crowns were put in the mouth for analysis of occlusal relation in maximum inter-cuspation and eccentric movements, evaluating contour and aesthetic parameters. After finishing laboratory procedures and glazing (Figure 4), crowns were placed onto the implant and tightened with the manual screw-driver. A resilient material, such as teflon tape was placed in the screw access channel and closed with a temporary filling. Within a week, the previous temporary filling was removed and the abutment screw was re-tightened to the recommended torque of 25 Ncm [8]. Teflon was placed again into the screw access channel and filled with a composite resin (Figure 5).

Study outcomes were analyzed with subjective and objective parameters six and twelve months after placing crowns onto the implants. Subjective parameters, such as esthetics and patient satisfaction with the restoration, were evaluated with standardized questionnaires on visual-analogue scales (VAS) [9]. This scale was presented as a line length of 10 cm, followed by verbal descriptions, where the beginning of the scale was defined with "totally unsatisfied," and the end as "totally satisfied" [9]. Patients were asked to vertically mark their opinion concerning the comfort, general chewing possibility, and aesthetics, and results were notified and measured from the null point to the marked line. Objective parameters in crown comparing were based on characteristics of soft tissues around dental restorations with a periodontal probe, done in observation periods at baseline, after six and 12 months. These clinical findings were recorded according to the following criteria [10]: 1) Modified Bleeding Index - MBI (0 – no bleeding on probing; 1 – isolated bleeding spots present; 2 – blood forms a red line on the gingival margin; and 3 – heavy profuse bleeding); 2) Modified Plaque Index (MPI) (0 – no detection of plaque; 1 – plaque only recognized by running a probe across the smooth marginal surface of the implant; 2 – plaque can be seen by the naked eye; 3 – the abundance of soft matter); and 3) peri-implant probing depth (PPD) measured by probing with a periodontal probe with millimeter graduation (Hu Friedy® periodontal probe) on all four sides of the osseointegrated implant, with the controlled force of 0.25N to resistance appearance.

Statistical analysis

All collected data were organized and evaluated using the dedicated software (SPSS Statistics, Version 17.0; SPSS Inc., Chicago, IL, USA) and were analyzed by descriptive statistical methods, by the measures of central tendency (mean and median), measure of variability (standard deviation and variation interval – minimum, maximum). Testing differences of numerical data between groups was done by the Mann–Whitney test (between two observed groups) and numerical data in each group during time by the Wilcoxon test (in one of the groups during observation periods). The level of significance was set at $p \leq 0.05$.

RESULTS

Clinical examination of the MBI and MPI at baseline, after six and 12 months among the observed groups did not show statistical significance in mean values (Tables 1 and 2). Additionally, analyzing mean values of MPI during observation time in the study group of crowns, statistical significance was registered at baseline (0.12 ±0.33; from 0 to 1) compared to the period after six months (0.35 ± 0.49; 0–1) – Table 1.

Table 1. The values of MBI during the time between groups of crowns

Clinical parameter	Study group of crowns		Control group of crowns		[b]p
	X ± SD; med (min–max)	[a]p	X ± SD; med (min–max)	[a]p	
MBI at baseline	0.12 ± 0.33; 0 (0–1)	(1:2) 0.046 (2:3) 0.705 (1:3) 0.083	0.06 ± 0.24; 0 (0–1)	(1:2) 0.317 (2:3) 1.000 (1:3) 0.317	0.551
MBI after six months	0.35 ± 0.49; 0 (0–1)		0.12 ± 0.33; 0 (0–1)		0.111
MBI after 12 months	0.29 ± 0.47; 0 (0–1)		0.12 ± 0.33; 0 (0–1)		0.210

MBI – modified bleeding index; X – mean value; SD – standard deviation; med – median; a – Wilcoxon test; b – Mann–Whitney test; p – significance;
* – statistically significant

Table 2. The values of MPI during the time between groups of crowns

Clinical parameters	Study group of crowns		Control group of crowns		[b]p
	X ± SD; med (min–max)	[a]p	X ± SD; med (min–max)	[a]p	
MPI at baseline	0.29 ± 0.59; 0 (0–2)	(1:2) 1.000 (2:3) 0.739 (1:3) 0.705	0.18 ± 0.39; 0 (0–1)	(1:2) 0.157 (2:3) 0.180 (1:3) 0.564	0.551
MPI after six months	0.29 ± 0.47; 0 (0–1)		0.06 ± 0.24; 0 (0–1)		0.111
MPI after 12 months	0.24 ± 0.44; 0 (0–1)		0.24 ± 0.44; 0 (0–1)		0.210

MPI – modified plaque index; X – mean value; SD – standard deviation; med – median; a – Wilcoxon test; b – Mann–Whitney test; p – significance;
* – statistically significant

Table 3. The values of PPD during the time between groups of crowns

PPD	Study group crowns		Control group of crowns		[b]p
	X ± SD; med (min–max)	[a]p	X ± SD; med (min–max)	[a]p	
At baseline	1.99 ± 0.70; 2 (1–3.25)	(1:2) 0.002 (2:3) 0.004 (1:3) 0.001	2.10 ± 0.85; 2 (1–4)	(1:2) 0.006 (2:3) 0.006 (1:3) 0.003	0.865
After six months	2.28 ± 0.73; 2.25 (1.25–3.75)		2.28 ± 0.85; 2 (1–4)		0.973
After 12 months	2.47 ± 0.73; 2.75 (1.25–3.75)		2.47 ± 0.88; 2.25 (1–4)		0.973

PPD – peri-implant probing depth; X – mean value; SD – standard deviation; med – median; a – Wilcoxon test; b – Mann–Whitney test; p – significance; * – statistically significant

Table 4. The values of visual analogue scale for esthetics and restoration satisfaction in both groups of crowns

Visual analogue scale	Study group of crowns	Control group of crowns	[a]p
	X ± SD; med (min–max)	X ± SD; med (min–max)	
Aesthetic outcome	9.95 ± 0.11; 10 (9.7–10)	9.84 ± 0.30; 10 (9.1–10)	0.357
Satisfaction with the restoration	9.88 ± 0.18; 10 (9.5–10)	9.37 ± 0.92; 9.7 (7.3–10)	0.002*

X – mean value; SD – standard deviation; Med – median; a – Wilcoxon test; b – Mann–Whitney test; p – significance; * – statistically significant

In terms of mean values of the PPD, there was no statistically significant difference between crown groups during the time (Table 3). However, statistical significance was found in intragroup comparison during the time both in the study and control group of crowns (baseline *vs*. after six months, after six months *vs*. after 12 months, and baseline *vs*. after 12 months) – Table 3.

The mean value of VAS testing for esthetic outcome in both groups of crowns showed no statistical difference (Table 4). The mean scores for VAS referring to satisfaction with the restoration indicate a statistically significant difference between groups, where study group restorations were valued by patients with the higher score (Table 4).

During the implant observation period, no implant was lost, resulting in an implant survival rate of 100%. The restoration cumulative survival rate in both groups was 100%. The fracture of the veneer material occurred in one single crown in the study group, while in the control group, two fractures were registered. The cumulative success rate was 94.12% for the study group of crowns and 88.23% for the control group of crowns.

DISCUSSION

In the conducted study, the split-mouth design was used for randomization, which is previously described as a very popular design in oral health research [11]. The advantage and the attractiveness of this study design compared to the whole-mouth design is that all variabilities within the subjects are removed [11]. On the other hand, some authors indicated disadvantages of this study design, referring to the problem of the patient recruitment due to the need for symmetrical patterns of randomization, and the "carry-cross effect," in which the main problem is that it compromises the possible confusion concerning treatment effect from one side to the other [12, 13, 14].

This study shows that patients were more satisfied with crowns made of PEEK material, which is very important parameter in the oral rehabilitation process and it should be used for the evaluation of the specific therapy [15]. Previous studies did not analyze patient's satisfaction with these types of crowns, but many of them refer to the efficiency of the implant therapy based on patient satisfaction, wherein most of the cases patients claimed that they were satisfied [16–20]. Chang et al. [21] established that patients have marked implant-retained crowns as "very satisfying" concerning esthetics, while clinicians were "less satisfied" with the same crowns. The findings in a three-year follow-up study showed no significant difference for VAS analysis of patient satisfaction about function and esthetic appearance, between the two groups – single implant screw-retained monolithic lithium disilicate and veneered zirconia crowns [22].

Our study has shown no statistically significant differences among the soft tissue parameters (MBI, MPI and PPD) between the observed groups of crowns, which is in correlation with the previous three-year follow-up study for the anterior implant screw-retained IPS e.max crowns, where similar results were demonstrated [23]. In the mentioned study, the mean values of MPI and MBI at baseline, after six months, one year and three years showed no statistically significant differences [23]. In addition, another study which compared clinical performances of screw-retained, monolithic, zirconia, and cemented porcelain-fused-to-metal implant crowns, showed no statistically significant difference between the study and the control groups in terms of the soft tissue parameters such as bleeding on probing and plaque index at the third, sixth, ninth, and 12th month after prosthetic loading [24].

Nevertheless, in our research, statistically significant difference was registered in terms of the mean values of MPI between baseline and after 6 months in the study group of crowns. Also, statistically significant intragroup differences were noticed in terms of PPD during the time, both in the study and control group of crowns, which is in correlation with the results of previous studies [23, 24, 25].

Suggested clinical parameters are commonly used as an evaluation method in the clinical trials for implant-retained restorations [26]. The peri-implant soft tissue is very important, and always must be evaluated, not only for the esthetics but also for the long-term stability of the implant-retained restorations. Our results of soft tissue parameters between the observed groups indicate that

the new system, that has been recently launched into the market (BioHpp®) can clinically perform as well as lithium disilicate material used in the For2press system (IPS e.max Press®), which has been marketed for many years.

The soft tissue around the implants has a similar role as soft tissue around natural teeth. Besides, dense soft tissue forms a protective barrier for crestal bone, as it creates contact with the abutment surface [27]. Previous studies have shown that there are some differences in anatomical characteristics of the soft tissue surrounding the implant and natural dentition; natural teeth are connected with perpendicular Sharpey's fibers, while the weaker connection is formed with parallel and circumferential fibers around the abutment surface [26, 28, 29].

CONCLUSION

The findings of this study showed that scores can be a reliable tool to rate the clinical outcome of implant-retained single crowns over time. MBI, MPI, PPD, and VAS scores can also be useful to monitor any possible early failure and to standardize follow-up recalls. Furthermore, the two materials tested in this randomized controlled trial showed comparable clinical performances, with a high success rate after one year of service. Nevertheless, future studies should be conducted to show clinical advantages or disadvantages referring to this new material for the solo crown in prosthodontics.

Conflict of interest: None declared.

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Литијум-дисиликатне и ПЕЕК имплантатно ношене шрафом ретиниране соло крунице – рандомизована, проспективна клиничка студија

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САЖЕТАК

Увод/Циљ Поређење два материјала под истим условима најбољи је начин за дефинисање разлика између њих. ПЕЕК је полимер који има много потенцијалних употреба у стоматологији, исто као и већ добро позната литијум-дисиликатна керамика.

Циљ овог истраживања био је да се, између два типа круница, упореди зарастање периимплантатног меког ткива у различитим периодима посматрања, процене естетска својства и задовољство пацијената зубним надокнадама, као и успех и стопа преживљавања.

Методе Студија је спроведена као клиничка, проспективна, рандомизована студија подељених уста на 17 пацијената са билатерално недостајућим горњим зубима исте врсте и уграђеним имплантатима у том пределу. Процена је извршена субјективним (визуелна аналогна скала) и објективним параметрима (модификовани индекс крварења, модификовани плак индекс и дубина сондирања).

Резултати Поређење резултата између ПЕЕК и литијум-дисиликатних круница показало је да нема статистички значајних разлика у погледу модификованог плак индекса, модификованог индекса крварења и дубине сондирања у посматраним периодима. Анализирајући модификовани плак индекс током периода посматрања у студијској групи круница, регистрована је статистичка значајност између почетне вредности и вредности након шест месеци. Такође, уочена је статистичка значајност у погледу дубина сондирања током времена, како у студијској тако и у контролној групи круница. Резултати визуелне аналогне скале за естетику нису показали статистички значајну разлику између група, док је визуелна аналогна скала за задовољство зубним надокнадама показала статистички значајну разлику.

Закључак Ова студија је показала да примењени субјективни и објективни параметри могу бити поуздан алат за оцењивање клиничког исхода појединачних круница на имплантатима током времена.

Кључне речи: литијум-дисиликат; ПЕЕК; соло крунице; имплантати

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The importance of anticoagulation in COVID-19-related acute kidney injury requiring continuous renal replacement therapy

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SUMMARY

Introduction/Objective In Serbia, the coronavirus disease 2019 (COVID-19) pandemic began in early March 2020.

The aim of this study is to summarize clinical experience in the treatment of COVID-19-associated acute kidney injury by methods of continuous renal replacement therapy (CRRT) with the focus on the amount of the administered dose of unfractionated heparin.

Methods The study covers 12 patients treated with CRRT at the Clinic for Infectious Diseases at the Clinical Center of Vojvodina from March 6 to May 20, 2020. Antithrombotic prophylaxis, risk of venous thromboembolism (VTE), applied therapy, biochemical parameters before and after CRRT, anticoagulation and other CRRT parameters were analyzed.

Results The mean age of the patients was 61.54 ± 10.37 years and seven (58.3%) were men. All the patients received standard thromboprophylaxis. Nine (75%) patients had Padua Prediction Score for Risk of VTE ≥ 4, but none developed a thrombotic event. Seven critically ill patients with multi-organic dysfunction developed acute kidney injury dependent on CRRT. The mean CRRT dose was 36.6 ml/kg/h, the mean bolus dose of unfractionated heparin was 3250 ± 1138.18 IU, and the continuous dose was 1112.5 ± 334.48 IU/kg/h. Discontinuation of CRRT due to the clotting circuit was necessary in only one patient. The values of leukocytes, AST, ALT, GGT, aPTT, PT were significantly higher after CRRT compared to urea, creatinine, potassium, chlorine and magnesium, whose values were significantly lower.

Conclusion In our COVID-19 patients who had high inflammatory parameters and D-dimer and an estimated risk of developing deep vein thrombosis, the implementation pre-dilution continuous veno-venous hemodiafiltration with antithrombotic membrane and $^1/_3$ to $^1/_2$ higher unfractionated heparin doses than the recommended one, the filter life lasted longer with no complications.

Keywords: COVID 19; continuous renal replacement therapy; acute kidney injury; thrombotic events

INTRODUCTION

Acute kidney injury (AKI) is frequently present in the critically ill patients, especially in patients with severe infections and it is related to significant morbidity and mortality rates [1].

A meta-analysis that included 20 journals and 6945 patients showed an 8.9% prevalence of AKI in patients with COVID-19, although statistical heterogeneity between studies was found [2]. According to previous studies, renal replacement therapy (RRT) is required by 25% of severely ill COVID-19 patients [3].

Several studies have shown that the course of COVID-19 can lead to diverse thrombotic complications caused by inflammation, hypoxia, disseminated intravascular coagulation as well as certain study drugs [4]. These drugs can be the cause of severe interactions with antithrombotic therapy or anticoagulants [5].

The most common hemostatic abnormalities in COVID-19 are mild thrombocytopenia and an elevated level of D-dimer, which is related to a higher possibility of the need for mechanical ventilation (MV), ICU admittance or lethal outcome [6]. It is believed that the severity of the disease is linked to a prolonged prothrombin time (PT) and international normalized ratio (INR), thrombin time (TT) and the shortened of activated partial thromboplastin time (aPTT) [4]. The latter consideration refers to the relation of hemostatic changes with the liver dysfunction in COVID-19 patients [7]. An elevated level of D-dimer is likely to cause thrombotic complications in COVID-19 patients [8].

Recent studies have reported the presence of venous thromboembolism (VTE) that are in fact pulmonary embolism found in 16.7–35% patients with cumulative frequency up to 49% in 14 days [9, 10].

Although RRT treatment can be related to a higher bleeding rate, a great prevalence of VTE supports the use of thromboprophylaxis in the absence of active bleeding or a severe thrombocytopenia [11].

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In the cases of AKI, continuous renal replacement therapy (CRRT) is a preferred treatment modality due to its lesser impact on hemodynamic stability and adequate volume control. However, the exposure of blood to the artificial circuit leads to blood clotting and it can cause thrombosis with a greater loss of blood, which results in the additional burdening of medical staff and increased expenses [12]. In order to diminish the risk of circuit thrombosis, regional anti-coagulation with citrate or heparin (unfractionated heparin (UFH) or low molecular weight heparin) or systemic anticoagulation (UFH, low molecular weight heparin, or prostacyclin) are used [13]. In case of frequent circuit clotting, national guidelines published in England suggest the following: vascular approach optimization, considering alternative/combined anticoagulant strategies including combined citrate and heparin (systemic or through circuit), heparin and epoprostenol or argatroban if other prothrombotic disorders are excluded [14].

The aim of this study is to summarize clinical experience in the treatment of COVID-19-associated AKI by modality of CRRT with the focus on the amount of the administered dose of UFH.

METHODS

The study included 276 patients with COVID-19 pneumonia who were treated at the Clinic for Infectious Diseases, Clinical Center of Vojvodina from March, 6 to May, 20 2020. Of those, 12 adult patients were treated with CRRT due to COVID-19-associated AKI. Seven of them (58.3%) developed AKI within multiorgan failure and were treated in ICU, while five (41.7%) were treated in the semi-intensive care unit.

The study has been approved by the competent ethics committee of the Clinical Center of Vojvodina.

We analyzed: demographic data; comorbidities; laboratory and clinical parameters 24 hours before and after CRRT; simplified acute physiology score (SAPS II) and modified early warning score (MEWS); presence of acute respiratory distress (ARDS) and secondary infections; the need for multiple organ support, invasive MV, non-invasive ventilation, high-flow nasal cannula; Padua Prediction Score for Risk of VTE, dose of thromboprophylaxis; onset of CRRT since admission, anuria before CRRT, CRRT modalities, type of adsorptive membrane, dose of CRRT (ml/kg/h), achieved ultrafiltration during CRRT (ml), bolus dose (IU) and continuous dose (IU/kg/h) of UFH during CRRT; number of procedures of CRRT; therapy received by patients, length of hospitalization and mortality.

The SAPS II score consists of 12 physiological variables and three disease-related variables collected in the first 24 hours of admission to the ICU. The SAPS II score may vary between 0 and 163 points (0–116 points for physiological variables, 0–17 points for age and 0–30 points for previous diagnosis). The MEWS score is based on four standard physiological variables and on the AVPU consciousness assessment (warning, voice response, pain response, no response). The primary purpose of the MEWS is to prevent delays in the intervention or transfer of the critically patients. A score ≥ 5 is statistically associated with an increased probability of lethal outcome or admission to the ICU.

Table 1. Patients' demographic and clinical characteristics

Variables		n (%)
Sex	Male	7 (58.3)
	Female	5 (41.7)
Mean age in years ± SD		61.54 ± 10.37
Comorbidities		
Hypertension		9 (30)
Diabetes mellitus		3 (10)
Myocardial infarction		2 (6.6)
Chronic pulmonary disease		1 (3.3)
Autoimmune diseases		2 (6.6)
Malignancy		2 (6.6)
Chronic kidney disease		5 (16.6)
Other		6 (20)
With acute respiratory distress		7 (58.3)
With secondary bacterial infection		7 (58.3)
Multiple organ support		
NIV		1 (8.3)
HFNC/NIV		1 (8.3)
MV and vasopressor support with norepinephrine		7 (35.8)
Supplemental oxygen		2 (16.7)
Extracorporeal membrane oxygenation		1 (8.3)
SAPS II/MEWS score 24 hours before CRRT		
SAPS II		7 (58.3)
SAPS II score (Mean ± SD)		39 ± 5.92
MEWS score		
1		3 (60)
3		2 (40)
Anuric patients 24h before CRRT		5 (41.7)
Start of CRRT from admission (days) (Mean ± SD)		9.17 ± 7.16
Padua Prediction Score for Risk of VTE		
< 4		3 (25)
≥ 4		9 (75)
Therapy		
Antibiotics		9 (20.9)
Hemomycin		12 (27.9)
Chloroquine		3 (6.9)
Antivirals		2 (4.6)
Corticosteroids		12 (27.9)
Intravenous immunoglobulins		2 (4.6)
Antifungal		3 (6.9)
Dose of Thrombophylaxis (IU)		
dalteparin-sodium 2500 IU/12h		11 (91.6)
dalteparin-sodium from 2500 IU to 10000 IU/12h		1 (8.3)
Nonsurvivors		9 (75)
Length of hospital stay (Mean ± SD)		14.92 ± 10.90

CRRT – continuous renal replacement therapy; HFNC – high-flow nasal cannula; SAPS II – simplified acute physiology score; MEWS – modified early warning score; MV – invasive mechanical ventilation; NIV – noninvasive ventilation; VTE – venous thromboembolism

The criteria for initiating CRRT according to Kidney Disease Improving Global Outcomes were the stages 2 or 3 AKI.

CRRT was performed on two devices each having its own filter: Multifilter (high-flux filter Kit8 CVVHDF 1000,

Table 2. Comparison of laboratory values between patients before and after CRRT

Variables	Before CRRT (IQR)	After CRRT (IQR)	p
Leukocytes ($10^9mm^3/l$)	12.9 (6.9–21.1)	18.3 (6.7–34.2)	**0.041***
Lymphocytes (%)	7.5 (6.4–11.8)	6.3 (2.5–11.4)	0.158
Hemoglobin (g/L)	84.5 (75.5–95.8)	88.0 (72.5–107)	0.475
Platelets ($10^9mm^3/l$)	191.0 (114.7–267)	170.5 (84.7–207.2)	0.065
CRP (mg/l)	178.9 (40.1–289.3)	176.2 (32.8–344.8)	0.859
PCT (ng/l)	2.1 (1.2–9.3)	1.45 (0.59–4.14)	0.346
Urea (mmol)	26.5 (21.1–36.3)	16.9 (9.3–23.3)	**0.005***
Creatinine (μmol)	486.0 (257.2–804)	308.0 (156.2–604.7)	**0.002***
Potassium (mmol)	4.9 (4.1–5.3)	4.3 (3.9–4.8)	**0.049***
Sodium (mmol/l)	144.0 (138.5–147)	139.0 (137.2–141.7)	0.065
Chlorine (mmol	106.0 (105–108.7)	102.5 (101–104)	**0.008***
Magnesium (mmol)	1.01 (0.79–1.07)	0.84 (0.76–0.97)	**0.021***
AST (U/L)	40.0 (35–104.5)	68.0 (43.7–110.2)	**0.003***
ALT (U/L)	42.0 (28.7–67)	74.0 (44.2–92.2)	**0.002***
GGT (U/L)	94.5 (43.2–115.7)	109.5 (73.7–141.2)	**0.002***
APTT (R)	1.33 (1.12–1.70)	2.96 (1.69–73.9)	**0.005***
PT (R)	1.15 (1.08–1.24)	1.43 (1.15–1.90)	**0.011***
Fibrinogen (g/L)	4.2 (2.40–5.27)	3.5 (2.37–5.25)	0.326
D-dimer (mg/L)	1680 (869.5–4373.2)	2278.5 (1075–5460.7)	0.182

CRRT – continuous renal replacement therapy; IQR – interquartile range; aPTT – activated partial thromboplastin time; PT – prothrombin time; R – ratio; ALT – alanine aminotransferase; AST – aspartate aminotransferase, GGT – gamma-glutamyl transferase; CRP – C-reactive protein; PCT – procalcitonin; aPTT – activated partial thromboplastin time; PT – prothrombin time; *p < 0.05 (*Wilcoxon test based on negative and positive ranks*)

Bad Homburg, Germany) and Prismaflex (high-flux filter ST150 Gambro, Deerfield, IL, USA). EMiC2 Hemofilter (Fresenius Medical Care, Bad Homburg, Germany, 1.8 m^2 surface area) and oXiris (Gambro, AN-69 based membrane, surface treated by polyethyleneimine and grafted with heparin) were administered in septic patients.

Statistical analysis

Descriptive and inferential statistical methods were used for the data analysis. Numerical characteristics are presented by the arithmetic mean, the median with interquartile range (IQR 25–75%) and the standard deviation, while the attributive characteristics are expressed by frequency and percentage. Given the sample size, i.e., the small number of frequencies to compare differences between the groups, the Wilcoxon test for paired samples was used, an alternative to the Student's t-test for two dependent samples. There was a statistical significance if $p < 0.05$, and a high statistical significance if $p < 0.001$. The IBM SPSS Statistical Package for Social Sciences 21 software package was used for statistical data processing.

RESULTS

The study included 12 COVID-19 patients with AKI (58.3% men), with a mean age of 61.54 ± 10.37 years of age. The most common comorbidity was hypertension in nine patients.

ARDS with secondary bacterial infection was found in seven (58.3%) patients who required MV and CRRT. Before CRRT, the average values of the SAPS II score were 39 ± 5.92 in 58.3% severely ill patients, while five of them (41.7%) were anuric. The average time for the start of CRRT from admission to the hospital was 9.17 ± 7.16 days. Nine patients had Padua Prediction Score for Risk of VTE ≥ 4. Median hospitalization time was 14.92 ± 10.90 days, mortality was 75%. The doses of thromboprophylaxis and the type of therapy used are also shown Table 1.

Table 2 shows the comparison in laboratory parameters before and after CRRT. Leucocyte count, hepatogram (AST, ALT, GGT), aPTT, and PT increased significantly after CRRT, in contrast to the levels of urea, creatinine, potassium, chloride and magnesium, which decreased, as expected.

The total of 20 CRRT procedures and six CRRT + extracorporeal membrane oxygenation (ECMO) were done, and the average number of procedures was 2.16 per patient. The most common modality was pre-dilution continuous veno-venous hemodiafiltration (CVVHDF) and the most commonly used membrane was highly adsorptive-oXiris membrane. The average duration of procedures in 11 patients was 24.8 h, with the CVVHDF + ECMO procedure performed in one patient lasting a total of 315.5 h. The median value of dialysate flow was 1558.3 ml/h, and median value of replacement flow was 1318.1 ml/h. The average CRRT dose in nine septic patients was 36.6 ml/kg/h and in the remaining patients 30 ml/kg/h. The average ultrafiltration per procedure in 11 patients was 4736.4 ml, while the total ultrafiltration in a patient who underwent CVVHDF and ECMO was 15.669 ml. The average bolus dose was 3250 ± 1138.18 IU while the continuous UFH dose was 1112.5 ± 334.48 IU/kg/h. The continuous dose of UFH during CRRT was increased by 1/3 in six patients (66.7%), while it was increased by 1/2 in (33.3%) patients. Discontinuation of CRRT was necessary in three patents (25%) – in the first case due to the clotting circuit, in the second case for technical reasons and in the third case due the hemodynamic instability and the fall of oxygen saturation. Table 3.

DISCUSSION

In addition to hemostatic disorders, immobility, and systemic inflammation, MV and central venous catheters contribute to the risk of VTE in ICU. Dietary deficiencies and liver dysfunction can also interfere with the synthesis of coagulation factors. Due to organ dysfunction, critically ill patients develop changes in pharmacokinetics, which may require adjustment of the anticoagulant dose [15]. Our patients had different levels of D-dimer depending on the severity of their clinical conditions as well as secondary infections. They had minor disorders of the hemostasis mechanism without developing of disseminated intravascular coagulation were verified, which corresponds to the results of a Dutch study [16]. Using the Padova Predic-

Table 3. Treatment parameters of CRRT

Variables	n (%)
Number of procedures CRRT	
1	5 (41.7)
2	4 (33.3)
3 or more	3 (25)
Types of CRRT modalities	
Predilution CVVHDF	8 (66.7)
Predilution CVVHDF + ECMO	1 (8.3)
CVVH	1 (8.3)
CVVHD	3 (25)
Type of adsorptive membrane	
EMIC2	2 (16.7)
OXIRIS	7 (58.3)
Kit 8	2 (16.7)
ST 150	1 (8.3)
Dose of CRRT	
≥ 35 ml/min/h	9 (75)
< 35 ml/min/h	3 (25)
Bolus dose of UFH (IU) (Mean±SD)	3250 ± 1138.18
Continuous dose of UFH (IJ/kg/h) (Mean±SD)	1112.5 ± 334.48
Increasing the continuous dose of UFH during CRRT	
1/3	6 (66.7)
1/2	3 (33.3)
Interruption of the CRRT	
Yes	3 (25)

CRRT – continuous renal replacement therapy; ECMO – extracorporeal membrane oxygenation; CVVHDF – continuous venovenous hemodiafiltration; CVVH – continuous venovenous hemofiltration; CVVHD – continuous venovenous hemodialysis; UFH – unfractionated heparin

tion Score for Risk of VTE, the risk ≥ 4 was determined in 75%, i.e., nine patients, (seven critically ill patients and two treated in semi-intensive care). Unlike other published studies, no patient developed a thrombotic event [16, 17, 18]. Namely, the authors of the Dutch study reported that 31% out of 184 COVID-19 patients had arterial and venous thrombotic events, although all patients had standard thromboprophylaxis [16]. The authors of another study also used the Padova score and showed that 40% of the patients were at risk for VTE, although the study did not provide the data on the use of VTE prophylaxis or an incident with VTE [18]. In two French ICUs, the overall rate of VTE in patients was shown to be very high at 69%, but only 31% of them were treated with prophylactic anticoagulation [17].

Our patients were at risk for developing AKI due to the presence of the most common comorbidities such as hypertension, chronic renal failure, diabetes and heart disease, use of diuretics and ACE inhibitors, which corresponds to the published results of other authors [19]. The onset of some CRRT methods was individually assessed based on clinical and laboratory parameters, in accordance with the current guidelines. Compared with traditional CRRT indicators in patients with an onset of AKI, the leading criterion was hypervolemia for the purpose of respiratory support. All patients had a double-lumen catheter placed in the right internal jugular vein, in accordance with the recommendations [20].

Depending on the availability of modalities, supply of dialysis material, adsorption membranes and cytosorber, the recommendation for critically ill patients is CVVH or CVVHDF targeting minimum delivery dose of 20–25 mL/kg/h [21]. In the study period, in COVID-19 confirmed patients requiring dialysis procedures, we were able to organize only the implementation of CRRT with heparin anticoagulation, with a predominance of pre-dilution CVVHDF and highly adsorbent membranes (oXiris, EMiC2) in nine (75%) patients with high proinflammatory parameters. In these patients CRRT dose was 35–40 mL/kg/h in order to eliminate inflammatory mediators, while other patients where the main goal was volume maintenance had CRRT 25–30 mL/kg/h. During the procedures, the doses of antibiotics were adjusted and the energy needs increased by 20–30 (kcal/kg.d), protein 1.5 ≤ 1.7 (g kg.d) and amino acids 1.5 ≤ 1.7 (g kg.d) according to the individual treatment regimen [22].

So far, papers on premature filter coagulation have been published frequently. In a multicenter French cohort of 150 patients, 29 of them were treated for RRT and 28 of them (97%) experienced a thrombosis circuit, with a shortened lifetime of the circuit [9]. The anticoagulation of the circuit has not been specifically analyzed, however, all the patients received at least thromboprophylaxis, and 30% of them had therapeutic doses of heparin. In a further study in one center with 69 critically ill patients with COVID-19, nine out of 11 patients had increased therapeutic UFH infusions due to thrombosis of recurrent circuits [23]. A third unicenter study reported filter coagulation in eight out of 12 severely ill patients with COVID-19 on hemofiltration, despite anticoagulation with prophylactic doses. Out of the four patients without filter clotting, three were on therapeutic UFH infusion due to existing thrombosis at the time of the hemofiltration onset [24].

The optimal anticoagulant strategy to prevent circuit coagulation and ensure CRRT efficacy is unknown in COVID-19. Since 75% of our patients had the Padua score ≥ 4, in order to prolong the filter life, pre-dilution CVVHDF with antithrombotic oXiris membrane was applied in 58.3% of critically ill patients with high inflammatory parameters and D-dimer. Wen et al. [25] have not determined the correlation between D-dimer values and shortened sustained low-efficiency dialysis sessions in around 30% patients, in contrast with the study done by Valle et al. [26], who proved that the higher levels of D-dimer indicate a higher rate of filter coagulation in CRRT in 46.6% patients. However, the results are not comparable due to the lower values of D-dimer, different treatment modalities and the lack of details on coagulation in the first study. Also, neither study monitored Anti-Xa and determined antithrombin III and the factor VIII. The correlation of higher values of CRP with shorter sustained low-efficiency dialysis duration was determined in the first study, which indicates the correlation between hyper-inflammation in COVID-19 patients and the coagulation of extracorporeal circuit. Elevated CRP levels in the acute phase are related to hyper viscosity, and the latter was diagnosed in severely ill COVID-19 patients [27, 28]. Our study did not analyze the

correlation between D-dimer and CRP with filter coagulation due to the proportion of the samples, and the fact that only one patient had clotting circuit.

The recommended start dose of UFH is 10–15 IU/kg per hour and the aPTT is 60–90 seconds [21]. In our study, in six patients (50%) the values of hemostasis parameters and platelets allowed the initial increase by 1/3 to 1/2 of the recommended bolus dose of UFH, and we increased the UFH dose until we reached the target values of aPPT ranging 180–220 seconds. Despite the administration of higher bolus doses of UHF, during CRRT all six patients required a dose increase, as well as the two patients (with malignant disease) treated in surgical intensive care unit in whom an adsorptive EMiC2 membrane was used. In case of using ECMO + CRRT blood flow was maintained at > 400 ml/min [29]. The patient who underwent ECMO + CVVHDF was prescribed UFH according to the guidelines of non-COVID-19 patients [30].

No bleeding, no heparin resistance, and no heparin-induced thrombocytopenia were found in any of the patients. However, CRRT was discontinued in one patient due to circuit clotting, therefore, the dose was increased to the upper limit of thromboprophylaxis to prevent recurrent circuit clots.

Until we obtain more precise recommendations on the amount of bolus and continuous doses of UFH for COVID-19 patients, one should take into consideration comorbidities, the doses of thromboprophylaxis, the type of RRT modalities and highly adsorptive membranes, the planned duration of the procedure and the level of ultrafiltration.

During the earliest period of the pandemic, two patients were treated with antiviral drugs (Lopinavir/Ritonavir), both of them took azythromicin and corticosteroids in the recommended doses [5]. Hydroxychloroquine was introduced in three patients. It is known that this drug can have an antithrombotic effect, especially on antiphospholipid antibodies, which we were not able to analyze during the epidemic. Two patients used antiplatelet and anticoagulant therapy for acute coronary syndrome and atrial fibrillation prior to COVID-19.

The average duration of hospitalization of our patients who required CRRT was 14.92 ± 10.90 days, similar to some published data [31]. The mortality was 75%, while in the studies done it ranges between od 63.3–90% [32, 33, 34].

There are some limitations associated with our study. This is a single-center study, covering a small number of patients during a short period of time. All our patients were treated with CRRT, there was no control group due to limited data availability, and we have no insight into the incidence of AKI in patients treated with conservative treatment.

CONCLUSION

Implementation of pre-dilution CVVHDF with antithrombin membrane and the UFH doses higher by 1/3 to 1/2 than the recommended ones, has extended the filter life without complications in our COVID-19 patients with high inflammatory parameters and D-dimer and an estimated risk of developing deep vein thrombosis. The need for a unified strategy in the diagnosis and optimization of AKI treatment with a better understanding of COVID-19 would contribute to determining the optimal approach to CRRT in these patients.

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Значај антикоагулације при континуираној замени функције бубрега код болесника са акутним оштећењем бубрега повезаним са ковидом 19

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САЖЕТАК

Увод/Циљ У Србији је пандемија вирусне болести корона 2019 (ковид 19) почела почетком марта 2020. године. Циљ овог рада је сумирање клиничког искуства у лечењу акутног оштећења бубрега повезаног са ковидом 19 методама континуиране замене функције бубрега (КЗФБ) са фокусом на висини примењене дозе нефракционисаног хепарина.

Методе Приказаћемо 12 болесника лечених КЗФБ-ом на Клиници за инфективне болести у Клиничком центру Војводине од 6. марта до 20. маја 2020. године. Анализирани су антитромботска профилакса, ризик од венске тромбоемболије, примењена терапија, биохемијски параметри пре и после КЗФБ-а, антикоагулација и други параметри КЗФБ-а.

Резултати Просечна старост болесника је била 61,54 ± 10,37 година и седам болесника (58,3%) било је мушког пола. Сви су примали стандардну тромбопрофилаксу. Падуа скор предикције ризика од венске тромбоемболије ≥ 4 имало је девет (75%) болесника, али ниједан није развио тромботски догађај. Акутно оштећење бубрега зависно од дијализе развило је седморо критично оболелих са мултиорганском дисфункцијом. Просечна доза КЗФБ-а је износила 36,6 *ml/kg/h*, просечна болусна доза нефракционисаног хепарина била је 3250 ± 1138,18 *IJ*, а континуирана доза 1112,5 ± 334,48 *IJ/kg/h*. Прекид КЗФБ-а због коагулације сета био је неопходан само код једног болесника. Вредности леукоцита, *AST*, *ALT*, *GGT*, *aPTT* и *PT* биле су значајно веће после КЗФБ-а у поређењу са уреом, креатинином, калијумом, хлором и магнезијумом, чије су вредности биле значајно мање.

Закључак Код наших болесника оболелих од ковида 19 са високим инфламаторним параметрима и Д-димером, као и процењеним ризиком од развоја тромбозе дубоких вена, примена предилуционе континуиране веновенске хемодијафилтрације са антитромботском мембраном и вишим дозама за ⅓ до ½ нефракционисаног хепарина у односу на препоручене дозе, омогућила је дужи век трајања филтера, без појаве компликација.

Кључне речи: вирусна болест корона 19; континуирана замена функције бубрега; акутно оштећење бубрега; тромботски догађаји

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Risk factors as outcome predictors of pulmonary rehabilitation in patients with chronic obstructive pulmonary disease

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SUMMARY

Introduction/Objective Chronic obstructive pulmonary disease (COPD) is a primary lung disease. Today, pulmonary rehabilitation (PR) is the basis for non-pharmacological treatment of these patients, with numerous confirmed effects on the most significant symptoms of the disease and the quality of life (QoL). The aim of this study was to determine the relationship between certain risk factors and the outcome of PR, as well as to determine the percentage of respondents who had a positive outcome of PR.

Methods The study included 500 patients with COPD, determined according to the Global Initiative for Chronic Obstructive Lung Disease guidelines, all stages (I–IV), in the stable phase of the disease, who completed the outpatient PR program. Disease stage, comorbidities, forced expiratory volume in the first second, six-minute walk test (6MWT), COPD Assessment Test (CAT), and Medical Research Council dyspnea scale, body mass index, airflow obstruction, dyspnea and exercise capacity (BODE) index, were measured before and after the program. The last four parameters have been observed as risk factors that affect the outcome of PR, but also as parameters by which we monitor the outcome of PR.

Results A successful outcome of PR was achieved by as many as 452 (90.4%) patients. The following were determined as independent predictors of a positive outcome of PR: lower number of comorbidities, absence of heart failure, higher BMI, and CAT ≥ 10.

Conclusions PR in our group of patients leads to statistically significant improvements in most of the examined subjective and objective parameters, in patients in all stages of the disease.

Keywords: COPD; comorbidity; respiratory rehabilitation; risk factors; treatment outcome

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a slow, progressive, primarily lung disease, but causes significant systemic consequences [1]. According to available data, 4–15% of the adult population in industrialized countries suffer from this disease. It is the only disease in the top 10 leading causes of death in the world, in which the prevalence and number of deaths continue to grow [2]. Inflammatory changes similar to those in the lungs also occur in the systemic circulation, and are thought to occur with a simple "spill-over" phenomenon, i.e., the overflow of the mediator of inflammation into the systemic circulation. Most likely, this concept is the key to understanding the systemic effects of COPD [3].

The first problems usually appear years after the first signs of inflammation and consequent damage to the respiratory function. Most often, rapid fatigue and dyspnea bring these patients to the doctor, because they consider coughing and expectoration to be a normal consequence of cigarette smoking. When it occurs, dyspnea is usually persistent and progressive [4].

The most significant systemic disorders include: skeletal muscle dysfunction, cardiovascular disease (CVD), diabetes, osteoporosis, depression [5]. Skeletal muscle dysfunction in COPD is a common occurrence. The pathophysiological mechanisms have not been precisely determined. One of the most important is the decline due to inactivity, because these patients avoid all efforts that lead to dyspnea [6]. One of the most significant comorbidities in COPD is CVD and it is a dynamic and progressive disorder that occurs by combining endothelial dysfunction and inflammation [7]. Recent studies provide evidence that inflammatory changes may be predictors of the development of diabetes (type 2) and impaired glucose tolerance [8]. Reduced lung function, systemic inflammation, corticosteroid therapy, reduced physical activity, which in turn causes reduced mechanical load on the bones, and it is one of the most important stimuli for bone building, contribute to the development of osteoporosis [9]. Depression and anxiety, have a significant impact on the course of COPD, the prognosis of the disease, and the quality of life (QoL) of the patients and their families. The prevalence of depression in patients with COPD is 10–40%, while it is 19% for anxiety [10].

The "gold diagnostic standard" is spirometry. Parameters necessary for the diagnosis of

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COPD are: forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1), and FEV1/FVC ratio [11]. Postbronchodilator values are recommended for the diagnosis and assessment of COPD severity. According to the severity of the obstruction, COPD is divided into mild (stage I), moderately severe (stage II), severe (stage III), and very severe (stage IV). A new classification of the disease was adopted – the ABCD classification, based on the assessment of disease symptoms, the degree of obstruction, and the risk of exacerbation. The assessment of disease symptoms is performed using the COPD Assessment Test (CAT) and the modified British Medical Research Council scale (mMRC) [12].

The CAT questionnaire is a practical test consisting of eight questions, which measure the impact of COPD on the health condition and the daily life of the patient. The total score ranges 0–40, higher values indicating poorer general condition of the patient. This test has been shown to reflect the impact of PR as well as recovery after exacerbations [13]. The mMRC scale is used to assess dyspnea by gradation 0–4, in relation to effort tolerance. Grade 4 indicates the appearance of dyspnea even during the lightest physical activities. This scale correlates well with other health parameters, clinical signs and pulmonary function, and provides an estimate of future mortality [14]. The body mass index, airflow obstruction, dyspnea and exercise capacity (BODE) index is a multidimensional index that consists of four parameters: body mass index (BMI), obstruction measured via FEV1, dyspnea, measured using mMRC, and exercise capacity expressed through the six-minute walk test (6MWT). This is an index whose values range 0–10, and the index with values ≥ 7 is an excellent predictive factor for mortality, and at the same time we can monitor the effects of PR [15].

Given that nowadays PR is a proven, very effective non-pharmacological method of treating patients with COPD, in this paper we wanted to determine whether and which risk factors affect the outcome of PR, as well as how successful the PR program is in treating patients with COPD.

METHODS

Material

This retrospective-prospective study included 500 patients diagnosed with COPD according to GOLD guidelines, stages I–IV, in the stable phase of the disease, who completed the program of outpatient PR during a two-year period. Data from associated diseases were taken from previous medical history, medical documentation, and based on the pharmacological therapy used by the patients. The PR consisted of 15 sessions, duration of each being 45 minutes, over a period of three weeks, and included strength exercises for the upper and lower extremities, endurance exercises on a stationary bike (symptom-limited), and diaphragmatic breathing exercises. All the patients underwent pre- and post-PR: 6MWT according to guidelines issued by the American Thoracic Association, FEV1-measured on a Master Scope PC spirometer (manufactured by JAEGER). Patients completed the CAT and mMRC questionnaires themselves, also before and after PR. Patients' body height and body weight are presented with BMI. Based on these parameters, we finally calculated the BODE index before and after the completion of the PR program. When it comes to the success of PR, our research determined the influence of individual risk factors on the successful outcome of PR (certain associated diseases, FEV1, 6MWT, CAT, and mMRC questionnaire, BODE index). The assessment of rehabilitation success was done on the basis of certain parameters that were observed as risk factors (individual improvement of these factors included an increase in distance travelled during 6MWT by ≥ 54 m, a decrease in the CAT questionnaire by 5 points, and in the mMRC questionnaire and BODE index by 1 point). The categories of success were the following: excellent (all four parameters improved), very good (three parameters improved), good (two parameters improved), sufficient (one parameter improved), and insufficient (without improvement of any parameter). The categories excellent, very good, good, and sufficient were considered a successful outcome of the PR.

Statistical analysis

The study used the measures of central tendency as methods of descriptive statistics. We used methods of identification of empirical distributions, methods for assessing the significance of differences: depending on the type of data distribution, independent t-test, Mann–Whitney U-test, Wilcoxon's test of equivalent pairs, χ^2 test, and Spearman's correlation test. To assess the significance of the relationship between input variables and outcomes, univariate as well as multivariate logistic regression analysis was used. Statistical analyses were performed using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA), with statistical significance level set at 0.05.

The study was approved by the Ethics Board of the Institute for Pulmonary Diseases of Vojvodina.

RESULTS

The study included 500 respondents, of whom 258 (51.6%) were male. The average age was 64.89 ± 9.02 years. The average BMI was 25.86 ± 4.25, while the average pack/years value was 42.09 ± 24.52. The average duration of the disease was 7.35 ± 6.03 years (Table 1).

Three or more associated diseases were registered in 189 (37.8%) respondents, 50 patients (10%) did not have any associated diseases, while the largest number of associated diseases (seven), was found in only one patient. The most common associated disease was arterial hypertension, found in 338 (67.6%) patients, followed by ischemic heart disease in 208 (41.6%) patients, and diabetes mellitus in 143 (28.6%) patients. The rarest associated disease was lung cancer, diagnosed in only six (1.2%) patients (Figure 1).

The mean distance travelled during 6MWT before PR was 421.76 ± 97.75, and after PR it increased on average by

Table 1. Descriptive parameters of patients in total and according to the outcome of pulmonary rehabilitation

Variables	Sum	Outcome of PR		p
		Successful	Unsuccessful	
Sex male (n, %)	258 (51.6)	231 (89.5%)	221 (91.3%)	0.498
Age (X ± SD)	64.89 ± 9.02	64.76 ± 9.04	66.10 ± 8.82	0.326
BMI (X ± SD)	25.86 ± 4.25	26.00 ± 4.26	24.59 ± 3.96	0.029
Pack-years (X ± SD)	42.09 ± 24.52	41.60 ± 24.81	46.73 ± 21.16	0.168
Length of disease years (median)	7.35 ± 6.03	6	3	0.103

BMI – body mass index; SD – standard deviation; X – mean

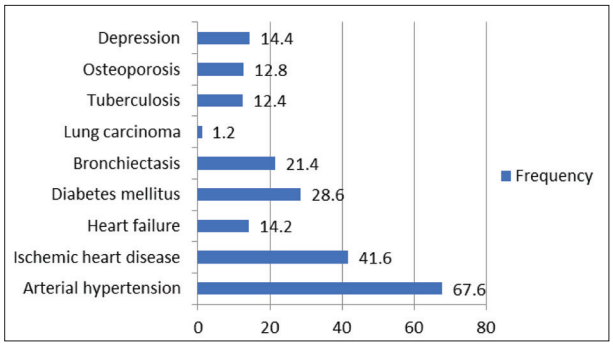

Figure 1. Frequency of comorbidities of respondents

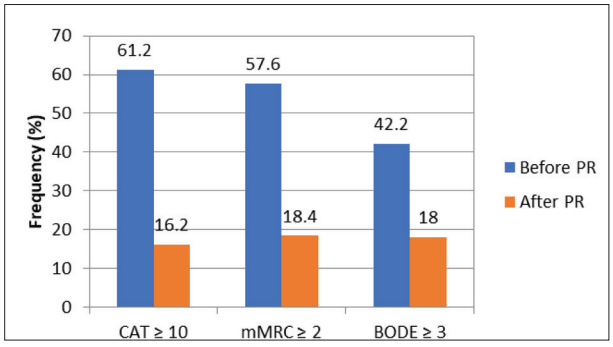

Figure 2. Frequency of cut-off values for Chronic Obstructive Pulmonary Disease Assessment Test (CAT), modified British Medical Research Council scale (mMRC), and Airflow Obstruction, Dyspnea and Exercise (BODE) capacity questionnaires before and after pulmonary rehabilitation

64.44 ± 35.07 ($p < 0.01$). The increase in distance during the 6MWT > 54 m was achieved by 314 (62.8%) respondents.

The mean value of FEV1 before PR was 58.15 ± 18.53, while after the PR program it increased on average by 3.05 ± 2.84 ($p < 0.01$).

The mean value of the CAT questionnaire before PR was 12.32 ± 6.38, and after PR it decreased by an average of 6.37 ± 3.11 ($p < 0.01$). The reduction of the CAT questionnaire by 5 points was achieved by 345 (69%) respondents. The mean value of the mMRC scale before PR was 1.75 ± 0.93, and after PR it decreased by an average of 0.71 ± 0.56 ($p < 0.01$). A decrease on the mMRC scale by 1 point was achieved by 329 (65.8%) respondents. The mean value of the BODE index before PR was 2.37 ± 2.05, and after PR it decreased by an average of 0.93 ± 0.95 ($p < 0.01$). The reduction of the BODE index by 1 point was achieved by 345 (69%) respondents.

When we observed these subjective parameters in relation to the proposed cut-off values for the categorization of mild and severe patients and symptoms (CAT questionnaire ≥ 10, mMRC questionnaire ≥ 2, BODE index ≥ 3) before and after PR, we obtained the results shown in Figure 2. Further determination of the correlation of these parameters revealed the existence of a statistically significant positive correlation between the values of the CAT questionnaire ($p = 0.006$) and the mMRC scale ($p = 0.014$) at the beginning of the study and the positive outcome of PR. No statistically significant correlation was found between the BODE index values at the beginning of the study and the PR outcome ($p > 0.05$).

Subjects with three or more associated diseases had a statistically significantly lower frequency of positive PR outcome compared to subjects with less than three associated diseases [163 (86.2%) *vs.* 289 (92.9%); $p = 0.014$]. There is a statistically significant negative correlation between the number of associated diseases and a positive PR outcome ($p = 0.008$), as well as three or more associated diseases and a positive PR outcome ($p = 0.014$). It was found that subjects with heart failure had a statistically significantly lower frequency of positive PR outcome compared to subjects without heart failure [59 (83.1%) *vs.* 393 (91.6%); $p = 0.024$]. There was no statistically significant difference in the frequency of positive PR outcome in subjects with ischemic heart disease [184 (88.5%) *vs.* 268 (91.8%); $p > 0.05$], arterial hypertension [302 (89.3%) *vs.* 149 (92.5%); $p > 0.05$], diabetes mellitus [128 (89.5%) *vs.* 324 (90.8%); $p > 0.05$], pulmonary tuberculosis [55 (88.7%) *vs.* 397 (90.6%); $p = 0.024$], lung cancer [5 (83.3%) *vs.* 447 (90.5%); $p > 0.05$], bronchiectasis [95 (88.8%) *vs.* 357 (90.8%); $p > 0.05$], osteoporosis [56 (87.5%) *vs.* 396 (90.8%); $p > 0.05$] and depression [62 (86.1%) *vs.* 390 (91.1%); $p > 0.05$] compared to patients without these comorbidities. There is a statistically significant negative correlation between cardiac failure and a positive PR outcome ($p = 0.024$), while there is no statistically significant association of the other examined comorbidities with a positive PR outcome ($p > 0.05$) (Figure 3).

When it comes to the success of PR, we must note that the evaluation of the success of rehabilitation was done on the basis of certain parameters that were observed as risk factors (6MWT, CAT questionnaire, mMRC questionnaire, and BODE index). Of the 500 patients included in the study, as many as 452 (90.4%) subjects achieved a successful PR outcome, while only 48 (9.6%) subjects were without improvement in any test parameter. Within the successful outcomes of PR, most respondents 142 (28.4%) were in the 'very good' category, followed by the categories 'good,' with 129 respondents (25.8%), 'sufficient,' with 102 respondents (20.4%), and the 'excellent' category,' with 79 (15.8%) respondents.

In our research, we tried to determine the predictive values of pre-determined risk factors. The results obtained by univariate logistic regression analysis showed that statistically significant univariate predictors of a positive PR outcome are the following: lower number of associated

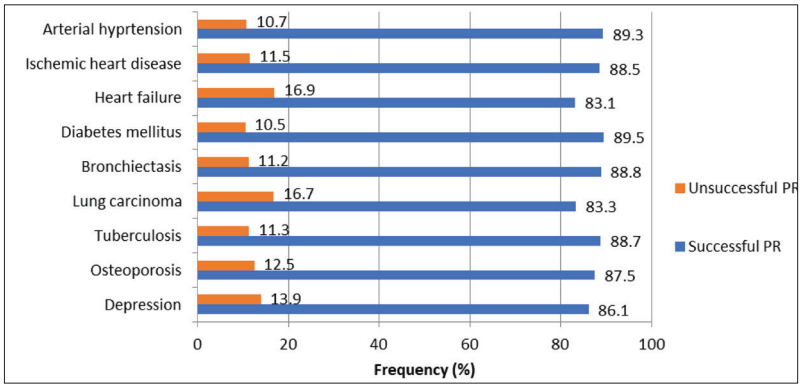

Figure 3. Pulmonary rehabilitation (PR) success rate by comorbidities

diseases [PR 0.74 95% CI (0.59–0.93); p = 0.011]; absence of heart failure [PR 0.45 95% CI (0.22–0.92); p = 0.027]; higher BMI [PR 1.84 95% CI (0.87–3.87); p = 0.03]; mMRC ≥ 2 [PR 2.73 95% CI (1.47–5.08); p = 0.002]; CAT ≥ 10 [PR 3.23 95% CI (1.74–6.02); p < 0.001].

Age, sex, smoking, pack-years, duration of illness, number of exacerbations during the previous year, ischemic heart disease, diabetes mellitus, arterial hypertension, osteoporosis, pulmonary tuberculosis, lung cancer, bronchiectasis, depression, 6MWT, BODE, FEV1, and GOLD stages are not statistically significant predictors of a positive PR outcome. Further data processing, multivariate logistic regression analysis showed that the independent predictors of a positive PR outcome are the following: lower number of associated diseases [PR 0.67 95% CI (0.52–0.88); p = 0.004]; absence of heart failure [PR 0.42 95% CI (0.19–0.93); p = 0.033]; higher BMI [PR 1.15 95% CI (1.05–1.25); p = 0.002]; CAT ≥ 10 [PR 4.99 95% CI (2.51–9.91); p < 0.001].

DISCUSSION

In our study, 500 patients with COPD were analyzed, with 258 (51.6%) being male. Today, it is known that comorbidities have a very significant impact on the health status of patients with COPD, but they also have a great impact on the burden on the entire health system. Comorbidities significantly worsen the patient's QoL and prognosis. The second revision of GOLD, for the first time, included comorbidities and exacerbations in the definition of COPD, thus confirming their importance. The prevalence of comorbidities is quite diverse, the most common data indicating that about two-thirds of patients with COPD have one or two comorbidities, although the results range 50–98.5%. Divo et al. [16] found in one of the largest comorbidity studies, which included 1,969 patients with COPD and 316 patients without COPD, they found that patients with COPD were more likely to have a larger number of comorbidities than patients without COPD.

In our study, 50 patients did not have comorbidities (10%), patients with comorbidities had a share of 90%. The number of comorbidities in the remaining 450 patients ranged from 1–7, and the average number of comorbidities was 2.1 ± 1.3. As a risk factor that can negatively affect the outcome of PR, we took the limit of ≥ 3 comorbidities, and a total of 189 patients had ≥ 3 comorbidities (37.8%). This factor proved to be statistically significant to the successful outcome of PR. In the group with ≤ 2 comorbidities, there were 311 patients, of which 289 had successful PR (92.9%), while in the group of patients with more then 3 comorbidities successful rehabilitation was reported in 86% of cases. These data correlate with the results from the references, even our prevalence of comorbidities is at the upper limit, compared to the results in the research published so far.

The comorbidity study within our paper included the following diseases: heart failure (present in 14.2%), ischemic heart disease (41.6%) and hypertension (67.7%), diabetes (28.6%), bronchiectasis (21.4), pulmonary tuberculosis (12.2), lung cancer (1.2%); osteoporosis (12.8) and depression (14.4%). The examination of the prevalence of comorbidities showed, as stated in the references, that CVD have the highest prevalence in people with COPD, and these values are compared with the results from the references. Prevalence values for other diseases also range within the values in the references, with the exception of lung cancer, where we had a much lower prevalence compared to data from the references, perhaps due to somewhat weaker screening in that direction, than osteoporosis, whose prevalence in the references is up to 35%, and depression, with a slightly lower prevalence compared to the references (about 25%). Only heart failure has a statistically significant impact on the success of PR. Its presence was more significant in the group with 'insufficient' PR success compared to all other success categories. These results also coincide with the results from the references. In addition to the impact of these comorbidities on the course and prognosis of COPD, they may also affect the success of PR. Studies indicate that patients with comorbidities, especially ≥ 2, have a higher degree of dyspnea, less tolerance to exertion, and a poorer QoL. Patients with CVD and COPD, according to Hornikx et al. [17], do not have worse values either before or after the PR program, when it comes to the assessment of dyspnea, but they have worse results related to the exercise tolerance and QoL.

In contrast, Carreiro et al. as well as Tunsupon et al. [18], received numerous positive changes in terms of symptoms, but also in terms of QoL, after completing the PR program in patients with this type of comorbidity [18, 19]. PR in these patients is more complex, difficult, and individualized, but these patients have more chances to progress and achieve better results. And just as these two views are opposed, so are the results of the studies that have been done on this topic in recent years.

The results of PR on the influence of the CAT test are very positive, and for these reasons it is used today as one of the main parameters for monitoring the effects of PR. Our results confirmed that PR significantly improved the

values of the CAT questionnaire, by far more than 2 points of minimum clinically important difference (MCID) value stated in the references. Also, the value of CAT showed that it has a statistically significant correlation with the success of PR, namely the initially worse the values of the CAT questionnaire are, the better the results of rehabilitation. For example, we must note that the group of patients with 'excellent' success had the highest mean CAT before the program (17.14 points), while the average correction for all categories of success was slightly more than 6 points. These results are in complete agreement with what Dodd et al. [20] officially confirmed in their prospective multicenter study, pointing out that it is a simple test which responds well to PR and that can distinguish categories in relation to the effects of this program very well. This author further examined the duration of changes in the values of the CAT and found that the CAT questionnaire responds to the PR program immediately, and that these effects last up to six months after PR.

Our work confirmed and pointed out the significant effect of PR to dyspnea. After PR, there was a statistically significant improvement of mMRC, and its statistically significant correlation with PR success was confirmed. In the case of mMRC, as well as in the case of CAT questionnaire, this correlation is negative, i.e., the higher the values of mMRC before PR, the better the success. The category with 'excellent' success initially had the highest values of mMRC (2.65 points), and the average improvement was by 1 point. A previous study has shown that PR leads to the improvement of dyspnea in all patients, although it is recommended that only patients with mMRC ≥ 2 should be included in the program. Rugbjerg et al. [21] found that all categories of patients, in relation to mMRC, have some improvement, and that it is weak in patients with mild symptoms, while in patients with more pronounced symptoms this improvement is statistically significant. Betancourt-Peña et al. [22] also contributed to this topic. They concluded that patients with mMRC 2 have the same improvement after the PR, when it comes to 6MWD and maximal oxygen uptake, as well as patients with mMRC¾, and added that all persons regardless of the degree of dyspnea should be referred to the PR.

Our research confirmed that the PR leads to a statistically significant improvement in 6MWT values and one of the best things about this test is that it reflects pulmonary and extrapulmonary manifestations of COPD. This improvement averaged 64.4 ± 35.1 m in our study, which is far more than all the mentioned values, which are referred to in the references as MCID (14–30.5 m). Today, it is assumed that the improvement in the 6MWT value could be clearly reflected in the increase in physical activity of the daily life of patients, measured by the number of steps taken during the day [23]. As with the aforementioned CAT and mMRC questionnaires, the lowest test values were in the group of patients with 'excellent' PR success (354.9 m). All this clearly indicates a strong correlation between CAT, mMRC and 6MWT parameters before and after PR, suggesting that patients who initially have poorer results of these parameters achieve better PR results.

CONCLUSION

Based on our results, we can conclude that PR should be a mandatory part of treatment of patients with COPD, regardless of the stage of the disease. It can also be performed in patients with numerous comorbidities, although we must note that a smaller number of associated diseases and the absence of heart failure in our work have been proven as independent predictors of a positive PR outcome. Patients with initially poorer CAT and mMRC questionnaire values had better PR scores. Baseline values ≥ 10 for the CAT questionnaire, in our study, also proved to be an independent predictor of a positive PR outcome. We proved that this program leads to statistically significant improvements in both subjective and objective parameters of the disease, and a successful outcome after the PR program was achieved by 90% of our patients.

Conflict of interest: None declared.

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Фактори ризика као предиктори исхода респираторне рехабилитације код болесника са хроничном опструктивном болешћу плућа

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САЖЕТАК

Увод/Циљ Хронична опструктивна болест плућа примарно је плућно обољење. Респираторна рехабилитација данас представља основу нефармаколошког лечења ових болесника, са бројним потврђеним ефектима на најзначајније симптоме болести и квалитет живота.

Циљ рада био је да се утврди повезаност одређених фактора ризика и исхода респираторне рехабилитације, као и проценат испитаника код којих је остварен позитиван исход респираторне рехабилитације.

Методе У истраживање је укључено 500 болесника са хроничном опструктивном болешћу плућа, утврђених према смерници *GOLD*, свих стадијума (I–IV), у стабилној фази болести, који су одрадили комплетан програм амбулантне респираторне рехабилитације. Стадијум болести, придружене болести, форсирани експиријумски волумен у првој секунди, шестоминутни тест хода, упитник за процену хроничне опструктивне болести плућа (*COPD Assessment Test – CAT*) и скала *mMMR* (*modified Medical Research Council*) за процену степена диспнеје, индекс *BODE*, мерени су пре и после завршеног програма. Последња четири параметра посматрана су и као фактори ризика који утичу на исход респираторне рехабилитације, али и као параметри помоћу којих пратимо исход респираторне рехабилитације.

Резултати Успешан исход респираторне рехабилитације остварила су чак 452 (90,4%) болесника. Као независни предиктори позитивног исхода респираторне рехабилитације утврђени су мањи број придружених болести, одсуство срчане слабости, виши индекс телесне масе и *CAT* ≥ 10.

Закључак Респираторна рехабилитација у нашој групи болесника доводи до статистички значајних побољшања већине испитиваних субјективних и објективних параметара код болесника у свим стадијумима болести.

Кључне речи: хронична опструктивна болест плућа; коморбидитет; респираторна рехабилитација; фактори ризика; исход терапије

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analysis of epidemiological characteristics and surgical treatment of patients with pressure ulcer

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SUMMARY

Introduction/Objective A pressure ulcer is a localized injury to the skin and/or underlying tissue, usually over a bony prominence. It appears as a result of pressure or the combination of pressure and shear. Pressure ulcers can be identified within a wide variety of patient subpopulations and epidemiological and etiological aspects play a major role in their treatment.

Methods A retrospective study of data analysis included 72 patients with pressure ulcers that were hospitalized and surgically treated in our institution over a five-year period. Main data features used in the analysis were sex, age, principal diseases, comorbidities, and biochemical indicators of malnutrition. The patients' data was obtained from the existing patients' records. Additionally, the study analyzed the method of treating pressure ulcers, types of reconstructive methods in surgical treatment, as well as the incidence rate of partial osteotomy.

Results A total of 72 patients with pressure ulcers were included in this study, with a mean age of 54.7 ± 16.1 years. Three times more patients injured in traffic accidents were male (75% *vs*. 25%), while most of the patients with multiple sclerosis were female (85.7%). More than 95% of patients who had pressure ulcers of stage III or IV were treated surgically with a reconstructive method of transposition or rotation myocutaneous flap. Patients with stage IV pressure ulcer were usually treated with partial osteotomy.

Conclusion Surgical reconstructive treatment with fasciocutaneous and myocutaneous flaps represents the gold standard for treating patients with pressure ulcers. These procedures provide reconstruction with adequate flap coverage and obliteration of dead space with well-vascularized tissue but with necessity of further implementation of antidecubitus measures.

Keywords: pressure ulcer; surgical treatment; osteotomy

INTRODUCTION

A pressure ulcer is a localized injury to the skin and/or underlying tissue, usually over a bony prominence. It appears as a result of pressure or the combination of pressure and shear. The increased pressure prevents the blood from circulating properly causing cell death, tissue necrosis, and consequently development of pressure ulcers [1]. According to some recent literature, hospitalizations related to pressure ulcers cost between $9.1 and $11.6 billion per year. The cost of individual patient care for a pressure ulcer may range from $20,900 to $151,700 [2, 3].

Understanding the challenges that pressure ulcers present both to the patient and health system, the education regarding their prevention and treatment is increasingly important.

Pressure ulcers are the most commonly developed complication in bedridden patients. They most frequently occur in intensive care unit patients. According to the existing data, 5.6–15.5% of hospitalized patients develop pressure ulcers [1, 3], while according to certain studies that number ranges 5–36.4% [1, 4]. This incidence rate is significantly higher with certain subpopulations and reaches 60% of quadriplegic subpopulation; 56% of elderly patients with thigh bone fractures, and as much as 33% in polytraumatized patients with prolonged medical treatment in intensive care units [2, 3, 5, 6]. A major cause of pressure ulcers in younger patients are spinal cord injuries (SCI). Pressure ulcer is an injury caused by body pressure at points of support. It is a tissue injury occurring after a longer period of lying or sitting, or caused by pressure of alloplastic materials such as cannula, oxygenation mask, nasogastric tube, endotracheal cannula, stoma, or other medical equipment during hospital treatment or physical rehabilitation [2, 4, 7]. Along with prolonged pressure and local ischemia, or some system factors, such as malnutrition, hypoproteinemia, hypoalbuminemia, anemia, vitamin deficiency, smoking, alcohol and drug abuse, other cardio-vascular and endocrine comorbidities represent etiological factors in the development of pressure ulceration [1, 5, 7, 8]. Socioeconomic factors and life quality also affect the development of pressure ulcers. The Braden scale as a clinically validated tool allows nurses and doctors to reliably score one's level of risk for developing pressure ulcers by assessing six subscales [3, 5, 9, 10].

Studies performed on animals showed that application of 70 mmHg pressure over a two-hour period can cause pathological changes. A similar study showed that 500 mmHg pressure

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applied during a two-hour period leads to the same degree of tissue injury as 100 mmHg pressure applied during a 10-hour period. Both of these situations result in laboratory animals' tissue necrosis. Both of these studies prove that muscular tissue is more susceptible to ischemia and necrosis than skin and hypodermis. Ischemic changes of muscular tissue and hypodermis were also recorded without any skin changes [1, 7, 11, 12].

Skin areas that cover protruding bony areas are prone to pressure ulcers. These areas are the following: occipital prominence, shoulder blades, vertebrae, elbows, sacrum, hips, and heels. However, pressure ulcers can occur on any body part if they are points of support or pressure for a longer period of time. Pressure ulcers can occur less frequently on: nasal ala due to the pressure caused by a nasogastric tube, nose dorsum due to the oxygenation mask pressure, trachea inner side due to endotracheal cannula pressure, disc pressure near stoma, as well as due to pressure applied by an inadequate part of prosthesis. There are also certain publications discussing unusual instances of pressure ulcers such as toes and shanks occurring due to prolonged use of compression stockings, but also vulva, perineum, and scrotum [2, 8, 12, 13].

Pressure ulcer care is a complex, long, and slow process. First of all, pressure must be relieved or removed by appropriate measures to prevent further injury. Also, an early and adequate rehabilitation should be applied. Nutrition is important in pressure ulcer healing. Pressure ulcer guidelines give a summary of nutritional intervention to enhance wound healing, such as the following: provide sufficient calories, provide adequate protein intake for positive nitrogen balance, provide and encourage adequate daily fluid intake in the interest of hydration, provide adequate vitamins and minerals. Pressure ulcer treatments can include partial necrectomy and debridement of devitalized tissue, frequent change of bandages, infection control and appropriate plastic and reconstructive surgical treatments, in order to compensate for the skin and muscular structure defects and bone prominence [1, 2, 12, 14, 15]. Pressure ulcers that are critically colonized or infected may show subtle signs of infection, such as delayed healing, change in odor, seriously increased exudate, absent or friable tissue granulation, new or increased pain. Necrotic or devitalized tissue in a wound signals the growth of bacteria and prevents it from healing. Debridement is the removal of nonviable tissue from a wound and is a natural part of the wound repair process.

The paper aims to give a thorough summary of both epidemiological characteristics of patients affected by pressure ulcers and morphological characteristics of pressure ulcers. Specific insight was given to the principal disease, comorbidity, evaluation of reconstructive method in surgical treatment, as well as partial osteotomy incidence.

METHODS

This retrospective study was performed in our institution. This research included 72 patients with pressure ulcers treated within the period from January, 2015 to January, 2020.

Study criteria included patients older than 18 years with pressure ulcers, who had been adequately prepared for surgical treatment. Study criteria excluded pressure ulcer patients younger than 18 years and patients with pressure ulcers and the American Society of Anesthesiologist score ≥ 3 and patients with stage I pressure ulcers. Preoperatively, the patients were examined by the team that consisted of a plastic surgeon, a radiologist, a cardiologist, and, when necessary, a neurologist. They were adequately prepared for surgery in general endotracheal, spinal, or local anesthesia depending on their neurological status and laboratory analyses that were within normal limits (toleration of 10–15% under normal limits for levels of proteins, albumin, white blood cells, and hemoglobin, which are normally expected in patients with pressure ulcers).

Main data features used in the analysis were sex, age, principal diseases, comorbidities, and biochemical indicators of malnutrition. The patients' data was obtained from the existing patients' records. Additionally, the study analyzed the method of treating pressure ulcers, types of reconstructive methods in surgical treatment, as well as the incidence rate of partial osteotomy.

Descriptive and analytical statistics methods were used for data processing and result presentation. The values of continuous variables are presented as an average value ± standard deviation, while the values of discontinuous variables are presented as frequency (n, %). The differences in the average value of continuous variables between the groups were tested using the analysis of variance (ANOVA) and Student's t-test. The χ^2 test was performed for the purpose of comparing discontinuous variables. The value of $p < 0.05$ was considered to have statistical significance.

This paper was planned in compliance with the Patient Rights Directive and ethical rules defined by the principles of the Declaration of Helsinki. The data used during the study is available upon reasonable request to the author.

RESULTS

Seventy-two patients with pressure ulcers were included in this study. Forty-one patients were male and 31 were female. The patients' mean age was 54.7 ± 16.1 years. The mean age of the male patients was higher than that of female patients. This difference had high statistical importance ($p = 0.003$) (Figure 1). Patients belonging to other age groups had similar incidence, but slightly higher incidence was noticed in age groups 40–49 years and 50–59 years (Figure 2).

In almost half of the cases, paraplegia was the main reason for long-lasting lying (immobility), and the cause of pressure ulcers (44.3%). Quadriplegia was the second most common cause of pressure ulcers (15.7%), immediately followed by hemiparesis and other causes (Figure 3).

Principal diseases, i.e., injuries resulting in immobility or impaired mobility of patients, are presented in Figure 4. It is clear that 50% of the cases refer to SCI. The most

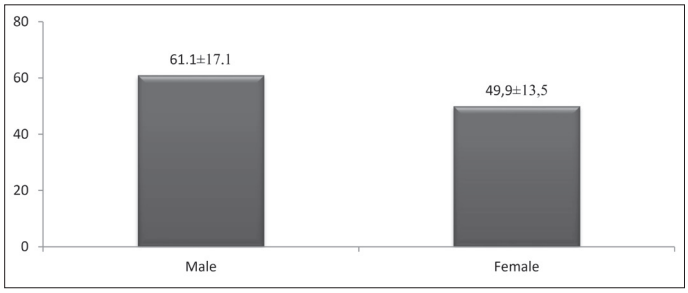

Figure 1. Average age of patients in comparison to their sex

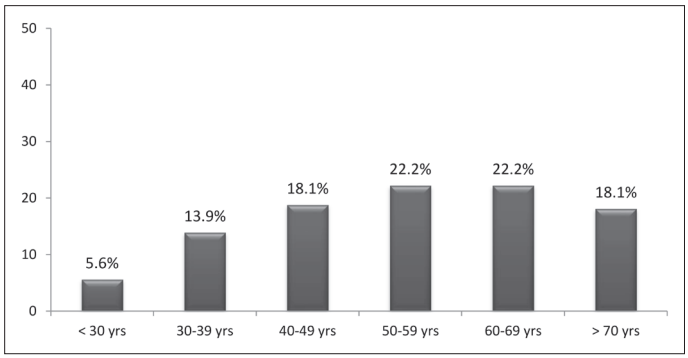

Figure 2. Patient distrubution according to the age group

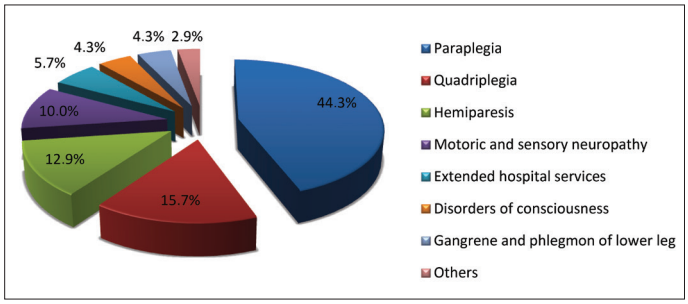

Figure 3. Causes of pressure ulcer development

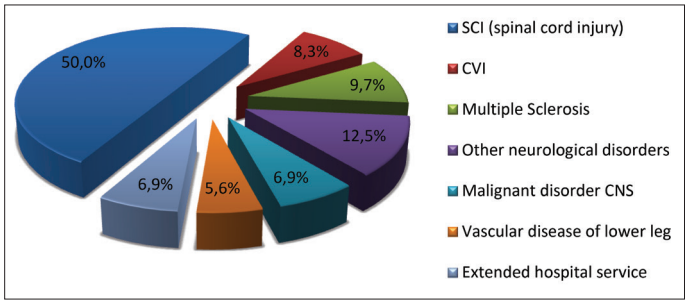

Figure 4. Principal diseases and injuries causing pressure ulcer development

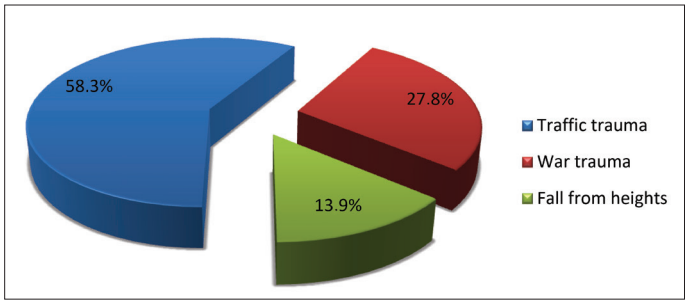

Figure 5. Main causes of spinal cord injuries

common causes of SCI are traffic accident trauma (58.3%), followed by war trauma (27.8%), and falls from height (13.9%) (Figure 5). It showed that there are three times more male patients injured in traffic accidents (75% *vs*. 25%), while 85.7% of the multiple sclerosis patients were female. Cerebrovascular insult and vascular diseases of the lower limbs equally affected both sexes. All other diseases were slightly more common in males.

Regarding male patients, 48.1% had SCI caused by a traffic accident trauma. As for the female patients, the result was significantly higher (88.9%). Only one female patient suffered from SCI due to war trauma (11.1%). There was no record of fall from heights. This difference in the incidence of injuries with different causes for male and female patients was statistically significant ($p = 0.02$).

Even though it was shown that the patients injured in traffic accidents were the youngest and those injured in falling accidents were the oldest, there is no statistically significant difference in the mean age of patients in relation to the cause of injury ($p = 0.379$) (Figure 6).

Traffic accident trauma is the predominant cause of injuries in all age groups, except for the fact that all patients younger than 30 years were injured in traffic accidents. Falling from height was recorded as an injury cause in patients aged 40–70 years. There were no patients older than 70 years among the ones with SCI.

The number of pressure ulcers localized in the sacral area was the highest (40.3%) ($\chi^2 = 37.33$; $p = 0.001$) (Figure 7). Most of the patients had only one pressure ulcer (72.2%), followed by patients with two pressure ulcers (26.4%), and only one patient with three pressure ulcers.

The highest percentage of patients had stage IV pressure ulcers (62.5%). The percentage of patients with stage III pressure ulcers was half as high (33.3%), and only 4.2% of patients had stage II pressure ulcers. Stage I pressure ulcers were not identified in any of the patients.

Hypoproteinemia and hypoalbuminemia were identified in almost half of our patients (47.2%), with levels of protein value less than 60 g/L, and albumin less than 40 g/L.

Apart from principal diseases and injuries causing pressure ulcers, our patients had numerous other comorbidities. According to their incidence rate (37.2%), cardiovascular diseases, such as hypertension, stenocardia, and myocardial infarction, are the first ones in ranking. Endocrine diseases come second in ranking, with 14% incidence rate, including type 2 diabetes mellitus and thyroid gland diseases as the most common. Incidence rate of psychiatric disorders and gastro-intestinal diseases is 10%, while the incidence rate of all other diseases is 27.9% in total.

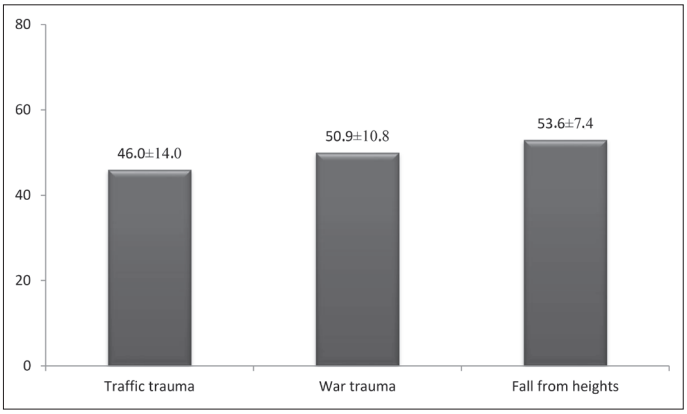

Figure 6. Mean age of patients with spinal cord injury

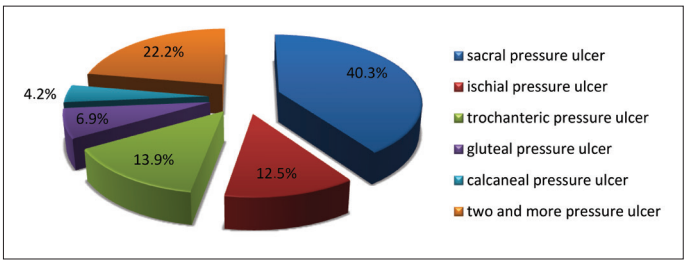

Figure 7. Pressure ulcer localization distribution

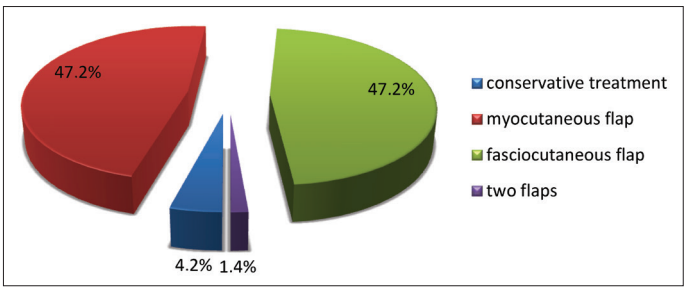

Figure 8. Reconstructive operative method

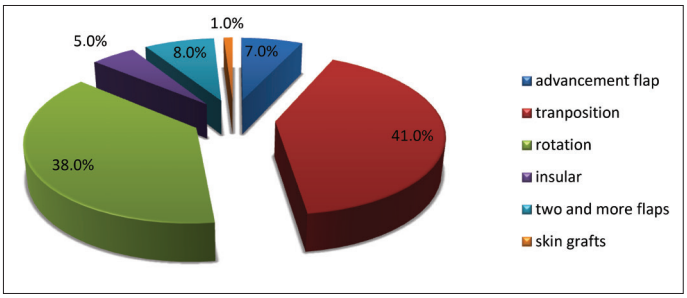

Figure 9. Distribution of different myocutaneous flap types

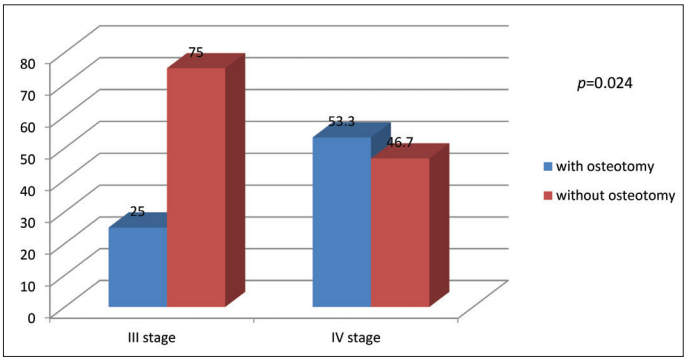

Figure 10. Distribution of partial osteotomy in comparison with pressure ulcer stage

More than 95% of pressure ulcer patients had surgical treatment. We used a myocutaneous flap in 47.2% and a fasciocutaneous flap in 47.2% of patients who were operated on; 1.4% of the patients had a reconstruction with two different flaps, while 4.2% of the patients were treated with skin grafts or conservative treatments. Myocutaneous flaps were used in almost half of the patients: transposition (41%) and rotation flaps (38%) the most frequently, following with advancement flaps (7%) and insular flaps (5%) or combination of two flaps. This differences in using specific myocutaneous flap type were statistically highly significant ($\chi^2 = 35.46$; $p = 0.001$). Proper flap selection was determined by the localization and the stage of the pressure ulcer, their number following the rules of not violating adjacent flap territories for possible future flap coverage and obligation to obliterate dead space with well-vascularized tissue. We analyzed the frequency of partial osteotomy in two groups of patients: the patients with stage III and those with stage IV pressure ulcer. We did not have any patients with stage I pressure ulcers, while those with stage II pressure ulcers underwent conservative treatment. Partial osteotomy was performed more frequently in the group of patients with stage IV pressure ulcers (53.3%) compared to the group of patients with stage III pressure ulcer (25%), $\chi^2 = 5.11$; $p = 0.024$ (Figure 10).

Patients involved in this study who were of higher age, with lower levels of serum proteins and albumins, and who were underweight, had only minor complications in postoperative period, such as small dehiscence, partial flap necrosis and hematomas (Clavien–Dindo grade I or II).

DISCUSSION

Research conducted in the past two decades has shown that the number of patients with pressure ulcers has increased by 80%, and that their leading cause was traffic accident trauma, followed by war trauma in our region. According to the National Pressure Ulcer Advisory Panel and European Pressure Ulcer Advisory Panel, an increasing tendency in the number of younger patients with pressure ulcers can also be noted, along with the higher heterogeneity of the primary disease, specifically SCI as the leading ones [3, 6, 7]. Many factors have an impact on the development of pressure ulcers, such as primary disease, comorbidities, sex, age, nutritional status, and hospital care [1, 2, 3].

Previous research, as well as our study, shows higher incidence rate of pressure ulcers in male patients [2, 6, 8]. However, that difference was not statistically significant. Majority of the studies show the same results –male patients with pressure ulcer are the dominant subpopulation group [6–9]. There

were no female patients in the patient group younger than 30. In the patient group over the age of 70 the percentage of females was 84.6%. In all other age groups, the number of males was higher. This difference in the patient sex and age distribution is statistically significant ($\chi^2 = 13.44$; $p = 0.02$). Other studies dealing with epidemiological characteristics of the pressure ulcer patients show the same or similar results [4, 6, 7].

The principal cause of pressure ulcers is immobility, most often due to paraplegia or quadriplegia. This coincides with the results of some other studies, which also indicated paraplegia as the principal cause of pressure ulcers [1, 2, 6, 12].

The male patients involved in this study (mean age: 61.1 ± 17.1 years) were older than the female patients (mean age: 49.9 ± 13.5 years) which is a statistically significant fact. Principal cause of pressure ulcers in our patients was a SCI occurring usually due to traffic accident trauma (58.3%), immediately followed by war trauma (27.8%), and fall from height (13.9%). Other studies indicate that SCI are a principal disease of patients with pressure ulcers, accounting for 80% of the cases. These injuries were most often caused by traffic accident trauma, while war trauma appears as a cause in a negligible number of cases. However, previous studies stated that after World War II, an increase in the number of pressure ulcers of 85% was recorded among war veterans [1, 12, 13]. There were three times more male patients injured in traffic accidents than female ones (75% *vs*. 25%). Also, 85.7% of multiple sclerosis patients were females. Other researches show an increase in the female population with neurological diseases. Cerebrovascular insult and vascular diseases of the lower limbs equally affect both sexes. All other diseases were slightly are common in men. Studies conducted in other countries also show that the principal disease, with the highest incidence rate in men, is SCI due to traffic accident trauma, while in women these are neurological diseases [1, 2, 3, 11].

Pressure ulcers in patients involved in this study were most often localized in the sacral area (40.3%), followed by the trochanteric and ischial areas. Pressure ulcers in patients involved in other research most often occurred in ischial area, followed by the trochanteric, and finally sacral area [2, 12]. This can be explained by early verticalization of patients, better physiotherapy, and more frequent use of wheelchairs when patients in developed countries are concerned.

The greatest number of our patients had stage III and IV pressure ulcers, which is also in conformity with some studies conducted in tertiary health institutions in European countries [10]. Our patients mainly had cardiovascular (37.2%) and endocrine diseases. All studies referring to pressure ulcers show that comorbidities which affect the patients with pressure ulcers are the following: cardiovascular diseases (41%), neurological diseases (27%), orthopedic injuries (15%), and endocrine diseases (15%) [2, 3, 4]. Normal nutritional status was found in 52.8% of the patients involved in this study, while 47.2% of the patients had hypoproteinemia and hypoalbuminemia. Other studies show a slightly higher percentage of patients with poor nutritional status, hypoproteinemia, and hypoalbuminemia, which also represent a risk factor for the development of pressure ulcers. During preoperative preparation, we had a tolerance of 10–15% lower than normal levels of proteins, albumin, white blood cells, and hemoglobin. All these lower levels are normally expected in patients with pressure ulcers. However, when patients have normal levels of protein and albumin, better surgical treatment outcome can be expected [1, 2, 3, 12].

Almost all patients in this study were treated surgically. Surgical treatment is the only method for stage IV pressure ulcer and sometimes for stage III. Stages II and III pressure ulcers can be treated in a conservative way with negative-pressure wound therapy [11, 16]. Stage I pressure ulcers were not included in this study because patients with pressure ulcer stage I are treated as outpatient patients with conservative treatment and advice. Almost one-half of pressure ulcer patients were treated surgically by using myocutaneous transposition (41%) and rotation flaps (38%) most frequently, following by advancement flaps (7%) and insular flaps (5%) or the combination of the two. The differences in using specific myocutaneous flap types were statistically highly significant and are strongly connected with the localization and the stage of the pressure ulcer. Proper flap selection is directly in correlation with the localization of the pressure ulcers, their stage, and their number. Moreover, one large retrospective study deals with flap selection type. Ischial pressure ulcers were covered with gluteal myocutaneous rotation flaps or posterior thigh/hamstring advancement flaps. Sacral pressure ulcers were covered with gluteal myocutaneous rotation flaps or gluteal fasciocutaneous V–Y advancement flaps. Trochanteric pressure ulcers were covered with tensor fasciae latae myocutaneous flaps [17, 18]. Other authors of the review article and guidelines summarize flap selection as follows: for sacral ulcer – lumbosacral flap; unilateral or bilateral gluteal fasciocutaneous flap versus myocutaneous rotation flap; unilateral or bilateral gluteal myocutaneous V–Y advancement flap; for ischial pressure ulcers – gluteal fasciocutaneous flap versus myocutaneous rotation flap; posterior hamstring myocutaneous V–Y advancement flap; for trochanteric pressure ulcer – tensor fasciae latae; tensor fasciae latae and vastus lateralis; Girdlestone procedure (proximal femurectomy and obliteration of dead space with vastus lateralis) [1, 3, 10, 19, 20]. Partial osteotomy was performed more frequently in the group of patients with stage IV pressure ulcers (53.3%) in comparison with the group of patients with stage III pressure ulcers (25%). Sometimes, during the operation, we touched the 'tip of the iceberg' and it was necessary to change the primary surgical plan with more extended debridement. Often, we had to use osteotomy when it was not primary planned to do so following extended reconstruction with the myocutaneous flap instead of the fasciocutaneous flap. The patients in this study were operated on by several different surgeons and the decision about osteotomy was not made only according to the pressure ulcer stage. Sometimes, that decision was made when osteomyelitis was suspected. Sometimes it is necessary to make decision about partial osteotomy to prevent bone

prominences to compromise flap vitality or to prevent new pressure ulcer in the future. Regardless of the bone status, in most of the cases osteomyelitis is not a contraindication to definitive surgery and can be treated definitively with decortication of the bone and appropriate soft-tissue coverage. Few studies analyze frequencies of partial osteotomy in surgical reconstructive treatment of pressure ulcer [9, 10, 11, 14]. However, all authors suggest partial osteotomy for stage IV pressure ulcer patients and when osteomyelitis is suspected [18–21]. Partial osteotomy has an important role in the prevention of osteomyelitis, but according to some authors partial osteotomy is an integral but tricky part of the surgical treatment of pressure ulcers [9, 12, 20, 21]. In the postoperative period, patients in this study had only minor complications, such as small dehiscence, partial flap necrosis, and hematomas (Clavien–Dindo grade I or II), but only in the group of patients who are of higher age, with hypoproteinemia and hypoalbuminemia or underweight. Furthermore, minor (Clavien–Dindo grade I) complications might not always be recorded in the medical records, and some minor complications might have been missed due to incomplete records. Authors in review studies have found that patients at higher age, with low serum albumin level, and who are over or underweight, were associated with an increased risk of complications [22, 23]. There is evidence that abnormal nutritional markers (e.g., anemia, serum protein, inflammatory markers) become normal after surgery. This study has limitations because it is a retrospective study and the follow-up was not long enough.

CONCLUSION

This study showed that the incidence rate of pressure ulcers is higher in male patients. SCI represents the most common principal disease in patients with pressure ulcers. These SCI are most often caused by traffic accident trauma. Pressure ulcers are most often localized in the sacral area, followed by the trochanteric area and the ischial area. The highest percentage of patients involved in this study had stage III and IV pressure ulcers. Surgical treatment represents the gold standard in pressure ulcer treatment especially in the group of patients with stage III or IV pressure ulcers. Preoperative findings help to prepare and make a decision about partial osteotomy, but the definitive decision is always made during surgery.

The most common comorbidities in patients involved in this study were cardiovascular, neurological, and endocrine diseases. Almost one-half of all patients involved were diagnosed with hypoproteinemia and hypoalbuminemia.

Understanding the challenges pressure ulcers present both to the patient and the health system, and the education regarding their prevention and treatment, is increasingly important. In the future, we can expect an increase of less usual localizations of pressure ulcers in the context of the COVID-19 pandemic, because prone position has been frequently used in intensive care units to improve the prognosis in patients with respiratory distress.

Conflict of interest: None declared.

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Анализа епидемиолошких карактеристика и хируршког приступа код болесника са декубиталним улцерацијама

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САЖЕТАК

Увод/Циљ Декубитална улцерација је рана настала изумирањем ткива под дејством прекомерног притиска који ствара само тело својом тежином на тачкама ослонца или у комбинацији са смицањем. Представља најчешћу компликацију код лежећих болесника која настаје изумирањем ткива под дејством притиска. Декубиталне улцерације представљају компликације током лечења различитих субпопулација болесника, а етиолошке и епидемиолошке карактеристике играју значајну улогу у одабиру адекватног третмана лечења.
Методе Урађена је ретроспективна анализа података 72 болесника са декубиталним улцерацијама који су хируршки лечени у нашој установи у петогодишњем периоду. Анализирана обележја посматрања су била пол, годиште, главна дијагноза, коморбидитети, биохемијски параметри малнутриције. Обрађени су одабир метода лечења, реконструктивни метод лечења као и учесталост остеотомије.
Резултати У студију су била укључена 72 болесника са декубиталним улцерацијама. Просечна старост болесника је била 54,7 ± 16,1 година. Три пута више болесника мушког пола је повређено у саобраћајном трауматизму (75% *vs*. 25%), док је већина болесника са мултиплом склерозом била женског пола (85,7%). Више од 95% болесника је било са улцерацијама III и IV степена и лечено је хируршки, реконструктивном методом транспозиције или ротације миокутаног режња, а остеотомија је најчешће примењена код болесника са декубиталном улцерацијом IV степена.
Закључак Хируршка реконструкција применом фасциокутаних и миокутаних режњева представља златни стандард за лечење болесника са декубиталним улцерацијама. Овим процедурама обезбеђује се адекватна надокнада изгубљеног ткивног волумена и коже квалитетним и добро васкуларизованим ткивом уз обавезну облитерацију мртвог простора, али уз неопходност даљег спровођења антидекубиталних мера.

Кључне речи: декубиталне улцерације; хируршко лечење; остеотомија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Safety and efficacy of surgical transobturator tape in the treatment of stress urinary incontinence in women – three years of follow-up

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SUMMARY

Introduction/Objective Stress urinary incontinence (SUI) is defined as the complaint of involuntary loss of urine in effort or physical exertion, or on sneezing or coughing. It is a common clinical condition affecting 50% of middle-aged and elderly women. Mid-urethral slings (MUSs) are the gold standard in the treatment of SUI.

The aim of this study was to investigate the success rate and complications of surgical treatment of SUI in women with transobturator tape (TOT) within the three years of follow-up.

Methods From January 2011 until January 2018, 86 women with predominantly SUI were operated by TOT procedure. In 61.6% of patients SUI was confirmed by preoperative urodynamic examination (cystometry, uroflowmetry, urethral presser profile) and in 38.4% of patients by clinical examination of stress test (cough provocation). All patients were invited for a follow-up examination six, 12, 24, and 36 months after surgery. The result of the operation is defined as cured, improved or without success.

Results The average age was 55 (32–72) years. The most common complications were tape erosion (3.5%), incision bleeding (2.3%), transient leg pain (3.5%), dyspareunia (2.3%), vaginal erosion (3.5%) and de novo urge (5.8%). After three years of follow-up, 82.6% patients were cured.

Conclusion TOT is a safe, effective and successful procedure with 82.6% of cured patients during a three-year follow-up.

Keywords: urinary incontinence; stress; trans-obturator tape; suburethral slings

INTRODUCTION

The International Urogynecological Association and the International Continence Society define stress urinary incontinence (SUI) as the complaint of involuntary loss of urine in effort or physical exertion, or on sneezing or coughing [1]. It is a common clinical condition affecting 50% of middle-aged and elderly women [2]. SUI negatively interferes with the quality of life and mental health [3].

In order to maintain urinary continence, it is very important that there is a synergy between the structures that make up the pelvic floor, the sympathetic and parasympathetic nervous systems and the motor fibers of the pudendal nerves. Involuntary loss of urine may occur as a result of an alteration in one or more components due to the inability of the urethra to counteract the increase in abdominal pressure. Predisposing factors for SUI are age, parity (especially with vaginal delivery) and obesity due to their influence on the weakening of pelvic floor structures, leading to urethral hypermobility. Parity can additionally lead to SUI through its effects on urethral and bladder innervation, triggered by the stretching or compression of nerves during the passage of the fetus through the birth canal [4].

Because of its safety and efficacy [5, 6], a surgical treatment is the method of choice, when conservative therapy fails. Until the introduction of mid-urethral slings (MUSs), the gold standard surgical treatment of SUI was the Burch retropubic urethropexy or Marshall-Marchetti-Krantz procedure through the retropubic routine [7]. Nowadays, the gold standard in the treatment of SUI is MUSs [8]. Since the report by Ulmsten and Petros in 1995, the tension-free vaginal tape (TVT) technique, thanks to its advantages such as shorter postoperative stay, minimal surgical trauma and long-term high sucess rate, has been the most commonly used surgical treatment for SUI [9]. Despite the fact that this technique has reached high success rates in the mid and long term, important complications, such as retropubic hematomas, bladder perforation and voiding dysfunction have also been described [9].

In 2001 a new technique was described, trying to reduce these complications, involving the placement of a synthetic mesh under the middle urethra through the transobturator route from the thigh to the vagina (transobturator tape outside-in [TOT]). De Leval presented a modification to the technique, in 2003, suggesting insertion of the mesh toward the opposite direction, from the vagina to the

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thigh (transobturator tape inside-out [TVT-O]) [10]. Both slings, TOT and TVT-O have shown high curative rate and lower number of complications [11, 12, 13]. Nevertheless, several researchers point at thigh and groin pain as the main complications.

The purpose of this study was to investigate the success rate and complications of surgical treatment of SUI in women with TOT outside-in technique with three years of follow-up.

METHODS

This was a retrospective study and presents the surgical results of treatment of SUI in women with monofilament polypropylene tape tension-free by transobturator approach, technique outside-in, in the period from January 2011 to January 2018 in a tertiary referral center at the Clinic for Gynecology and Obstetrics, Clinical Center of Vojvodina in Novi Sad, Serbia. This study was approved on February 21, 2019 under number 00-187/1 by Ethics Committee of the University of Novi Sad, Faculty of Medicine, Serbia.

A total of 86 women, who had SUI or mixed urinary incontinence, with a predominantly stress component, underwent surgery. The exemption criteria were the absence of urodynamic changes associated with the SUI, findings indicating infravesical obstruction and detrusor overactivity, coagulopathy, pregnancy, history of sensitivity of a foreign body (i.e., polypropylene), acute cystitis, vulvovaginitis, previous surgery to treat SUI and history of pelvic radiotherapy treatment.

In the preoperative preparation, a detailed anamnesis, previous medical history, clinical urogynecological examination, laboratory analyzes, negative urine culture findings, provocative cough tests were taken.

In our study, we used clinical and functional terminology that is in accordance with the standardization of the International Society for Continence. Urinary stress incontinence was confirmed by preoperative urodynamic examination (cystometry, uroflowmetry, urethral presser profile) or clinical examination of stress test (cough provocation) with full bladder in standing and lying position. Two surgeons, trained in urogynecological surgery, performed all operations according to the original Delorme technique (TOT outside-in) using monofilament polypropylene tape. Patients underwent surgery under general or spinal anesthesia. The Foley catheter was removed on the first postoperative day. The patients were discharged home in 1–4 days. Before surgery, all patients signed written consent for surgery and postoperative follow-up. All patients were invited for a follow-up examination six, 12, 24, and 36 months after surgery, which consisted of asking the patients about postoperative satisfaction, gynecological examination, urine and urine culture analysis and performing provocative cough tests with a full bladder in standing and lying down. The result of the operation is defined as cured, improved or without success. Cure was defined as the absence of subjective complaint of urine leakage, and the absence leakage on cough stress testing. Patients were considered improved when they had a decrease of stress incontinence. Other cases were considered as without success.

RESULTS

A total of 86 women, who suffered from SUI or mixed urinary incontinence, with a predominantly stress component, underwent surgery by placing a monofilament polypropylene tape tension-free by transobturator approach, technique outside-in. Preoperative urodynamic examination (cystometry, uroflowmetry, urethral presser profile) was performed in a total of 53 (61.6%) patients. These were 21 patients who suffered from SUI, eight patients with history of previous abdominal hysterectomy, three patients with history of previous vaginal hysterectomy as well as 14 patients who had mixed urinary incontinence and seven patients who had initial anterior vaginal wall prolapse (Figure 1). In 33 (38.4%) patients, who were not subjected to urodynamic examination, SUI was confirmed by a clinical trial of a stress test (cough provocation) with a full bladder in standing and lying position.

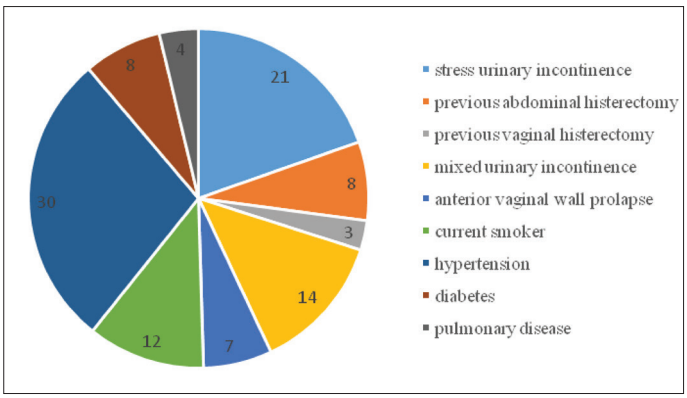

Figure 1. Graphic representation of comorbidities of patients

All patients in the preoperative preparation had a positive cough test and a negative urine culture.

The age of the patients ranged 33–72 years, with an average of 55 years. 12 patients (14%) were current smokers. More than a quarter, 30 (35%) patients had hypertension. Almost 10% (eight) of patients had diabetes, while four patients (5%) had pulmonary disease (Figure 1).

The length of hospitalization was 1–4 days. The urinary catheter was removed on the first postoperative day. 69 patients (80%) underwent surgery in general anesthesia and 17 patients (20%) under spinal anesthesia. Complications such as tape erosion occurred in three patients (3.5%), incision bleeding in two patients (2.3%), transient leg pain in three patients (3.5%) and dyspareunia in two patients (2.3%). No perforations of the bladder or urethra were observed, as well as intestines or blood vessels. Vaginal erosion occurred in three patients (3.5%). Erosion occurred in

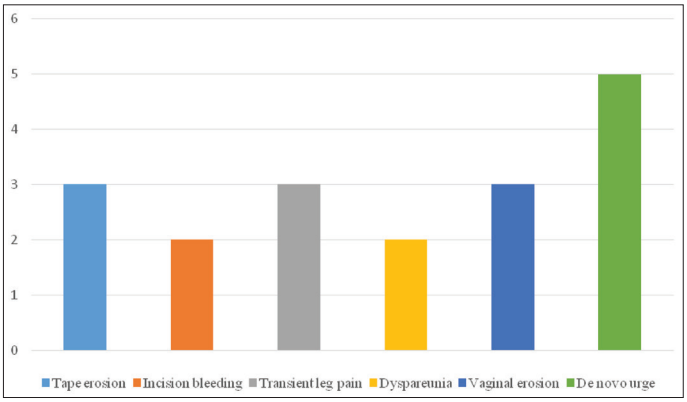

Figure 2. Graphical representation of the type and frequency of complications

two patients six months after surgery, and in one patient 12 months after surgery. In two patients, the solution was achieved by repeated sutures, and in one patient the tape was cut. Transient leg pain occurred in three patients and lasted less than 10 days. Bleeding from the incision site was treated conservatively (tamponade) and resolved during the first postoperative day. Dyspareunia, which manifested as a late complication in both patients, occurred one year after the operation. One patient underwent conservative treatment, the other had a tape cut. In five patients (5.8%) de novo urgency was registered as a late complication (Figure 2).

Table 1. Results of realistic valuation of cure determined on the basis of postoperative assessment

Period after surgery (months)	Objective cure rate n (%)		
	Cured	Improved	Without success
6	80 (93%)	/	6 (7%)
12	77 (89.5%)	(1.2%)	8 (9.3%)
24	73 (84.9%)	4 (4.6%)	9 (10.5%)
36	71 (82.6%)	(6.9%)	9 (10.5%)

In our study, 71 (82.6%) patients who underwent surgery with the TOT outside-in technique, after three years of follow-up, were cured (Table 1).

DISCUSSION

SUI is a widespread, global disease that affects women around the world and is often underestimated. Most frequently it occurs among middle-aged and elderly women, as shown by the results of our study in which the average age of women was 55 years [2].

There are several advantages, such as minimal morbidity, short operation time, rapid convalescence, and long-term efficacy that make MUSs considered the gold standard for treating SUI [6, 7, 10].

TOT is an effective and safe method for treating SUI and is the method of choice in many centers [14]. Nevertheless, the reports show different success rates for the procedure [15]. In the current study, TOT was very successful because more than three-quarters of patients 71 (82.6%) were relieved of symptoms after the three-year follow-up.

Synthetic MUSs is the first-line surgical procedure for SUI according to the 2017 position statement from the European Urogynaecological Association with a success rate of > 80% [16] what we also proved in our research using only polypropylene tape with a success rate of 82.6% and improvement rate of 6.9% after three years of follow-up. One year after surgery, the success rate in our study was observed in 89.5% of patients, improvement in 1.2% of patients, while 8.9% of patients reported no success after the treatment. After two years, success rate was 84.9%, improvement was observed in 4.6% of patients, while 10.5% of patients were without success after the treatment. The results of our research are correlated with literature data in several studies [3, 11, 16].

In ours, as in other studies, patients were operated under general or under spinal anesthesia. The anesthesiologists made a decision whether to use general or spinal anesthesia taking into account the patient's condition and comorbidities. The type of anesthesia had no effect on the outcome of the operation although it was previously thought that the use of spinal anesthesia was important to achieve the adequate tensioning of the sling and control of continence performing the cough test during the procedure [17].

Delorme described TOT procedure in the attempt to minimize TVT complications such as bladder perforation, retropubic hematomas and voiding dysfunction. However, there are still complications, and the most common intraoperative complications are bladder and vaginal perforations and hemorrhage [10]. There were no significant intraoperative complications in our study, as evidenced by Abrar et al. [18] who conducted a cross-sectional study of 162 patients who underwent surgery for SUI with Burch colposuspension (n = 40), tension free vaginal tape (TVT) (n = 59) or TOT (n = 63), from 2006 to 2014 at the Aga Khan University Hospital in Karachi.

Tape erosion is directly associated with biomechanical properties, wound healing, local factors as infection and also surgical technique. Tape erosion occurred in only three patients (3.5%), which correlated with the results of various studies [19].

After TOT surgery, transient leg pain which lasted for less than 10 days, occurred in three patients (3.5%) and it is in accordance with the existing literature where it is stated that the incidence of leg pain reach up to 15.5% [20].

In our study dyspareunia is manifested as a late complication in two patients (2.3%) and occurred a year after the operation, which indicates that this rate was almost ten times lower than that of Karakeçi et al. [20]. One patient underwent conservative treatment, while the other had the tape cut.

Individual factors, as well as surgical technique and sling material are significant contributing factors to the development of vaginal erosion. Vaginal erosion can also

occur as a result of inadequate suturation of the vaginal incision, infection, rejection of the sling material, early sexual intercourse and vaginal perforation. A study by Afflar et al. reported that 3.3% of the patients who underwent the TOT operation, developed vaginal erosion complication what is almost exactly the same rate as in our study 3.5% [21]. Erosion occurred in two patients six months after the surgery, and in one patient 12 months after the surgery. The solution was achieved by repeated sutures in two patients and in one patient the tape was cut.

As a complication, de novo urge incontinence effects the life quality negatively but occurs rarely after the TOT operations and it proves that TOT operation has a minimal obstructive effect. Rougmeguere et al. as well as Krauth et al. reported de novo urge incontinence rate as 2.5% and 5.2% after three months follow-up. In the study of Afflar et al. de novo urge incontinence rate in the TOT group was low (4.2%). Göynümer et al. established de novo urge incontinence in 3% of the cases [21]. In our study, in the medium-term follow-up, in five patients (5.8%) de novo urge incontinence occurred as a late complication and this rate was similar with the literature [21].

Gynecological surgeries and procedures significantly increased the risk for de novo urge incontinence, so that eight patients with the history of previous abdominal hysterectomy and six patients with the history of previous vaginal hysterectomy in our study had twice the risk of developing de novo urge incontinence. In one patient with a history of previous vaginal hysterectomy and diagnosed SUI, there was no successful outcome treated at the first follow up after six months. The second patient was continental even after the third year of follow-up with the appearance of urge incontinence one year after the operation. The third patient was continental and subjectively satisfied after the third year of follow-up.

The surgeon's experience is essential for the success of a surgical procedure so that the low complication rate and the high success rate of surgical treatment in this study can be explained by the adequate surgical training and experience of the surgeons who performed the operations [22].

CONCLUSION

Our study confirms that TOT is a safe procedure in the short and medium term with very few intraoperative, early and late postoperative complications. It is also an effective and successful procedure in the treatment of SUI with 82.6% of cured and 6.9% of improved patients during a three-year follow-up. Further evaluation of the procedure requires studies with a longer follow-up.

Conflict of interest: None declared

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Сигурност и ефикасност хируршке трансобтураторне траке у лечењу стресне уринарне инконтиненције код жена – трогодишње праћење

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САЖЕТАК

Увод/Циљ Стресна уринарна инконтиненција (СУИ) дефинише се као невољно испуштање мокраће приликом напора, физичког напрезања, кијања или кашљања. То је често клиничко стање које захвата 50% жена средње и старије животне доби. Субуретрални слингови су златни стандард у третману СУИ.

Циљ ове студије је био да истражи стопу успешности и компликација хируршког третмана СУИ код жена трансобтураторном траком (ТОТ) унутар трогодишњег праћења.

Методе У периоду од јануара 2011. до јануара 2018. године, 86 жена са предоминантном СУИ оперисане су процедуром ТОТ. Код 61,6% болесница СУИ је преоперативно потврђена уродинамским испитивањем (цистометрија, урофлоуметрија, профил уретралног притиска), а код 38,4% болесница клиничким испитивањем теста осетљивости на кашаљ. Све болеснице су позване на контролни гинеколошки преглед шест, 12, 24 и 36 месеци после операције. Резултат операције је дефинисан као излечен, побољшан или без успеха.

Резултати Просечна старост болесница је била 55 година (32–72). Најчешће компликације су биле ерозија траке (3,5%), крварење из места инцизије (2,3%), пролазна бол у нози (3,5%), диспареунија (2,3%), ерозија вагине (3,5%) и *de novo* хитност (5,8%). После трогодишњег праћења 82,6% болесница је било излечено.

Закључак ТОТ је сигурна, ефикасна и успешна процедура са 82,6% излечених болесница током трогодишњег праћења.

Кључне речи: уринарна инконтиненција; стрес; трансобтураторна трака; субуретрални слингови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The pharmacogenomics of vincristine-induced peripheral neuropathy in pediatric acute lymphoblastic leukemia patients in Serbia – a single center experience

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SUMMARY

Introduction/Objective Vincristine (VCR) is one of the key drugs in current treatment protocols for pediatric acute lymphoblastic leukemia (ALL). By destabilizing microtubules, VCR arrests cells in metaphase, inducing apoptosis of malignant cells. VCR also causes axonal degradation and impairment of axonal transport, which leads to VCR-induced peripheral neuropathy (VIPN).
This study aimed to investigate the association of five variants in pharmacogenes involved in VCR metabolism with VIPN in Serbian ALL children. We also wanted to discover candidate pharmacogenomic markers of VIPN in Serbian population.

Methods PCR and sequencing-based methodology was used to detect variants in *CYP3A5, CEP72, ACTG1, MIR3117*, and *MIR4481* genes. Statistical analyses were performed for investigating their association with VIPN in 56 pediatric ALL patients. Population VCR pharmacogenomics analysis of 17 pharmacogenes from in-house next-generation sequencing data was also done. Data on allele frequency distribution for the European population were extracted from public databases.

Results During the treatment, 17.86% of patients developed VIPN. Association analyses have shown that none of the genetic variants contributed to the occurrence of VIPN in our study. Population pharmacogenomics study did not reveal valid candidate pharmacovariants for VIPN. Our results suggested that pre-emptive pharmacogenetic testing for VCR is not applicable presently.

Conclusion More comprehensive approaches are needed to identify the panel of genes that could explain the VIPN development after VCR administration in ALL patients. Utilizing better designed genome-wide association studies and more robust artificial intelligence-based tools would provide a panel of pharmacogenes for pre-emptive tests of VIPN to individualize therapy for ALL in children.

Keywords: acute lymphoblastic leukemia; pharmacogenomics; vincristine; vincristine-induced peripheral neuropathy (VIPN)

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy, comprising about one-fourth of all cancers in children. The cure rate for childhood ALL reached 85%, but about 75% of all patients experience treatment side effects, and 1–3% of all children with ALL die due to the treatment toxicity [1]. Application of the principles of pharmacogenomics could lower the number and intensity of drug-induced side effects.

One of the key drugs in treatment protocols for pediatric ALL is vincristine (VCR). VCR binds to tubulin, preventing the polymerization of microtubules and inducing apoptosis in cancer cells. However, the affinity of VCR for tubulin makes the microtubules in nerve fibers a likely target of VCR action, leading to axonal degradation, impairing axonal transport, and causing the development of VCR-induced peripheral neuropathy (VIPN). VIPN is a major side effect of VCR administration, manifesting as muscle weakness, areflexia, neuropathic pain, sensory loss, or autonomic polyneuropathies [2]. VIPN often results in dose reduction, treatment delays, and further withdrawal. The occurrence of VIPN in children is determined by multiple factors [3, 4], with most of the recent studies focusing on genetic influences [5].

The most comprehensive candidate gene and genome-wide association studies (GWAS) pointed to the possible involvement of following pharmacogenes: *CYP3A5, CEP72, ACTG1* [6–10]. Also, several variants in genes encoding miRNAs were shown to be potential pharmacogenomic markers of VIPN [11]. *CYP3A5* is the most important metabolizer of VCR. The rs776746 variant in the third intron introduces a premature stop codon which leads to low or no expression of this enzyme [6]. *CEP72* gene encodes a centrosomal protein important for microtubule formation and stability of the centrosome. Variant rs924607 in the promoter region

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of this gene could be associated with a higher risk of development and severity of VIPN [11], by possibly introducing a binding site for transcriptional repressor NKX-6.3, lowering expression on *CEP72* mRNA [7]. Alterations in the interactions between the gamma isoform of actin (*ACTG1*) and microtubules are involved in signal transduction to the actin cytoskeleton. The variant *ACTG1* rs1135989 was associated with higher risk and more frequent episodes of high-grade neurotoxicity, as well as a lower tolerated VCR dose [10]. A recent study identified variants in genes encoding miRNAs related to VIPN [11]. The variant *MIR3117* rs12402181 reduces the risk of VIPN by decreasing the degradation and increasing the expression of *ABCC1* and *RALBP1* mRNAs, thus increasing the efflux of VCR from the axons. The variant *MIR4481* rs7896283 increases the stability of the premature miR-4481, lowering expression of proteins in the axon guidance pathway that may affect peripheral nerve regeneration [11]. Several studies have indicated additional pharmacogenes potentially involved in the development of VIPN [8, 9, 12–15].

The aim of this study was to determine if the selected genetic variants *CYP3A5* rs776746, *CEP72* rs924607, *ACTG1* rs1135989, *MIR3117* rs12402181, and *MIR4481* rs7896283 are associated with the development of VIPN in ALL children treated with VCR in Serbia. Additionally, we aimed to perform an analysis of clinical exome sequencing data for population pharmacogenomics study to discover candidate pharmacogenomic markers of VIPN in the Serbian population.

METHODS

Subjects

This study included 56 children diagnosed with ALL between 2010 and 2018 at the University Children's Hospital, Belgrade, Serbia. It was approved by the University Children's Hospital Ethics Committee and performed according to the Declaration of Helsinki. Informed consent was obtained from the parents or legal guardians of each patient.

All patients were treated according to the ALL Intercontinental Berlin-Frankfurt-Munster (IC-BFM) 2009 protocol, divided into the usual phases: remission induction and early intensification, consolidation, reinduction and maintenance. The patients were stratified into three risk groups: standard risk (SR), intermediate risk (IR), and high risk (HR). Patients in the IR and HR groups were randomized during the early intensification phase in arm 1 (IR-1 and HR-1) and arm 2 (IR-2 and HR-2). Stratification and randomization of the patients resulted in patients receiving different number of VCR doses (1.5 mg/m^2 per dose) during the treatment (Table 1) [16].

The patients were assessed for VIPN using the National Cancer Institute Common Toxicity Criteria [17]. A patient was diagnosed with VIPN if exhibiting one or more symptoms of VCR-related neurotoxicity at the end of the reinduction phase.

Data for the European population frequencies of the investigated variants were extracted from the Genome Aggregation Database, GnomAD.

Genetic variants detection

Detection of variants investigated in this study (*ACTG1* rs1135989, *CEP72* rs924607, *MIR3117* rs12402181, *MIR4481* rs7896283, and *CYP3A5* rs776746) was performed using PCR and sequencing-based methodology as described elsewhere [18]. Primer sequences and annealing conditions used for amplification of each variant are available upon request. Allele frequency for *CEP72* rs924607 in healthy controls of Serbian descent was determined using the same methodology.

Population pharmacogenomics study

We have searched the literature on the PubMed database before April 2021 using the terms "vincristine" AND "pharmacogenomics" OR "pharmacogenetics" AND "GWAS" OR "candidate gene" OR "vincristine-induced peripheral neuropathy." Studies were identified from the titles and abstracts by the primary (BR) and the secondary reviewer (BZ).

In-house database of 154 TruSight One Illumina sequenced clinical exomes belonging to individuals of Serbian descend was used to search for variants in 17 genes found during literature search that could be related to VCR metabolism: *CYP3A4, CYP3A5, ABCB1, PON1, ABCA4, ABCG1, CY51A1, SLCO1C1, ABCC1, SLC5A7, TTPA, ABCC2, SYNE2, COCH, TUBB1, TUBB2B*, and *TUBB3*.

The criteria for pharmacogenomics relevance of a variant were allele frequency higher than 5% and assigned annotation in PharmGKB database [19].

Assignment of a Level of Evidence by the PharmGKB annotation scoring system for clinical and variant annotations enables easier identification of significant pharmacovariants. The clinical annotation score represents the sum of the scores of all attached variant, guideline, and drug label annotations. Variant annotations are scored depending on the following: phenotype category, p-value, cohort size, effect size, and weighting by study type or by association and significance [19].

Statistical analysis

All variants were tested for the Hardy–Weinberg equilibrium (HWE) using an exact test.

The association of age of the patients and occurrence of VIPN was tested using logistic regression. The associations of immunophenotype, sex, and the number of doses administered were tested for association with VIPN using Fisher's exact test.

The association between the genotyped variants and VIPN was tested using the multiplicative genetic model. Multivariate analysis was adjusted for the number of VCR doses or sex.

Table 1. Characteristics of pediatric acute lymphoblastic leukemia patients

Characteristics	Patients without VIPN	Patients with VIPN	Total	p[1]
Age (years)				0.913[2]
Average	7	6.8	7	
Median	5.5	3.7	5.3	
Range	0.7–17.9	1.0–17.1	0.7–17.9	
Sex (n/%)				0.171[3]
Male	26 (56.5%)	3 (30%)	29 (51,8%)	
Female	20 (43.5%)	7 (70%)	27 (49,2%)	
Immunophenotype [n (%)]				1[3]
B-lineage	42 (91.3%)	10 (100%)	52 (49,2%)	
T-lineage	4 (8.7%)	0 (0%)	4 (7,1%)	
Risk group [n (%)]				0.346[3]
SR; 8 VCR doses	10 (21.7%)	1 (10%)	12 (21.4%)	
IR-1; 8 VCR doses	18 (39.1%)	2 (20%)	19 (33.9%)	
IR-2; 12 VCR doses	5 (10.9%)	3 (30%)	8 (14.3%)	
HR-1; 12 VCR doses	10 (21.7%)	3 (30%)	13 (23.2%)	
HR-2; 16 VCR doses	3 (6.5%)	1 (10%)	4 (7.2%)	

VIPN – vincristine-induced peripheral neuropathy; VCR – vincristine; SR – standard risk; IR – intermediate risk; HR – high risk;
[1]p-value refers to statistical testing the difference between groups of patients with and without VIPN;
[2]Logistic regression;
[3]Fisher's exact test

Association analyses were performed using SPSS (version 21, IBM, Armonk, NY, USA).

Probabilities lower than 5% were considered statistically significant.

RESULTS

Demographic and clinical characteristics of the subjects

This study encompassed 56 children with ALL, with slight predominance of male sex (n = 29; 51.8%). The median age at diagnosis was 5.3 years, ranging 0.7–17.9 years. Risk stratification revealed the following distribution: SR – 11 (19.6%), IR – 28 (50%), and HR – 17 (30.4%).

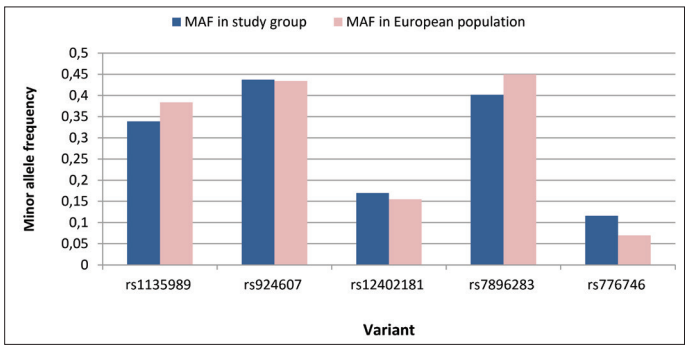

Figure 1. Minor allele frequencies (MAFs) of analyzed genetic variants in the study group and European population; all investigated variants were in Hardy–Weinberg equilibrium (HWE) (for rs1135989 HWE was 0.768, for rs924607 it was 0.790, and for the other variants 1); the data for MAF in the European population was extracted from the GnomAD database

Ten patients (17.86%) developed VIPN during the therapy, with high predominance of girls (70%). The median age at diagnosis of the patients that developed VIPN was 3.7 years, ranging 1–17.1 years. Nine out of ten patients were stratified into the IR and HR groups (IR – 5, HR – 4).

Occurrence of VIPN was higher in girls, though statistical significance has not been demonstrated (0.171, Fisher's exact test, OR = 3.03). A trend of higher occurrence of VIPN in patients who received more VCR doses was observed, but without statistical significance. However, patients who received more than 10 doses of VCR were 3.6 times more likely to develop VIPN than patients who received less than 10 doses (OR = 0.363; CI = 0.83–15.89; p = 0.092; Fisher's exact test) (Table 1).

Association of pharmacogenetic markers with VIPN

The HWE testing showed that genotype frequencies corresponding to all variants investigated in this study were in equilibrium. Frequencies of investigated variants in the Serbian pediatric ALL patients and the control group of European origin and results of HWE testing are presented in Figure 1.

We analyzed the association between the investigated variants and VIPN. For each variant, we evaluated the contribution associated with each additional minor allele to the probability of developing the neuropathy using a multiplicative genetic model [20]. In univariate analysis, no variant showed a statistically significant association with the VIPN. Applying logistic regression, the following p-values associated with variants in *ACTG1*, *CEP72*, *MIR3117*, *MIR4481*, and *CYP3A5* genes were obtained: 0.917, 0.898, 0.788, 0.310, and 0.577, respectively. In univariate or multivariate analysis (adjusted for the number of VCR doses or sex), no variant showed a statistically significant association with VIPN (Table 2).

Population pharmacogenomics study

In-house database of clinical exome sequences of 154 Serbian individuals was searched for variants in 17 genes with possible influence to VCR metabolism. Ten variants in six genes have been detected with allele frequency higher than 5%. Allele frequencies for most of them were similar to the ones detected in the European population (Table 3).

Only two variants were worthy to be further analyzed as potential pharmacogenetic markers of VIPN in the Serbian population. Although a variant *CYP3A4* rs4986910 is assigned to have a 2A level of evidence, its allele frequency in the Serbian population is very low (0.97%), similarly to European populations (0.73%). Therefore, it is not considered to be an appropriate candidate for pre-emptive pharmacogenomic testing.

Variant *CEP72* rs924607, considered to be the best candidate pharmacogenomic marker for VCR, is present in the Serbian population with 60% compared to 43.4% in European populations.

Table 2. Association of analyzed variants and vincristine-induced peripheral neuropathy (VIPN)

Gene	dbSNP	Genotype	n (%)	Patients without VIPN	Patients with VIPN	p	p[1]	p[2]
ACTG1	rs1135989 C>T	CC CT TT	25 (44.6%) 24 (42.9%) 7 (12.5%)	21 (45.7%) 19 (41.3%) 6 (13%)	4 (40%) 5 (50%) 1 (10%)	0.917	0.837	0.886
CEP72	rs924607 C>T	CC CT TT	17 (30.4%) 29 (51.8%) 10 (17.9%)	14 (30.4%) 24 (52.2%) 8 (17.4%)	3 (30%) 5 (50%) 2 (20%)	0.898	0.909	0.791
MIR3117	rs12402181 G>A	GG GA AA	38 (67.9%) 17 (30.4%) 1 (1.8%)	31 (67.4%) 14 (30.4%) 1 (2.2%)	7 (70%) 3 (30%) 0 (0%)	0.788	0.963	0.605
MIR4481	rs7896283 T>C	TT TC CC	20 (35.7%) 27 (48.2%) 9 (16.1%)	15 (32.6%) 23 (50%) 8 (17.4%)	5 (50%) 4 (40%) 1 (10%)	0.310	0.188	0.500
CYP3A5	rs776746 A>G	AA AG GG	0 (0%) 13 (23.2%) 43 (76.8%)	0 (0%) 10 (21.8%) 36 (78.2%)	0 (0%) 3 (30%) 7 (70%)	0.577	0.702	0.360

[1]Adjusted for the number of vincristine doses;
[2]adjusted for sex

Table 3. Allele frequencies of pharmacogenes related to vincristine-induced peripheral neuropathy in Serbian population (MAF > 5%)

Gene	dbSNP[1]	PharmGKB LoE[2]	MAF in Serbian population (%)	MAF in European population (GnomAD) (%)
CEP72	rs924607	3	60	43.3
ABCC2	rs3740066	3	27.27	37.02
ABCC2	rs2273697	3	17.85	19.75
ABCC2	rs17222723	3	5.19	5.61
SLC5A7	rs1013940	VA	8.44	8.01
PON1	rs854560	4	37.01	36.7
PON1	rs662	3	22.4	28.06
COCH	rs1045644	VA	54.87	63.49
TUBB1	rs6070697	VA	15.26	17.94
TUBB1	rs463312	VA	6.17	5.26
ABCC1	rs246221	VA	31.82	30.5

MAF – minor allele frequencies; VA –variant annotation; LoE – level of evidence;
[1]reference single nucleotide polymorphism (SNP) ID number (rs number) of SNPs that map an identical location assigned by the National Center for Biotechnology Information;
[2]PharmGKB level of evidence, score of pharmacogenomics clinical (level 1: the highest, level 4: the lowest evidence association) and variant relevance

Despite high allele frequency in the Serbian population, this marker is not an applicable pharmacogenetic marker in pediatric ALL since our study demonstrated that it has the same distribution in pediatric ALL patients with and without VIPN.

DISCUSSION

Efforts towards treatment individualization of patients experiencing side effects of drugs are made constantly. Previous studies analyzed pharmacogenomics of some essential drugs used for treatment of pediatric ALL patients in Serbia [18, 21, 22].

We analyzed the correlation of five variants in pharmacogenes involved in VCR metabolism in pediatric ALL patients with VIPN. A total of 56 patients treated according to the BFM protocol were included in the study. Ten patients (17.86%) developed VIPN during the treatment. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

Our results have shown a trend of higher occurrence of VIPN in girls as in several studies [7], while others reported no influence of patients' sex on VIPN development [12, 23]. We have also observed a trend of higher occurrence of VIPN in patients who received more than 10 doses of VCR during therapy, as they were 3.6 times more likely to develop VIPN. Several studies reported significant association between VCR dose and VIPN [7, 14], which is in contrast to some other studies [12, 24]. Assessment of cumulative dose effect of VCR to VIPN development has also shown conflicting results. There are reports of the absence of association between VIPN and cumulative VCR dose [24], as well as reports showing that cumulative dose of VCR is associated with VIPN [25].

Variant *CEP72* rs924607 was identified in GWAS study as key pharmacogene relevant for VIPN in ALL children [7], and those findings were confirmed in meta-analysis of pharmacogenomic data from over 500 patients [8]. However, several studies did not confirm this association [9, 23, 26]. Our results do not support the association of *CEP72* rs924607 with VIPN in pediatric ALL patients. The frequency of rs924607 T allele in our group of ALL patients with VIPN was almost the same as in ALL patients without VIPN (45% and 43.5%, respectively). The frequency of the same allele in our healthy population was rather high (60%), similar to frequency of this variant in the European population (43.4%). Therefore, we conclude that *CEP72* rs924607 pharmacomarker was not shown to be of pharmacogenomic relevance for the Serbian population.

Variants *ACTG1* rs1135989 and *CYP3A5* rs776746 have also been reported to contribute to VIPN susceptibility [6, 10]. Furthermore, analysis of the SNPs in miRNAs which could regulate VCR-related genes in a large cohort of pediatric ALL patients identified the *MIR3117* rs12402181 and *MIR4481* rs7896283 as variants significantly associated with VIPN [11]. In our study, none of the aforementioned

variants have shown statistically significant association with VIPN in pediatric ALL patients.

As additional variants have been indicated to contribute to the development of VIPN, we have performed the pilot population pharmacogenomics study in order to assess if the molecular genetics study of additional potential pharmacogenes would be beneficial and informative. The population pharmacogenomics study encompassed 17 relevant, literature-reviewed pharmacogenes present in in-house NGS database sequences of Serbian individuals: *CYP3A4*, *CYP3A5*, *ABCB1*, *PON1*, *ABCA4*, *ABCG1*, *CY51A1*, *SLCO1C1*, *ABCC1*, *SLC5A7*, *TTPA*, *ABCC2*, *SYNE2*, *COCH*, *TUBB1*, *TUBB2B*, and *TUBB3* [8, 9, 12–15]. Also, for these pharmacogenes our population pharmacogenomics study did not reveal valid candidate pharmacovariant for VIPN. A GWAS study identified *PNPLA3* rs735409 as potential pharmacovariant relevant for VCR use [27]. Further study confirmed that the *PNPLA3* rs735409 variant was associated with hepatotoxicity induced by asparagine administration [28], and we decided not to include it in our study. Our results have shown that pre-emptive pharmacogenetic testing for VCR is not presently applicable either in pediatric ALL patients or in patients of Serbian descent to whom VCR needs to be administered.

VCR metabolic pathway is complex. Many transporters and enzymes have an important role in the VCR pharmacokinetics and pharmacodynamics and so far, data have not shown a single universal VCR pharmacogenomic marker [29]. This is another example of the failure of predictions that have resulted from GWAS studies. A lack of consistency in neuropathy assessment, grading systems, and the choice of end points make it difficult to interpret results between studies [4, 29]. Treatment regiments, including number of VCR doses administered and lengths of VCR treatment, differ between treatment protocols used in different studies [30]. Furthermore, population-specific genetic variants could have relevance or other expression quantitative trait loci of the genes in question for VIPN assessment.

The main limitation of our study is a small number of patients included. However, we have tried to overcome the limitations of GWAS studies by analyzing the patients treated with the same protocol and by establishing the comparative groups with similar VCR administration.

CONCLUSION

Our results have shown that pre-emptive pharmacogenetic testing for VCR is not presently applicable to neither pediatric ALL patients nor to patients of Serbian descent to whom VCR needs to be administered. Association analyses have shown that none of the genetic variants were significant for the occurrence of VIPN in our cohort.

More comprehensive approaches are needed to identify a panel of genes that could explain the VIPN development in ALL patients. Extending genome-wide research to larger, well-characterized and more diverse patient cohorts and development of more robust artificial intelligence bioinformatics tools, including machine learning, statistical learning, and soft-computing approaches, should be done. This would provide a panel of pharmacogenes that could be used for pre-emptive tests of VCR side effects leading to therapy individualization in pediatric ALL patients.

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Conflict of interest: None declared.

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Фармакогеномика винкристином индуковане периферне неуропатије код деце са акутном лимфобластном леукемијом у Србији – искуство једног центра

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САЖЕТАК

Увод/Циљ Винкристин је један од кључних лекова у протоколима лечења дечје акутне лимфобластне леукемије (АЛЛ). Винкристин доводи до дестабилизације микротубула, чиме се ћелија зауставља у метафази и индукује апоптоза. Такође доводи до деградације аксона и поремећаја аксонског транспорта, узрокујући винкристином индуковану периферну неуропатију (ВИПН).

Циљ ове студије био је да истражи повезаност пет варијанти у фармакогенима укљученим у метаболизам винкристина код деце оболеле од АЛЛ која су развила ВИПН, у Србији. Такође, циљ нам је био да откријемо кандидате за нове фармакогеномске маркере ВИПН-а у српској популацији.

Методе Детекција варијанти гена *CYP3A5*, *CEP72*, *ACTG1*, *MIR3117* и *MIR4481* изведена је методологијом заснованом на ПЦР-у и секвенцирању. Статистичким методама је испитана њихова асоцијација са ВИПН-ом код 56 педијатријских болесника оболелих од АЛЛ. Урађена је и популациона винкристин фармакогеномска анализа 17 фармакогена из постојећих података добијених секвенцирањем нове генерације у српској популацији. Подаци о дистрибуцији фреквенција алела за европско становништво преузети су из јавних база података.

Резултати Током лечења, 17,86% болесника је развило ВИПН. Асоцијативне анализе показале су да ниједна генетичка варијанта није била повезана са ВИПН-ом у нашој студији. Наше популационо фармакогеномско истраживање није открило валидне фармаковаријанте за ВИПН. Наши резултати не препоручују превентивно фармакогенетичко испитивање винкристина у Србији.

Закључак Потребан је свеобухватнији приступ како би се идентификовао панел гена којим би се могао објаснити развој ВИПН-а после примене винкристина код педијатријских болесника оболелих од АЛЛ. Боље осмишљене студије асоцијација на нивоу генома (*GWAS*) и робуснији алати који користе вештачку интелигенцију довели би до дизајнирања панела фармакогена за превентивно тестирање предиспозиције за развој ВИПН-а, доприносећи индивидуализацији и унапређењу терапије деце оболеле од АЛЛ.

Кључне речи: акутна лимфобластна леукемија; фармакогеномика; винкристин; винкристином индукована периферна неуропатија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association between flat foot prevalence and nutritional status in schoolchildren

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SUMMARY

Introduction/Objective The aim of this study was to examine the association between flat feet and socio-demographic factors and nutritional status in children aged 7–14 years of the Province of Vojvodina, Serbia.

Methods The research was conducted as a cross-sectional study. The survey instrument was a questionnaire, and anthropometric measurements were done using standardized procedures. To determine the impact of socio-demographic factors and nutritional status as independent variables on the flat feet in schoolchildren as a dependent variable, a multivariate logistic regression model was implemented. A multivariate model was adjusted for age, sex, type of settlement, and material status.

Results This study included 1376 children (685 boys and 691 girls). Significant differences were observed in the frequency of flat feet between normal weight, overweight, and obesity (p = 0.006), where obese children were rated highest in the flat foot category. Overweight children had a 1.76 times higher chance to have flat feet than those with normal weight (OR = 1.76; 95% CI 1.08–2.88), while obese children were 1.88 times more likely to have flat feet than those with normal weight (OR = 1.88; 95% CI 1.14–3.11).

Conclusion The research showed that nutritional status was significantly associated with the presence of flat feet in schoolchildren. The high prevalence of flat feet and obesity in schoolchildren should be accepted as a warning sign, and many public health policies should be undertaken to solve these issues.

Keywords: children; flat feet; BMI; Vojvodina

INTRODUCTION

The most important factors shaping a child's foot are the beginnings of locomotion and increasing loading of the lower extremities. The foot has two longitudinal (medial and lateral) arches and transverse arch. Among the arches of the foot, the medial arch plays a significant role in shock absorption upon contact with the ground. It achieves this by transmitting the vertical load on the foot through deflection of the arch, thereby lessening the impact on the foot as it hits the ground. For patients with flat feet, however, this arch stretches out to an abnormal limit, flattening out completely on the ground during gait and resulting in a postural deformity of the foot. Lowering of the foot arch in children and development of static flat foot is the result of muscle weakness and deficiency in the locomotors apparatus [1]. Obesity is one of the leading causes of flat feet and excessive weight is a factor distorting the foot shape in children. Extreme body weight significantly contributes to abnormal motor development, agility, and overall coordination of movements, and may consequently result in postural defects [2].

There are numerous studies corroborating harmful effect of increased body weight on foot loads and accompanying deformities [3, 4, 5]. As per Dowling et al. [6], while standing, obese children created higher forces essentially over a larger foot area and experienced fundamentally higher plantar pressing factors contrasted with their nonobese counterparts. Likewise, while walking, obese children produced higher forces altogether over all spaces of their feet, except the toes. The essentially lower plantar arch height found in the overweight and obese youngsters recommends that their flatter feet might be brought about by a bringing down of the medial longitudinal arch, most presumably brought about by their feet consistently bearing excess mass. Overweight and obesity can be associated with a generalized lack of foot functionality as a weight-bearing structure as a result of longitudinal medial arch collapse [5].

This study's objective was to identify and establish the prevalence of flat feet and its relationship with socio-demographic factors and nutritional status in schoolchildren aged 7–14 years from the Province of Vojvodina, Serbia.

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METHODS

The study was carried out on the schoolchildren population in the Province of Vojvodina, the northern part of Serbia, within the national study entitled "National Health Survey in Serbia" in 2013, conducted as a cross-sectional study on the representative population sample of the Republic of Serbia. The Ministry of Health of the Republic of Serbia allowed the use of the National Study Database, therefore enabling the realization of this study. A specially created face-to-face questionnaire was used as a research instrument, and anthropometric measurements were done using standardized procedures.

Ethical standards applied in this study comply with the international standards [Helsinki Declaration – World Medical Association Declaration of Helsinki and the Directive of the European Parliament on the protection of individuals with regard to the processing of personal data and on the free movement of such data (Directive 95/46/EC)] and specific legislation in Serbia. All interviewed members of households signed an informed consent, and parents/guardians signed for their children. The survey was conducted by trained interviewers whose work was supervised by licensed supervisors. The process of the data collection was standardized and performed by the methodological guidelines. Anthropometric measurements, including height and weight, were performed while the subjects were without shoes and in light clothing.

This study analyzed the data on 1376 children aged 7–14 years, 685 boys (49.8%) and 691 girls (50.2%). Variables included socio-demographic characteristics [age, sex, type of settlement, material status (Wealth Index)], nutrition status, and foot deformities. According to the Wealth Index (Demographic and Health Survey Wealth Index), the respondents were classified into three socioeconomic groups or quintiles: rich (richer and the richest class), middle, and poor class (poorest and poorer class) [7]. The children were divided into two age groups (7–10 years and 11–14 years). Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca, Chiba, Japan). Body height was measured to the nearest 0.1 cm using a stadiometer (Seca GmbH & Co. KG, Hamburg, Germany) as the child stood erect against a vertical wall. Body mass index (BMI) was calculated as the ratio of the body weight to the square of body height (kg/m^2) and was classified into four categories to assess nutritional status as underweight (< 5th percentile), normal weight (≥ 5th – < 85th percentile), overweight (≥ 85th – < 95th percentile), and obese (≥ 95th percentile) [8].

Statistical analysis was done using descriptive and inferential statistics. The results are given as mean ± SD and proportion. Univariate and multivariate logistic regression analysis was used to assess the association of flat feet with nutritional status and socio-demographic factors. Selected socio-demographic factors and nutritional status among schoolchildren aged 7–14 years according to the prevalence of flat foot were first examined using χ^2 tests and the Student's t-test. Then, to determine the impact of socio-demographic factors and nutritional status as independent variables on flat foot as a dependent variable, a multivariate logistic regression model was implemented. The dependent variable (flat foot) was transformed into dichotomous variables. The model was adjusted for age, sex, type of settlement, and material status. The data was weighted to be more representative of the Vojvodina population in 2013. We calculated the association through the odds ratio (OR) with 95% confidence intervals (95% CI). The probability of $p < 0.05$ was taken as the minimum level of significance. All the statistical analyses were performed using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA).

Table 1. Socio-demographic characteristics, nutritional status, and flat foot prevalence of children aged 7–14 years

Variable	Sex				Total	
	Male		Female			
	n	%	n	%	n	%
Age (years)						
7–10	352	51.4	358	51.8	710	51.6
11–14	333	48.6	333	48.2	666	48.4
Nutritional status						
Underweight	82	12.2	80	11.8	162	12
Normal weight	400	59.2	433	63.9	833	61.5
Overweight	96	14.2	98	14.5	194	14.3
Obese	98	14.5	67	9.9	165	12.2
Type of residence						
Urban	386	56.4	396	57.3	782	56.8
Rural	299	43.6	295	42.7	594	43.2
Material status						
Poor	256	37.4	230	33.3	486	35.3
Middle	152	22.2	138	20	290	21.1
Rich	277	40.4	323	46.7	600	43.6
Flat foot						
Yes	75	11	59	8.6	134	9.8
No	609	89	631	91.4	1240	90.2
Age (years), M ± SD	10.5 ± 2.3		10.3 ± 2.3		10.4 ± 2.3	
Height (cm), M ± SD	147.6 ± 15.6		146.9 ± 15.6		147.2 ± 15.6	
Weight (kg), M ± SD	42.4 ± 15		42.0 ± 14.1		42.2 ± 14.5	
BMI (percentiles), M ± SD	58.6 ± 30.9		58.2 ± 29.5		58.4 ± 30.2	

M ± SD – mean ± standard deviation

RESULTS

A total of 1376 children, 7–14 years old, were voluntarily recruited into this study. Out of the total sample, 685 (49.8%) were boys, while 691 (50.2%) were girls. The participants were divided into two age groups: 7–10 years old (n = 710; 51.6%) and 11–14 years old (n = 666; 48.4%). The average age of the children was 10.4 ± 2.3 years. The survey covered a larger number of children from the urban (56.8%) than from the rural areas (43.2%). The average weight recorded in this study amounted to 42.4 ± 15 kg in the boys and 42 ± 14.1 kg in the girls. The average BMI of the boys was 58.6 ± 30.9 percentiles and that of the girls was 58.2 ± 29.5 percentiles.

Most of the children in this study were with normal nutritional status (61.5%). Underweight was observed in 12% of the children, while overweight and obesity appeared in

14.3% and 12.2% of the cases, respectively. The sample sizes for children by sex, age group, nutritional status, type of settlement, material status (Wealth Index), and flat foot prevalence are shown in Table 1.

The prevalence of obesity was higher in children aged 7–10 years (14.4%) and in boys (14.5%) than in children aged 11–14 years (9.5%) and girls (9.9%), while overweight was higher in children aged 11–14 years (15.1%) and girls (14.5%) as compared to children age 7–10 years (13.4%) and boys (14.2%). There are significant differences in the nutritional status regarding age groups ($\chi^2 = 13.943$; $p = 0.003$), but sex showed no significant relationship to the nutritional status ($\chi^2 = 6.726$; $p = 0.081$).

Flat foot prevalence in the study population was 9.8% (n = 134), and the children with flat feet had significantly higher mean values of BMI compared to children without flat feet (65.9 *vs.* 57.6 percentiles; $t = 3.019$; $p = 0.003$). The prevalence of flat feet among boys (10.8%) was not significantly different than among girls (8.7%). Age was found to have a significant relationship to flat feet ($p = 0.049$), where 7–10 years old children (11.3%) were rated higher than 11–14 years old children (8.2%) in the flat foot category. Significant differences were observed ($p = 0.007$) in the frequency of flat feet between underweight (6.8%), normal weight (8.4%), overweight (13.4%), and obese groups (15.4%). The participants who were more overweight had flatter feet. The material status was found to have a significant relationship to flat feet ($p < 0.001$). Flat foot prevalence was the highest (13.6%) among rich participants and the lowest (5%) among the poor. The type of settlement showed no significant relationship to flat feet (Table 2).

Table 2. Association between socio-demographic characteristics, nutritional status, and prevalence of flat foot of children aged 7–14 years

Variable	Flat foot				p*
	Yes		No		
	n	%	n	%	
Sex					
Male	76	10.8	630	89.2	0.193
Female	58	8.7	610	91.3	
Age (years)					
7–10	78	11.3	611	88.7	0.049
11–14	56	8.2	629	91.8	
Nutritional status					
Underweight	11	6.8	150	93.2	
Normal weight	70	8.4	767	91.6	0.007
Overweight	26	13.4	168	86.6	
Obese	25	15.4	137	84.6	
Type of residence					
Urban	88	10.8	727	89.2	0.113
Rural	46	8.2	514	91.8	
Material status					
Poor	24	5	454	95.0	
Middle	28	9.6	264	90.4	< 0.001
Rich	82	13.6	522	86.4	

Data is presented in frequency and percentages;

*χ^2test

In the multivariate logistic regression model, nutritional status was singled out as a predictor of flat foot. Overweight children had a 1.76 times higher chance to have flat feet compared to those with normal weight [OR = 1.76; 95% CI (1.08–2.88); $p = 0.023$], while obese children were 1.88 times more likely to have flat feet compared to those with normal weight [OR = 1.88; 95% (CI 1.14–3.11); $p = 0.014$] (Table 3).

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for the presence of flat feet depending on socio-demographic factors and nutritional status

Nutritional status	Multivariate model*	
	OR (95% CI)	p*
Normal weight	1	
Overweight	1.76 (1.08–2.88)	0.023**
Obese	1.88 (1.14–3.11)	0.014**

*Model adjusted for sex, age, material status, type of residence; dependent variable: flat foot (ref. children with flat foot);
**$p < 0.05$

DISCUSSION

This study presented a high prevalence of overweight (14.3%) and obesity (12.2%) in children 7–14 years old, with significant differences in the nutritional status regarding age groups (greater number of obese is present in younger age group), but without significant differences regarding sex. The high prevalence of obesity in schoolchildren in Vojvodina is still lower than the reported rate from Vietnam for 2014 (19.1%), but is very similar to the reported data from a Montenegro study from 2013 (10.3%), Mexican study from 2012 (14.6%), Iranian study from 2011 (14.9%), and a Chinese study from 2014–2017 (11.7%) [9–13].

The results showed significant differences in the prevalence of flat feet, depending on some socio-demographic variables (age and material status) and nutritional status. Alsancak et al. [14] and Yin et al. [15] showed that three variables had a significant relationship with the prevalence of flat foot: age, sex, and weight. Our study demonstrates that the prevalence of flat feet in schoolchildren is not influenced by sex, although the percentage distribution of flat feet was higher in boys (10.8%) than in girls (8.7%). A few published studies also affirmed that the percentage distribution of flat feet in boys is marginally greater than that in girls, yet without any significant statistical difference [16, 17, 18].

Opposite to our results, a study on 6992 children in Poland aged 8–12 years reported a significant positive correlation between sex and incidence of flat feet, where flat feet were more frequent in boys (6.2%) than in girls (3.3%) [4]. Also, in a Taiwanese study implemented on 5–13-year-old children, boys (35%) had significantly higher frequency of flat feet than girls (20%) [19]. On the other hand, according to Sadeghi-Demneh et al. [20], the prevalence of flat feet (children aged 7–14 years) in girls (11.3%) is slightly greater than that in boys (10.3%) but without any statistically significant difference.

Similar to the results of other studies, we also detected a decreasing trend in the prevalence of childhood flat feet with increasing age [14, 16, 17]. Statistically significant differences in the prevalence of flat feet (greater number is present in earlier grades) were found between younger (11.3%) and older (8.2%) schoolchildren. Studies carried out in Spain reported different results – that no significant relationship was observed between the prevalence of flat foot and the age in a population of 6–12-year-old students [21].

In our study, increasing weight status was also significantly associated with a higher prevalence of flat foot. The prevalence of flat foot was the highest in the obese (15.4%) group and the lowest in the underweight group (6.8%). Close to our findings, Sadeghi-Demneh et al. [20] reported the significantly highest prevalence of flat foot in the obese group (36.1%) and the lowest in the normal weight group (7.9%) of children aged 7–14 years from Iran. Essentially, Taiwanese examination of 1024 children 5–13 years old tracked down a huge expansion in the commonness of flat foot in overweight and obese children [19]. Several published studies in the same context showed similar findings [15, 22, 23]. As per Dowling et al. [6], obese children showed fundamentally lower footprint angles contrasted with their non-obese counterparts. They proposed that these underlying foot changes were related to contrasts in plantar pressures between obese and normal weight children.

Our research results indicate that overweight children had a 1.76 times higher chance to have flat feet than those with normal weight, while obese children were 1.88 times more likely to have flat feet than those with normal weight. These numbers are lower than those from the findings reported in an Ethiopian study in which children who were overweight or obese were found to be 3.77 and 4.16 times more likely to have flat feet than those underweight [23]. As per Suciati et al. [24], overweight/obese children were found to be 4.5 times more likely to have flat feet than those of normal weight.

CONCLUSION

The present study indicates that the overall prevalence of obesity in the whole sample was 26.5%, of which 14.3% were overweight, and 12.2% were obese schoolchildren. Flat foot prevalence in the study population was 9.8%, and the children with flat feet have statistically significantly higher BMI values than children without flat feet. Children aged 7–10 years, obese, and children who belonged to the rich class have a significantly higher frequency of flat feet. The research showed that nutritional status was significantly associated with the presence of flat feet. The high prevalence of flat foot and obesity in schoolchildren should be accepted as a warning sign, and strategies that promote healthy weight and physical activity among children should be adequately developed and applied.

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Повезаност преваленције равних стопала и нутритивног статуса код школске деце

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САЖЕТАК

Увод/Циљ Циљ рада је био да се испита повезаност преваленције равних стопала са социодемографским факторима и нутритивним статусом деце узраста 7–14 година у Војводини, Србија.

Методе Истраживање је спроведено као студија пресека. Као инструмент истраживања коришћен је анкетни упитник, а антропометријска мерења су вршена стандардизованим поступцима. Да би се утврдио утицај социодемографских фактора и нутритивног статуса као независних променљивих на присуство равних стопала код школске деце, примењен је мултиваријантни модел логистичке регресије. Мултиваријантни модел прилагођен је старости, полу, типу насеља и материјалном статусу.

Резултати Овом студијом обухваћено је 1376 деце (685 дечака и 691 девојчица). Истраживањем је утврђена значајна разлика у учесталости равних стопала између деце са нормалном телесном масом, прекомерном телесном масом и гојазне деце ($p = 0,006$), при чему су гојазна деца имала највећу учесталост равних стопала. Деца са прекомерном телесном масом су имала 1,76 пута већу шансу да имају равна стопала од деце са нормалном телесном масом (*OR* = 1,76; 95% *CI* 1,08–2,88), док су гојазна деца имала 1,88 пута већу вероватноћу да имају равна стопала од деце са нормалном телесном масом (*OR* = 1,88; 95% *CI* 1,14–3,11).

Закључак Истраживање је показало да је код деце школског узраста нутритивни статус значајно повезан са присуством равних стопала. Високу преваленцију равних стопала и гојазности код школске деце треба схватити као знак упозорења, за чије је решавање потребно унапређење постојећих и креирање нових јавноздравствених програма.

Кључне речи: деца, равна стопала; *BMI*; Војводина

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Measurement properties of New Mobility Score to evaluate functional recovery in the elderly following total hip arthroplasty

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SUMMARY

Introduction/Objective The aim of this study is to identify and evaluate the use of New Mobility Score (NMS) in estimating functional recovery three months after total hip arthroplasty (THA).

Methods In total, 70 patients, aged > 60 years, underwent THA. Treatment group was subjected to the comprehensive rehabilitation program and control group to the standard one. Primary outcome was assessed with Harris Hip Score (HHS) and NMS, and secondary one by Medical Outcomes Health Survey (Short-Form Health Survey – SF-36). Questionnaires were collected before and three months after hip surgery.

Results Treatment group showed significant improvement three months postoperatively. The correlation in both groups between HHS and NMS was very strong (r > 0.700). Treatment group following surgery showed strong correlation between Recovery through Personal Care Services (PCS) and HHS and NMS (r > 0.700), moderate to strong between pain categories and HHS (r = 0.380; r = 0.583) and NMS (r = 0.424). Control group showed strong correlation between PCS and HHS (r = 0.704), and NMS (r = 0.568) and moderate to pain categories and HHS (r = 0.546; r = 0.466). The area under the curve (AUC) described the inherent validity of all measurement used $AUC_{NMS} = 0.724$, p = 0.001, $AUC_{HHS} = 0.788$, p = 0.000 and $AUC_{PCS} = 0.747$, p = 0.001.

Conclusion The NMS could be successfully used in routine clinical assessment of elderly patients following THA.

The trial is registered in ISRCTN Register with https://doi.org/10.1186/ISRCTN73197506.

Keywords: Harris Hip Score; New Mobility Score; SF-36; outcome assessment; hip arthroplasty; rehabilitation; ROC curve

INTRODUCTION

Total hip arthroplasty (THA) is the most commonly performed surgical procedure undertaken to relieve pain and restore function in elderly people with end-stage hip osteoarthritis [1]. With the projected increase in the number of the elderly undergoing THA over the next two decades, it becomes even more critical to develop effective rehabilitation strategies, individually adapted, which can contribute most benefit. Surgery alone fails to fully restore physical function and address longstanding impairments associated with chronic joint disease [2, 3].

Despite the increased interest in evaluating outcomes following hip arthroplasty, challenges remain in ensuring that such assessments of outcome are accurate, reliable, and relevant [4]. Generic patient-reported outcome measures (PROMs) describe a patient's global health status, and numerous comprehensive specific PROMs instruments are available for patients with hip problems [5]. Health-related quality-of-life data are valuable as they can provide relevant health-status information to health professionals and should be used as rationale for implementing the most adequate standard of health care [6]. There are many different tools available to measure an outcome, each with its advantages and drawbacks. Pain assessment is a crucial component of joint specific and generic self-assessment instruments because it influences physical functioning (PF) [7]. After surgery, outcome measures are generally conveyed as the quality-of-life score, and joint-specific tools focus on disability relating to a particular joint irrespective of the underlying pathology [7, 8]. Therefore, there is a need for guidance in defining criteria for the most useful outcome measures, using the International Classification of Functioning, Disability, and Health (ICF) model to conceptualize joint replacement outcomes [9, 10].

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The meta-analysis from 2019. distinguishes results on patient-reported function, hip-related pain, and health-related quality of life after total hip replacement and suggests focusing on early rehabilitation [11, 12].

The aim of this study is to identify and evaluate the use of New Mobility Score (NMS) together with already confirmed Harris Hip Score (HHS) and the medical outcomes study Short-Form Health Survey (SF-36) in estimating functional recovery three months after primary hip arthroplasty.

METHODS

Study design, participants, and ethics

This study was designed as a prospective randomized controlled study. The data were collected preoperatively and three months after THA to evaluate the effectiveness of a comprehensive rehabilitation program compared to the standard one after hip arthroplasty. Recruited patients were enrolled between 2013 and 2015, prior to surgery at the orthopedic surgery department. The inclusion criteria were older than 60, end-stage primary hip osteoarthritis, and primary unilateral total hip replacement. The exclusion criteria were postoperative complications, cognitive impairment (assessed clinically), history of congenital hip dislocation, bilateral hip disease or inflammatory arthritis, significant neuromuscular disease (e.g., Parkinson's disease), lower extremity fractures, or paralysis.

A randomization sequence was created using a computer-generated list of numbers in block sizes of four. Those who qualified for the trial underwent a hip replacement, by posterior-lateral approach, performed by the same surgery team. Both groups received a standard exercise program guided by a physiotherapist, starting on the first post-surgery day. Participants in the treatment group were given a comprehensive program with additional physical exercises for the arm and upper body. Both program sessions were performed twice a day, five days a week, during a two-week stay at the hospital. The patients were supervised in an inpatient rehabilitation center (for four weeks) and finally at home, unsupervised (for six weeks).

All participants gave their voluntary written consent according to approval by the Regional Committee for Medical Research Ethics (n.29/V-17). The trial is registered in ISRCTN Register with ISRCTN73197506.

Patient characteristics

Before surgery, a questionnaire including anthropometric characteristics (age, sex, body height and weight, comorbidities) was completed for all patients.

Patient assessment

The primary outcome was changed in the lower limbs' hip function and physical performances, assessed by HHS and NMS, from baseline and after three months. HHS is a multidimensional assessment of the results of hip surgery [2, 13]. The domains covered by the HHS are pain and daily living activities, and hip function assessment (limping), absence of deformity, and range of motion. The final score ranges from 100 points (no disability) to 0 (maximum disability).

NMS is a composite score of the patient's ability to perform: indoor walking, outdoor walking, and shopping, providing a score between 0 and 3 (0 – not at all, 1 – with help from another person, 2 – with an aid, 3 – no difficulty) for each function, resulting in a total score from 0 (no walking ability at all) to 9 (fully independent) [14, 15, 16].

Secondary outcomes were estimating and measuring functional physical recovery after primary hip arthroplasty and quality of life by SF-36. The SF-36 is a common general health scale evaluating physical and mental health (MH), which includes one multi-item scale that assesses eight health concepts: 1 – limitations in physical activities because of health problems; 2 – limitations in social activities because of physical or emotional problems; 3 – limitations in usual role activities because of physical health problems; 4 – bodily pain (BP); 5 – general MH (psychological distress and well-being); 6 – limitations in usual role activities because of emotional problems; 7 – vitality (energy and fatigue), and 8 – general health state. It has been tested for its psychometric properties. Each subscale score is converted from 0 to 100; the higher the score, the better the quality of life [4, 17].

Statistical analysis

Baseline data on patients' characteristics were examined for differences between the two groups by chi-square test for categorical variables or Student t-test and Mann–Whitney U test for continuous variables.

In this paper, we used norm-based scoring for all domains of SF-36. Norm-based scoring generates Physical Component Summary (PCS) and Mental Component Summary (MCS) scores, using Scoring Software 4.5™ [18]. The response to 36 questions is transferred to 0–100 worst/best scale, according to which 50 points score corresponds to a generally healthy population.

Correlations between specific and generic tests in two measurement periods were assessed with the Pearson linear correlation coefficient. The degree of correlation was defined as low if the coefficient was less than 0.3, moderate if it was between 0.3 and 0.5, and reliable if it was more significant than 0.5 [19].

Receiver operating characteristic (ROC) curve plots were generated for all used outcome measurements. The point closest to the upper left corner of the curve represents the optimal trade-off between sensitivity and specificity for detecting clinical improvement. The area under the curve can be interpreted as the probability of the test to identify an improvement in patients correctly. An AUC of 1 demonstrates an ideal test with a 100% sensitivity and specificity, while an AUC of less than 0.5 indicates that the test is less useful. Cut-off points are defined by positive-to-negative (P/N) ratios [20].

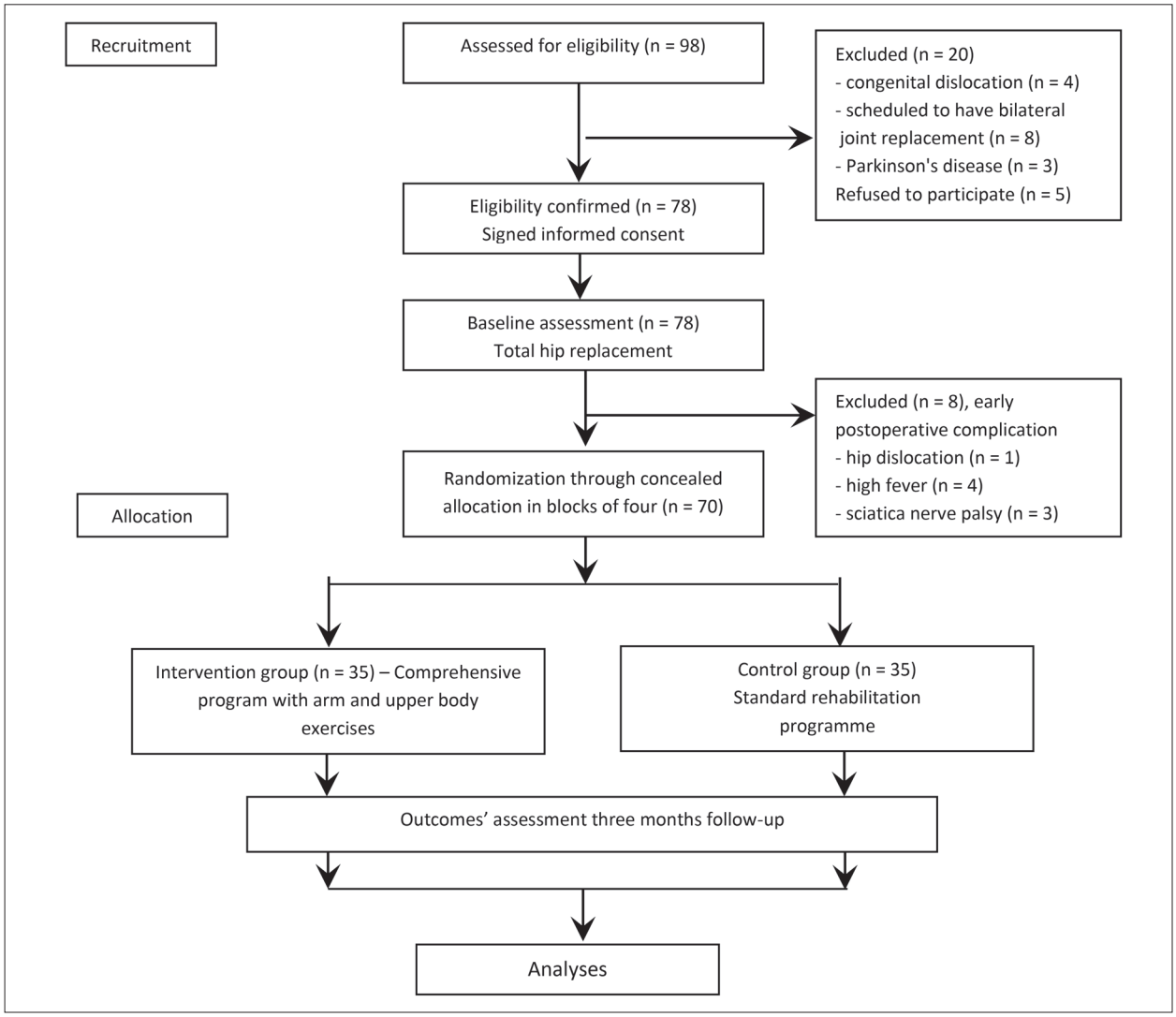

Figure 1. Flow diagram of the randomized clinical trial

IBM Statistical Package for Social Science for Windows (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses.

RESULTS

In total, 98 patients were eligible for participation in the study from January 1, 2013, to June 30, 2015. After exclusion criteria had been implemented, 70 participants underwent randomization and did not change groups until the end of the research.

The treatment group consisted predominantly of females, 63% (n = 22) as well as in the control group, 77% (n = 27). There were no significant pre-treatment differences between the groups, suggesting that the randomization procedure produced well-balanced and comparable groups at baseline. The average age of participants was 69 (SD = 6.3 years) in both groups.

A flow diagram of the trial progression (recruitment, randomization, intervention allocation, follow-up, and data analysis) is shown in Figure 1. The average baseline characteristics of both groups are listed in Table 1.

Table 1. Socio-demographic and clinical characteristics of the participants

Characteristics	Study group n = 35	Control group n = 35	p
Age in years, mean (SD)	69.2 (6.29)	68.1 (6.35)	0.725
Sex			0.192
Male, n (%)	13 (37.1)	8 (22.9)	
Female, n (%)	22 (62.9)	27 (77.1)	
BMI in categories, n (%)			0.408
Normal, n (%)	7 (20)	11 (31.4)	
Overweight, n (%)	17(48.6)	12 (34.3)	
Obese, n (%)	11 (31.4)	12 (34.3)	
Comorbidities, mean (SD)	2.77 (1.8)	3.34 (2.38)	0.138
ICED score			0.405
Mild, n (%)	1 (2.9)	0 (0)	
Moderate, n (%)	2 (5.7)	2 (5.7)	
Severe, n (%)	30 (85.7)	33 (94.3)	

ICED – Index of Coexistent Disease; BMI – body mass index;
According to χ^2, t-test, or Mann–Whitney U-test where appropriate

The only significant difference between treatment and control study groups before the intervention was detected in the Vitality (VT) domain of the SF-36 questionnaire.

Table 2. Mean scores of NMS, SF36, and HHS questionnaires before and 3 months after the intervention in treatment and standard groups

Time	Questionnaire, mean (SD)	Groups		p*
		Treatment n = 35	Control n = 35	
	HHS	34.6 (10.56)	35.5 (9.3)	0.693
	HHS-pain	11.71(3.82)	12.86(4.58)	0.261
Before intervention	Physical Functioning	25.7 (4.93)	24.0 (3.73)	0.121
	Role-Physical	30.4 (6.95)	28.4 (10.3)	0.362
	Bodily Pain	29.9 (5.52)	27.7 (5.55)	0.106
	General Health	53.9 (9.06)	52.5 (8.84)	0.511
	Vitality	45.7 (10.68)	39.7 (10.29)	**0.019**
	Social Functioning	29.8 (10.63)	27.4 (9.22)	0.309
	Role-Emotional	35.1 (11.1)	36.3 (12.17)	0.670
	Mental Health	38.2 (13.45)	34.1 (12.09)	0.183
	Physical Component summary	32.9 (5.44)	30.4 (4.93)	0.054
	Mental Component summary	41.3 (12.31)	38.8 (12.62)	0.402
	NMS	3.9 (1.82)	3.9 (1.14)	1.000
Time	Questionnaire, mean (SD)	Groups		p*
		Treatment n = 35	Control n = 35	
	HHS	88.3 (4.62)	82.4 (5.51)	**< 0.001**
	HHS-pain	42.6(1.92)	41.1 (1.83)	**0.002**
3 months after intervention	Physical Functioning	49.3 (6.9)	44.2 (7.8)	**0.005**
	Role-Physical	51.9 (5.17)	47.0 (5.42)	**< 0.001**
	Bodily Pain	60.3 (4.54)	55.4 (5.43)	**< 0.001**
	General Health	58.9 (7.04)	56.0 (7.09)	0.096
	Vitality	61.8 (7.04)	56.6 (7.55)	**0.004**
	Social Functioning	55.8 (4.83)	52.2 (5.37)	**0.005**
	Role-Emotional	54.1 (5.88)	51.4 (6.92)	0.086
	Mental Health	56.3 (7.96)	52.4 (7.13)	**0.032**
	Physical Component summary	54.0 (5.70)	49.1 (5.62)	**0.001**
	Mental Component summary	57.9 (7.08)	54.9 (6.37)	0.067
	NMS	8.2 (1.09)	7.1 (1)	**< 0.001**

HHS – Harris Hip Score; NMS – New Mobility Score;
*According to the t-test

A higher score of VT was detected among treatment patients (45.7 *vs.* 39.7).

After the intervention, there were significant differences in favor of treatment group as regards HHS (88.3 *vs.* 82.4), pain category of HHS (42.63 *vs.* 41.14), NMS (8.2 *vs.* 7.1), and SF-36 domains PF (49.3 *vs.* 44.2), Role-Physical (RP) (51.9 *vs.* 47.0), BP (60.3 *vs.* 55.4), VT (61.8 *vs.* 56.6), Social Functioning (SF) (55.8 *vs.* 52.2), MH (56.3 *vs.* 52.4), as well as PCS (54 *vs.* 49.1), but for MCS (p = 0.067) there were no statistically significant differences between groups (Table 2).

Correlations prior to THA were given in Table 3.

In the treatment group, a strong statistically significant correlation was found between HHS and NMS. Moderate to strong positive statistically significant correlation was found between SF-36 domains: PF, RP, BP, SF, RE, PCS, HHS, and NMS. A moderate statistically significant correlation was found between the pain category of HHS and total HHS.

In the control group, a strong statistically significant correlation was found between HHS and NMS. Moderate to strong positive statistically significant correlation was found between SF-36 domains: PF, BP, VT, PCS, pain category of HHS, and total HHS and NMS. A moderate level of statistically significant correlation was found between SF-36 domains: BP, SF, and NMS, as well as VT and HHS.

Correlations three months after THA were given in Table 3.

In the treatment group, a strong significant correlation was found between HHS and NMS. Moderate to strong positive statistically significant correlation was found between SF-36 domains: PF, RP, GH, VT, PCS, pain category of HHS, and total HHS and NMS. A moderate significant correlation was present between BP and HHS.

In the control group, a strong significant correlation was found between HHS and NMS. Moderate to strong positive statistically significant correlation was found between SF-36 domains: PF, RP, GH, VT, and PCS and both HHS, as well as SF-36 domains: BP, SF, RE, MH, MCS, pain category HHS and total HHS. (Figure 2, Table 4)

AUC for NMS was 0.724 (CI 95% 0.598–0.849) p = 0.001, cut-off 7.5, with sensitivity of 80% and specificity of 71%.

AUC for HHS was 0.788 (95% CI 0.683–0.894) p = 0.000, cut-off 85.5, with sensitivity of 71% and specificity of 74%.

AUC for PCS SF-36v2 was 0.747 (95% CI 0.628–0.867) p = 0.001, cut-off 51.8, with sensitivity of 77% and specificity of 77%.

DISCUSSION

Previous studies have found that NMS was only used for functional assessment of patients with hip fractures [14, 15, 16]. In our study, NMS was used for the first time to evaluate physical functional recovery after primary hip arthroplasty in the elderly and recorded the same significant improvement as estimated by HHS and SF-36. Many papers assessed the PF of patients undergoing hip replacement surgery using different PROMs. They provided a shortlist of the most promising generic and joint-specific instruments [4, 5, 11, 12]. In Gagnier et al. [4], seventy-three studies were investigated, and 26 instruments were included, one of the most frequently assessed instruments being HHS. This study opted for physician-administered HHS, a widely used important instrument for evaluating outcomes and predicting early revision surgery after THA [6, 13, 21]. HHS and SF-36 are highly valid and reliable outcome measurement instruments, which we also used in this study. Mariconda et al. [22] presented that HHS was the essential determinant of SF-36 PCS and PF scale scores, showing that hip functionality is critical in determining the patients' general functioning. The most important findings of the systematic review and meta-analysis are that mid-term health-related quality of life following THA is superior to preoperative levels in a broad range of SF-36 domains and results in patient satisfaction and specific functional gains [23]. Our study has proven statistically significant functional

Table 3. Correlation coefficients between HHS, NMS, and SF-36 domains before and three months after the intervention in treatment and control groups

Before intervention				3 months after intervention	
Group		HHS	NMS	HHS	NMS
Treatment group		r = 0.737; p < 0.001		r = 0.718; p < 0.001	
SF-36	Physical Functioning	r = 0.715 **p < 0.001**	r = 0.626 **p < 0.001**	r = 0.733 **p < 0.001**	r = 0.757 **p < 0.001**
	Role-Physical	r = 0.659 **p < 0.001**	r = 0.467 **p = 0.005**	r = 0.543 **p = 0.001**	r = 0.515 **p = 0.002**
	Bodily Pain	r = 0.708 **p < 0.001**	r = 0.454 **p = 0.006**	r = 0.380 **p = 0.024**	r = 0.305 p = 0.079
	General Health	r = 0.201 p = 0.246	r = 0.196 p = 0.259	r = 0.616 **p < 0.001**	r = 0.659 **p < 0.001**
	Vitality	r = 0.321 p = 0.060	r = 0.186 p = 0.284	r = 0.635 **p < 0.001**	r = 0.513 **p = 0.002**
	Social Functioning	r = 0.674 **p < 0.001**	r = 0.470 **p = 0.004**	r = 0.323 p = 0.058	r = 0.263 p = 0.133
	Role-Emotional	r = 0.378 **p = 0.025**	r = 0.146 p = 0.404	r = 0.258 p = 0.135	r = 0.209 p = 0.236
	Mental Health	r = 0.322 p = 0.059	r = 0.125 p = 0.473	r = 0.292 p = 0.089	r = 0.212 p = 0.229
	Physical Component summary	r = 0.567 **p < 0.001**	r = 0.556 **p = 0.001**	r = 0.714 **p < 0.001**	r = 0.757 **p < 0.001**
	HHS-Pain	r = 0.527 **p = 0.001**	r = 0.218 p = 0.208	r = 0.583 **p < 0.001**	r = 0.424 **p = 0.011**
	Mental Composite score	r = 0.396 **p = 0.019**	r = 0.159 p = 0.363	r = 0.214 p = 0.217	r = 0.130 p = 0.463
Control group		r = 0.695; **p < 0.001**		r = 0.733; **p < 0.001**	
SF-36	Physical Functioning	r = 0.676 **p < 0.001**	r = 0.522 **p = 0.001**	r = 0.603 **p < 0.001**	r = 0.519 **p = 0.001**
	Role-Physical	r = 0.319 p = 0.062	r = 0.337 **p = 0.048**	r = 0.608 **p < 0.001**	r = 0.504 **p = 0.002**
	Bodily Pain	r = 0.797 **p < 0.001**	r = 0.603 **p < 0.001**	r = 0.546 **p = 0.001**	r = 0.264 p = 0.125
	General Health	r = 0.243 p = 0.160	r = 0.224 p = 0.195	r = 0.627 **p < 0.001**	r = 0.436 **p = 0.009**
	Vitality	r = 0.398 **p = 0.018**	r = 329 p = 0.053	r = 0.625 **p < 0.001**	r = 0.501 **p = 0.002**
	Social Functioning	r = 0.458 **p = 0.006**	r = 0.380 **p = 0.024**	r = 0.530 **p = 0.001**	r = 0.223 p = 0.198
	Role-Emotional	r = 0.280 p = 0.103	r = 0.195 p = 0.262	r = 0.393 **p = 0.020**	r = 0.263 p = 0.126
	Mental Health	r = 0.280 p = 0.103	r = 0.117 p = 0.503	r = 0.542 **p = 0.001**	r = 0.302 p = 0.077
	Physical Composite score	r = 0.632 **p < 0.001**	r = 0.575 **p < 0.001**	r = 0.704 **p < 0.001**	r = 0.568 **p < 0.001**
	HHS-Pain	r = 0.715 **p < 0.001**	r = 0.474 **p = 0.004**	r = 0.466 **p = 0.005**	r = 0.228 p = 0.187
	Mental Component summary	r = 0.292 p = 0.089	r = 0.175 p = 0.305	r = 0.483 **p = 0.003**	r = 0.249 p = 0.149

HHS – Harris Hip Score; NMS – New Mobility Score

Table 4. Area Under the Curve (AUC) and P/N ratio cut-off points

Test result variable(s)	AUC	p	95% CI lower limit	95% CI upper limit	Cut-off	Sensitivity	Specificity
PCS postoperative	0.747	**0.000**	0.628	0.867	51.8	77	77
HHS postoperative	0.788	**0.000**	0.683	0.894	85.5	71	74
NMS postoperative	0.724	**0.001**	0.598	0.849	7.5	80	71

PCS – Physical Component Summary of SF-36; HHS – Harris Hip Score; NMS – New Mobility Score

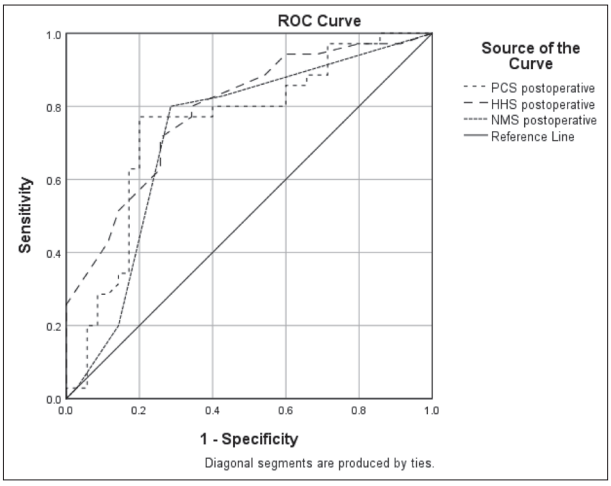

Figure 2. ROC curves three months postoperatively

improvement, predominantly in the treatment group, three months postoperatively, assessed with all used measurements: NMS, HHS and in virtually all domains SF- 36: PF, RP, BP, SF, VT, MH, and physical summary component. We found that correlation in treatment and control groups between HHS and NMS was very strong. The correlation in both groups between preoperative physical performances and pain was strong. Three months after arthroplasty, the correlation between the treatment and control group was strong to very strong between assessed physical performances. Following surgery and both physical exercise programmes (comprehensive and standard), we found a moderate correlation in treatment group and moderate to strong in control one between pain domain SF-36, pain category HHS and functional ability HHS.

Pain and physical function represent different but related health concepts and interventions [7, 23]. Therefore, separate assessments of these attributes were recommended at the Outcome Measures in Arthritis Clinical Trials conference [9, 10]. Results of the Terwee et al. [24] study confirmed that self-report measures of PF are more

influenced by the amount of pain experienced than performance-based measures of PF. We have established a connection between pain and PF following hip replacement measured by specific HHS, NMS, and generic SF-36.

Elibol et al. [25] found moderate to strong correlations between HHS and performance-based tests in evaluating patients with THA. In contrast, our study presented a strong correlation between outcomes assessment HHS and NMS in evaluating patients with THA.

ROC curves synthesized information on the sensitivity and specificity to discriminate significant functional improvement in the treatment group, on the one hand, and functional improvement in the control group. The AUC is an effective and combined measure that describes all measurements' inherent validity used HHS, NMS, and SF-36 [20]. The ROC curves of NMS with HHS and PCS of SF- 36 were located closer to each other in "ROC space," which confirmed validity for NMS. Hoeksma et al. [26] also used ROC curve to determine the ability of HHS and SF-36, walking speed, and pain to measure clinically relevant improvement after exercise therapy. In summary, they showed that HHS could detect a small improvement in hip function and recommended that it be used in rehabilitation interventions that focus on the improvement of functional ability in patients with OA of the hip.

Kristensen et al. [27] suggest that NMS is a valid and easily applicable score that provides a predictive value of the short-term potential of the patient's independence in functional mobility during admission and discharge status. Prieto-Moreno et al. [28] confirmed that NMS is a reliable and valid outcome measure to assess the pre-fracture functional status and cognitive impairment in older patients with hip fracture in Spain. We agreed that the NMS is an easy-to-use and quick-to-complete score that can be used for all patients with hip surgery, based on the information provided by the caregivers for the patients with functional status [28]. We also found that a strong correlation between used outcome measurements confirms that NMS is useful and important instrument for fast and relevant clinical assessment and evaluation of functional recovery after primary hip arthroplasty in the elderly in Serbia.

Consensus on which combination of measures will best assess physical function in people with hip OA still does not exist [9]. We related physical functional performances to "the ability to move around "and "the ability to perform daily activities "in THA patients unified in NMS, HHS, and SF-36. These functional activities were classified using the ICF model WHO [29].

There are potential limitations of this study, the first being using only self-reported generic and specific instruments. The second limitation is that the patients undergoing hip replacement were evaluated for a short follow-up time.

The strong points of this study are that we have created a randomized control study for assessing outcomes three months following primary hip arthroplasty in the elderly and that we have used valid outcome assessment.

Our further research will focus on the correlation between self-reported generic, specific, and performance-based outcome measurements to evaluate the effectiveness of a comprehensive rehabilitation programme over a more extended follow-up period.

CONCLUSION

In conclusion, we strongly support the use of joint self-reported specific and generic measurements in the assessment of impact of pain experience on PF after THA. We believe the findings of a strong correlation with all used outcome measurements confirm that NMS is useful and important for fast and adequate clinical evaluation of functional abilities after primary hip arthroplasty over a short follow-up time. The NMS can be successfully used in routine clinical practice to assess functionality outcomes after hip replacement in elderly patients.

Conflict of interest: None declared.

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Мерна својства Новог упитника за процену мобилности у функционалном опоравку старијих особа после тоталне артропластике кука

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САЖЕТАК

Увод/Циљ Циљ ове студије је да се идентификује и испита употреба Новог упитника скоровања мобилности (НУС) у процени функционалног опоравка три месеца након тоталне артропластике кука.

Методе У истраживање је укључено укупно 70 пацијената, старијих од 60 година, који су били подвргнути тоталној артропластици кука. Испитивана група је подвргнута свеобухватном програму рехабилитације, а контролна група стандардном. Примарни исход функционалног опоравка је оцењен Харисовим упитником за кук (ХУК) и НУС, а секундарни Општим упитником о здрављу (*SF-36*). Упитници су попуњавани преоперативно и три месеца постоперативно.

Резултати Испитивана група је у односу на контролну показала значајније побољшање три месеца после артропластике кука. Корелација у обе групе између ХУК и НУС је била врло јака ($p > 0,700$). У испитиваној групи је три месеца постоперативно показана јака повезаност између укупног физичког опоравка (УФО) *SF-36*, ХУК и НУС ($r > 0,700$), умерена до јака између категорија бола, ХУК ($r = 0,380$; $r = 0,583$) и НУС ($r = 0,424$). У контролној групи је показана јака корелација између УФО *SF-36*, ХУК ($r = 0,704$) и НУС ($r = 0,568$) и умерена између категорија бола и ХУК ($r = 0,546$; $r = 0,466$). Подручје испод криве (*AUC*) показало је валидност свих коришћених мерних инструмената: $AUC_{НУС} = 0,724$, $p = 0,001$, $AUC_{ХУК} = 0,788$, $p = 0,000$ и $AUC_{УФО} = 0,747$, $p = 0,001$.

Закључак НУС може успешно да се користи у рутинској клиничкој процени функционалног опоравка старијих пацијената после тоталне артропластике кука.

Истраживање је регистровано у регистру *ISRCTN* (https://doi.org/10.1186/ISRCTN73197506).

Кључне речи: Харисов упитник за кук; Нови упитник скоровања мобилности; *SF-36*; мерење исхода; артропластика кука; рехабилитација; *ROC* крива

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Association between bipolar affective disorder, use of antidepressants and osteoporosis

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SUMMARY

Introduction/Objective Osteoporosis is one of the most common comorbid disorders in depressive mood disorder. The aim of this study was to assess the association between the use of antidepressants and osteoporosis in patients with bipolar affective disorder (BPAD).

Methods The study included 73 inpatients, aged 50–72 years, male and female, hospitalized with a depressive episode of BPAD from 2016 to 2020 at the Clinic of Psychiatry, Clinical Centre of Vojvodina, divided into two groups: a) the first group (40) was treated with selective serotonin reuptake inhibitors (SSRIs) in combination with mood stabilizer (lithium carbonate/lamotrigine); b) the second group (33) was treated with mood stabilizer only. Study included two control groups as well. Clinical measurements of bone mineral density at lumbar spine and hip was made using dual energy X-ray absorptiometry. CrossLaps and levels of calcium and vitamin D were collected from blood samples. The data was analyzed by the analysis of variance and the Kruskal–Wallis test.

Results Osteoporosis was registered in 25% of patients in the first group and in 18% of patients in the second group, while osteopenia was observed within 40% of patients in the first group and in 37% of patients in the second group. There was significant difference in value of CrossLaps, and the level of 25(OH)D vitamin between the control groups and the first two groups, as well as in prevalence of osteoporosis and osteopenia.

Conclusion Depressive episodes in BPAD is connected with higher prevalence of osteoporosis. Patients treated with SSRIs have higher prevalence of osteoporosis than patients treated with mood stabilizers only.

Keywords: bone mineral density; depression; selective serotonin reuptake

INTRODUCTION

Depression has been reported as the most common mental disorder in the 21st century based on its incidence and prevalence that have been increasing constantly over the last decades, not only in Serbia but in the majority of other countries with valid health statistics [1, 2]. Besides the incidence rate, depression is significant because numerous metabolic disorders can result from an untreated or inadequately treated mental disorder. Osteoporosis is one of the most common comorbid disorders, especially in bipolar and unipolar mood disorders. Nowadays, there is a risk of osteoporosis becoming a "silent epidemic," just like mood disorders, which are both metabolic disorders. According to the official statistics, every third woman and every sixth man over 60 is affected by osteoporosis [3]. The risk of these health problems is growing substantially considering the common comorbidity of mood disorders and osteoporosis [4, 5]. Well-known risk factors are biological predisposition, sex, age, positive family history, low body weight and bad habits (smoking, consuming alcohol, fast food, greasy and poor-quality food, lack of physical activity) as well as the use of certain medications (e.g., corticosteroids) [6, 7]. In addition to those risk factors, it is not clear whether the same pathophysiological processes take place in mood disorders and osteoporosis [1, 2].

Common facts that prove the existence of common pathophysiological processes in both disorders are considered to be hypercortisolemia, increased activity of the hypothalamic-pituitary-adrenal axis, increased cytokine activity (interleukin-6 (IL-6) and tumor necrosis factor (TNF)), and a decrease in anti-inflammatory interleukin activity (IL-10, IL-13) as well as an increase in oxidative stress factors, an increase in parathyroid hormone levels with consecutive decrease of 25(OH)D vitamin, and the decrease in estrogen levels in plasma [6, 8–11].

Although the association between depression and osteoporosis has not been clearly explained, recent studies suggest that depression should be considered as an official risk factor for osteoporosis [12]. Depressive disorder can be represented as unipolar (depressive episodes only), or within bipolar disorder, when depressive symptoms are replaced or can overlap with manic/hypomanic symptoms. Few clinical studies have been focused on monitoring the risk for osteoporosis within bipolar patients [13].

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The aim of this study was to assess the association between developing osteoporosis and taking antidepressants in patients with the diagnosis of bipolar affective disorder.

METHODS

The study included 73 patients, aged 50–72 years, both male and female, hospitalized with diagnosis of bipolar affective disorder [middle, recurrent, depressive episode, more than 20 on the Hamilton Depression Scale (HAMD)], 2016–2020 at the Clinic of Psychiatry, Clinical Centre of Vojvodina. Also, two control groups were included: a) the first control group (30) included depressive patients without psychopharmacotherapy; b) the second control group (30) was formed of healthy volunteers.

Patients with psychopharmacotherapy (73) were divided into two groups: a) the first group (40) was treated with selective serotonin reuptake inhibitors (SSRIs) antidepressants (escitalopram, fluoxetine, sertraline) in combination with mood stabilizers (lithium carbonate and/or lamotrigine). Antidepressants were added to therapy due to non-reactive depression; b) the second group (33) was treated with mood stabilizers only, without antidepressants.

Sociobiographic and sociodemographic data were collected, including information about all medication. Diabetes mellitus, hypothyroidisms, hyperlipidemia, and hypertension were the most common comorbidities. The patients were under medication control. The diagnosis of osteoporosis was based on the ICD 10 code. Clinical measurements and assessment of BMD at lumbal spine (L2–L4) and hip was made, using dual energy X-ray absorptiometry (DEXA). Also, CrossLaps and vitamin D and calcium levels were ascertained from blood samples [14].

All the patients signed the consent according to the Declaration of Helsinki. The study was approved by the competent ethics committee of the Clinical Center of Vojvodina, and conforms to the legal standards. For the purpose of statistical analysis, IBM SPSS Statistics, version 21.0 (IBM Corp., Armonk, NY, USA) and JASP, version 0.14.1.0 (University of Amsterdam, Amsterdam, the Netherlands) were used. Results were statistically analyzed by one-way analysis of variance – ANOVA and Tukey's Honest Significant Difference post-hoc test as parametric method of testing and by Kruskal–Wallis test with post-hoc Mann–Whitney U-tests as a non-parametric method of testing. The presence of osteoporosis or osteopenia was ascertained using the analysis of contingency tables. A p-value less than 0.05 was considered statistically significant.

RESULTS

The average age in the first group was 56.2 ± 7.8 years, and it was 55.9 ± 4.3 years in the second group. In both control groups, the average age was similar (55 ± 3.3 years in the first control group and 56.1 ± 4.1 years in the second control group). Women were represented in a larger percentage in all groups: 66% *vs.* 34% and 67% *vs.* 33% (control groups: 68% *vs.* 32% and 75% *vs.* 25%). Mood stabilizers such as lithium carbonate and lamotrigine were included: 7.8 ± 3.6 years in the first group, and 8.4 ± 3.2 years in the second group. Patients with comorbid physical disorders (hypothyroidism, diabetes mellitus, and arterial hypertension) were also included in this study. All comorbidities were adequately treated. Forty patients were administered with SSRI antidepressants, which are indicated in the treatment of moderate depressive episodes in combination with a mood stabilizer. Thirty-three patients were treated with mood stabilizers as monotherapy. The following SSRI antidepressants were administered: sertraline in 68% of patients in an average dose of 75 mg/day, escitalopram in 20% of patients in an average dose of 10 mg/day, and fluoxetine in 12% of patients in an average dose of 20 mg/day. The average length of SSRI was 72 days (Table 1).

In Table 2 are presented values of indicators of osteoporosis in the patients of all four groups: osteoporosis was registered in 25% of patients treated with mood stabilizers and SSRI antidepressants and in 18% of patients treated with mood stabilizers, while osteopenia was observed

Table 1. Type of psychopharmacotherapy

Patients		First group SSRI and mood stabilizers in therapy (± SD)	Second group Mood stabilizers in therapy (± SD)	Third group Control group of patients with depressive disorder, without SSRI or mood stabilizers in therapy (± SD)	Fourth group Control group of healthy volunteers (± SD)	p
n = 133		n = 40	n = 33	n = 30	n = 30	No significant difference
Age		56.2 ± 7.8	55.9 ± 4.3	55 ± 4.3	54.2 ± 3.2	No significant difference
Sex	Male	34%	33%	32%	25%	No significant difference
	Female	66%	67%	68%	75%	No significant difference
Duration of therapy with mood stabilizers (in years)		7.8 ± 3.6	8.4 ± 3.9	-	-	No significant difference
Dose of SSRIs: sertraline (68%) escitalopram (20%) fluoxetine (12%)		75 mg/day 10 mg/day 20 mg/day	-	-	-	-

SSRI – selective serotonin reuptake inhibitor

Table 2. Parameters of bone metabolism

Bone metabolism parameters	First group	Second group	Third group	Fourth group	p
	SSRI and mood stabilizers in therapy (n = 40) (± SD)	Mood stabilizers in therapy (n = 33) (± SD)	Control group of patients with depressive disorder, without SSRI or mood stabilizers in therapy (n = 30) (± SD)	Control group of healthy volunteers (n = 30) (± SD)	
25(OH)D vitamin (ng/ml)	24.5 ± 6.7[a]	29.1 ± 8.5[a]	37.5 ± 7.2[b]	51.1 ± 11.4	< 0.001*
Ca++ (mmol/l)	1.1 ± 0.1[c]	1 ± 0.1[a]	1.1 ± 0.1	1.1 ± 0.1	< 0.001*
β-CrossLaps (ng/l)	760.9 ± 129.4[a]	780.5 ± 84[a]	583.7 ± 48.3[b]	380 ± 84.9	< 0.001*
Osteopenia	40%[d]	37%	32%	20%	< 0.01*
Osteoporosis	25%[e]	18%	16%	10%	< 0.01*

SSRI – selective serotonin reuptake inhibitor;
[a]p < 0.001 compared with the third and fourth group;
[b]p < 0.001 compared with the fourth group;
[c]p < 0.001 compared with the second group;
[d]p < 0.01 compared with the fourth group;
[e]p < 0.01 compared with the second, third, and fourth group;
*significant difference

within 40% of patients treated with the combination of SSRI and mood stabilizers and in 37% of patients treated with mood stabilizers only. In the first control group, osteoporosis was detected in 16% of patients and osteopenia was present in 32% of the patients. In the group of healthy volunteers, osteoporosis was presented in 10% of examinees, while osteopenia was detected in 20% of examinees. There was no significant difference in the value of CrossLaps and 25(OH)D vitamin between the first two groups of patients. There was a significant difference between both control groups and other two groups in the prevalence of osteoporosis and osteopenia as well as in the value of CrossLaps and 25(OH)D vitamin.

Smokers were highly represented in all the patient groups (98% in patients treated with SSRI *vs.* 99% in patients treated just with mood-stabilization medicaments). In the control groups, smokers represented 86.5% of the sample.

DISCUSSION

Numerous results indicate strong relationship between use of antidepressants and osteoporosis. In addition to undoubtedly common comorbidity of depression and osteoporosis, the measures for prevention and early diagnosis need to be taken in order to have an early detection, treatment and lowering of the complication and mortality rate of both disorders [1, 4, 10, 11, 14].

According to the World Health Organization reports, mood disorders represent a huge health problem in comorbidity with osteoporosis and there is no doubt that unrecognized and untreated symptoms of depression, as well as anxiety symptoms, extensive use of certain types of antidepressants have been associated with an increased risk of osteoporosis in the last decade [4, 15, 16]. Osteoporosis is related to higher incidence of hip fracture in women over 60 years old treated with therapeutic doses of SSRI in comparison with depressive patients treated with other antidepressants [17, 18]. The most supported assumption in literature nowadays is that depression, as a separate mood disorder, triggers the development of osteoporosis through neuroendocrine and immune mechanisms as well as through bad habits (poor nutrition, alcohol intake, smoking, lack of physical activity) [3–5, 7, 15, 16]. The results have been controversial up to now, even though there is still a large number of studies that do not support the assumption that SSRI antidepressants play neuroendocrine role in bone metabolism. However, all the data require adequate choice of antidepressants for each individual patient together with consideration of all comorbidities and received therapies.

Bone architecture and the risk of osteoporosis can be assessed in a timely manner during therapy by measuring the bone density, determining vitamin D status in bones, as well as ionized calcium and other parameters of osteoporosis. Vitamin D is very important for physical and mental health. It is one of the key hormones in the regulation of bone metabolism. Vitamin D deficiency could increase the risk for low bone mineral density, or osteoporosis. Over recent years there have been studies that point out that vitamin D plays an important role in depression vulnerability [10, 15, 17]. It is interesting that an insufficient level of 25(OH)D (less than 50 mmol/l) could be associated with depressive disorder. Interestingly, lower level of vitamin D is found in 40–50% of depressive patients. This is probably due to lifestyle in depression. Depressive patients are very often heavy smokers, they sometimes abuse alcohol or other psychoactive substances. Such behavior may lead to hypovitaminosis [6, 19]. On the other hand, according to some studies, people with lower level of vitamin D could be at a greater risk of developing depressive disorders [20, 21]. Until now, the relationship between hypovitaminosis D and depression remains unclear. The association between low level of D vitamin and depression probably lies in homeostatic, trophic, and immunomodulatory effects of vitamin D [22, 23, 24]. New investigations also show that vitamin D receptors are identified in the same area of the brain associated with depression. In any case, hypovitaminosis D may represent an underlying biological vulnerability for depression [1, 17, 18, 23]. Although there is still no evidence that treatment with vitamin D supplements can reveal depressive symptoms, there is a possibility that some subgroup of depression may greatly

benefit from the treatment with vitamin D. Be as it may, it is clear that vitamin D has a prominent role in the treatment of depressive patients with low level of vitamin D. In such patients, long-term supplementation of vitamin D and calcium can increase bone mass and prevent fracture and long-term invalidity. Depressive patients with osteopenia/osteoporosis, benefit the most from the combination of adequate antidepressant therapy, psychotherapy, and, if indicated, therapy with vitamin D supplements. Also, there are studies suggesting that more intensive depression is associated with a lower level of vitamin D [23, 24]. In the present study, there was no relationship between the level of vitamin D and the severity of depressive symptoms measured with HAMD. In the future, an additional study should be made on a larger sample of patients to investigate if there is a connection between the low level of vitamin D in the blood and the severity of depressive symptoms.

The benefits as well as possible adverse effects of received therapy should be examined in every patient and useful advice should be given to them on their way of lifestyle and physical activity. In case it is necessary, bisphosphonates can be used for treating osteoporosis without any risk of interaction with SSRI antidepressants. Schweiger et al. [25] argue that osteoporosis of the spine has been diagnosed in almost 15% of the patients who suffer from depression – unipolar or bipolar – while numerous recent studies show a considerably higher incidence of depressive symptoms in women with vertebral and hip fractures [2, 8, 9, 25]. In the adolescent population, girls who suffer from anorexia and mood disorders are significantly more affected by osteoporosis than the general population. For the purpose of screening for osteoporosis, the National Osteoporosis Foundation recommends using the DEXA technique in women, aged 65 years and above and men aged 70 years and above or in people who are older than 50 years and are at an increased risk of fracture [1, 3, 4, 9, 10].

Hypercortisolemia in depression is meant to be the possible neurobiological base for such hypothesis [8, 9, 19]. Depression causes the activation of the hypothalamic–pituitary–adrenal axis and this alteration, which could be the crucial factor for the increased risk of osteoporosis in depressed patients [1, 10]. Actual hypothesis considers that corticotropin-releasing hormone and persisting high level of cortisol in depressed patients lead to secondary hypogonadism, which present one of the crucial risk factors for bone loss [1, 8, 14, 15, 21]. Such negative influence could be responsible for higher incidence of osteoporosis in patients with depressive symptoms, both in unipolar and bipolar affective disorders compared to the general population [1, 15, 17, 22]. According to the definition provided by the World Health Organization, osteoporosis is a progressive systemic skeletal disease characterized by reduced bone mineral density and bone microarchitecture alteration, which contributes to the risk of fracture and disability [1, 2]. The incidence of osteoporosis is 8–10%, but it is 10 times more common in women who reach menopause – the osteoporosis has been diagnosed in almost 22 million women and about 5.5 million men in the European Union [6]. The reduced bone mass and demineralization cause the bone to lose its strength and elasticity, which significantly increases the risk of fracture even with minimal trauma. According to the majority of the world's statistics, it is considered that almost 70% of fractures occur due to osteoporotic bones. Bone demineralization occurs as a result of bone remodeling process due to increased catabolic processes (increased osteoclast function and reduced osteoblast function). The osteoporosis is diagnosed by using the bone densitometry or DEXA scan and bone mineral density (BMD) described as a T-score and Z-score. A T-score represents standard deviation of a patient's BMD from the average value of BMD of a person of the same sex and constitution aged 20–30 years. The T-score between -1 and -2.5 is classified as osteopenia and the score lower than -2.5 is classified as osteoporosis [3, 20].

In addition to depression being considered a risk factor for osteoporosis, a considerable controversy has been caused by the results of certain studies in the last 10 years that show a possibility that certain types of antidepressants can cause osteoporosis as well. This assumption refers to the SSRIs type of antidepressants [12]. The mechanism of the action of these "newer" antidepressants is based on preventing the reuptake of serotonin at the presynaptic membrane, which leads to an increase in the level of serotonin in the synaptic cleft and its reuptake by receptors located at the postsynaptic membrane. The SSRIs achieve their antidepressant effect by binding to the serotonin transporter (SERT) in the central nervous system. The possibility that these antidepressants trigger osteoporosis is based on the discovery that functional serotonin receptors such as SERT (for which SSRIs are bound to at allosteric sites) are identified in osteoblasts, osteoclasts, and osteocytes. The second possibility is that SSRIs lead to a decrease in testosterone levels and an increase in prolactin level in both sexes, which represents a risk for osteoporosis. The following risk factors have a significant effect on the increased incidence of osteoporosis: chronic diseases – thyroid and parathyroid function disorders, hypogonadism, Cushing's and Addison's disease, insulin-dependent diabetes, neurological disorders and digestive disorders. [1, 14, 19, 21, 22].

At risk from osteoporosis are not only the middle age and elderly patients, but also the adolescent population – girls who suffer from anorexia and mood disorders are significantly more affected by osteoporosis than the general population. With regard to undoubtedly common comorbidity of depression and osteoporosis, the measures for prevention and early diagnosis need to be undertaken in order to have early detection, treatment, and lowering of the complication and mortality rate of both disorders [1, 4, 10, 21, 22, 26].

CONCLUSION

The results of this investigation indicate that a middle intensity depressive episode in bipolar affective disorder is connected with a higher prevalence of osteoporosis. Patients treated with SSRIs have higher prevalence of osteoporosis than patients treated with mood stabilizers only,

without antidepressants. In the future, larger cohorts of patients should be included in this kind of study.

Prevention is undoubtedly better than treatment of osteoporosis, which, in case of treatment of depressive symptoms, implies the selection of adequate group of medicines with respect to the age, initial status of the patient's skeleton, especially in patients already diagnosed with osteoporosis or other familiar risk factors pertaining to its development.

Conflict of interest: None declared.

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Повезаност биполарног афективног поремећаја, употребе антидепресива и остеопорозе

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САЖЕТАК

Увод/Циљ Остеопороза је један од најчешћих коморбидних поремећаја међу болесницима са депресивним поремећајем.

Циљ овог истраживања је био да се процени повезаност употребе антидепресива са остеопорозом код болесника лечених од биполарног афективног поремећаја.

Методе Ова студија је обухватила 73 болесника, стара 50–72 године, оба пола, хоспитализована због депресивне епизоде биполарног афективног поремећаја, између 2016. и 2020. године, на Клиници за психијатрију Клиничког центра Војводине. Болесници су подељени у две групе: а) прва група (40) лечена је селективним инхибиторима поновног преузимања серотонина у комбинацији са стабилизатором расположења (литијум-карбонат/ламотригин), б) друга група (33) третирана је само стабилизатором расположења. Студија је такође обухватила и две контролне групе испитаника. Клиничка мерења минералне густине кости на лумбалној кичми и куку изведена су методом апсорпциометрије рендгенских зрака двоструке енергије. Измерени су нивои калцијума и Де-витамина из узорака крви болесника. Подаци су статистички обрађени анализом варијансе и Краскал–Волисовим тестом.

Резултати Остеопороза је регистрована код 25% болесника у првој и код 18% болесника у другој групи, док је остеопенија установљена код 40% у првој и код 37% болесника у другој групи. Постоји статистички значајна разлика у вредностима нивоа *CrossLaps* и нивоа 25(*OH*) Де-витамина, као и у заступљености остеопорозе и остеопеније у односу на контролне групе.

Закључак Депресивна епизода у биполарном афективном поремећају је повезана са већом преваленцом остеопорозе у односу на контролну групу. Болесници лечени селективним инхибиторима поновног преузимања серотонина имају већу преваленцу остеопорозе у односу на болеснике лечене само стабилизатором расположења.

Кључне речи: минерална густина кости; депресија; селективно преузимање серотонина

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Expression and distribution of β amyloid precursor protein immunomarkers in the detection of diffuse axonal injury

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SUMMARY

Introduction/Objective The diffuse axonal injury has a very important place in clinical and forensic aspects of neurotraumatology. A special challenge is proving it in situations of short survival (less than two hours) after a craniocerebral injury.

The aim of this study was to determine the efficacy of beta-amyloid precursor protein (βAPP) immunohistochemical staining in postmortem diagnosis of axonal injuries in head injury survival shorter than two hours, its expression, and distribution through the brain tissue of the deceased.

Methods 36 adult fatalities, both sexes, injured by acceleration-deceleration mechanisms were divided into two groups: died up to two hours and died more than two hours after the injury. Immunostaining of brain tissue samples (frontal parasagittal white mass, genu and splenium of the corpus callosum and rostral pons) was used to register βAPP positivity. Data were processed by methods of descriptive and inferential nonparametric statistics, and $p < 0.05$ was considered statistically significant.

Results The βAPP immunopositivity was shown in 88.9% of cases (82.3% of ≤ two hours group *vs*. 94.7% of > two hours group). βAPP expression was enhanced towards the posterior structures of the brain. The shortest survival period with detected βAPP immunopositivity was 20–25 minutes, in three cases. There was an association of βAPP expression in the brainstem and interhemispheric/perimesencephalic subarachnoid hemorrhage ($p = 0.035$).

Conclusion βAPP immunohistochemical staining is effective in proving diffuse axonal injury in casualties that survived less than half an hour. Interhemispheric/perimesencephalic subarachnoid hemorrhage may indicate a more severe form of axonal injury.

Keywords: craniocerebral trauma; diffuse axonal injury; fatal outcome; amyloid beta-peptides

INTRODUCTION

Diffuse axonal injury (DAI) is one of the most common forms of diffuse brain injury. It is often present in traffic traumatism and can also be seen after falls from height, and blows to the head. Regardless of the mechanism of injury, the basic biomechanical principle of DAI is the acceleration-deceleration mechanism with elements of head rotation [1, 2]. The injury begins with the direct action of mechanical forces that cause the primary axonal injury followed by the pathophysiological cascading process of secondary axonal injuries that develops over time (for hours and days). The primary axonal injury occurs less frequently and to a lesser extent, while the secondary axonal injury is the dominant pathophysiological event [1, 3]. Clinically DAI is characterized by a prolonged comatose state (over six hours) in the absence of increased intracranial pressure or ischemic processes. Modern magnetic resonance imaging (MRI) techniques are sensitive enough to register the presence of an axonal injury in patients who have survived craniocerebral trauma long enough. Unfortunately, MRI is not a routinely applicable diagnostic method in the acute phase of diagnosis, immediately after injury. The reason is the incompatibility of the technical and procedural properties of MRI and the unstable condition of the traumatized patient who requires different types of support for vital functions in the initial phase of care. In this phase, computed tomography (CT) is still an indisputable radiological diagnostic method, but unfortunately it is insufficiently powerful in the detection of axonal injuries. Macroscopic pathomorphological manifestation of the axonal injury is often absent and not visible during autopsy. Pathomorphologically DAI is manifested by irregular axonal thickening, swelling (so-called varicosities) of partially damaged axons, and axonal bulbs at the ends of complete axonal tears [4]. These lesions are disseminated and scattered mainly along with the central brain structures: the parasagittal white matter of the frontal lobes, corpus callosum, internal capsule, thalamus, brainstem, and sometimes in the white matter of the cerebellum. Besides βAPP there are also other biomarkers in DAI diagnostic: N-acetylaspartate, glial fibrillary acidic protein, S100 calcium-binding

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protein B, ubiquitin C-terminal hydrolase, neurofilament-light etc. [5, 6, 7].

DAI is not exclusively of traumatic origin, but can also occur as a part of the development of brain edema, cerebral hypoxia, intoxication, etc. [8, 9]. Therefore, the term DAI is often changed to the more specific name of a traumatic axonal injury, which emphasizes its traumatic etiology. In literature, the classification of axonal injuries according to Adams is generally accepted [10]:

- grade 1: microscopically verified axoplasmic damage in the white matter of the cerebrum, corpus callosum, or brainstem;
- grade 2: grade 1 + focal lesions (bleeding) in the corpus callosum;
- grade 3: grade 1 + grade 2 + focal lesions in the rostral part of the brainstem and the upper cerebellar peduncles.

Special forensic significance is the fact that DAI very often ends in death, sometimes in a short period of several hours. In these situations, the primary axonal injury and the rapid decay of axons in certain, vital regions of the brain play an important role. This is especially true in cases where the axonal injury is the only intracranial pathological change. Its presence proves the viability of the injury (vital reaction), determines the exact cause of death and the possible mechanism of the injury, which are all crucial forensic issues.

The aim of this study was to test the efficacy of βAPP immunohistochemical staining in casualties that survived less than two hours after the injury, to examine the prevalence, distribution, and characteristics of the axonal injury of the deceased.

METHODS

Brain tissue was collected during regular forensic autopsies at the Institute for Forensic Medicine of the Republic of Srpska in Banja Luka, in the period from June 2017 to the end of 2019. The study included fatalities in acceleration-deceleration mechanisms (traffic accidents, fall from height, blows to the head), whose autopsies were performed up to 24 hours after death. A total of 36 brains of the deceased, both sexes, aged 18–81 years were collected. Brain tissue samples from the parasagittal white matter of the frontal lobe, anterior (genu) and posterior (splenium) parts of the corpus callosum, the rostral part of the pons with the upper cerebellar peduncles were taken for the study. The sex, age distribution, and the mode of injury (drivers/passengers in the vehicle, pedestrians, cyclists, motorcyclists, falls from height and blows to the head), brain mass, the Glasgow Coma Scale (GCS), fractures of cranial bones, focal lesions, and the presence of microbleeds in the brain tissue were observed. Exclusion criteria were: deceased under 16 years of age, and cases with confirmed neurodegenerative, inflammatory, or global ischemic-hypoxic changes of the central nervous system. The control group (10 subjects) consisted of brain tissue of deceased who died from causes not related to neurotrauma, without verified neurodegenerative, hypoxic-ischemic, hypoglycemic, and inflammatory changes of the central nervous system. The police reports and medical documentation determined the exact time and manner of the injury, the time of death, and the period of survival. During autopsy, all macroscopic craniocerebral injuries were carefully registered. The brain was examined on 1 cm thick cross-sections while brainstem and cerebellum were analyzed on 0.5 cm thick horizontal sections. Then, tissue blocks were taken from: the parasagittal white matter of the frontal lobe, the genu and splenium of the corpus callosum, and the rostral part of the pons. After fixation in a buffered solution of 10% formalin, standard molding and preparation of histological specimens was performed. Hematoxylin-eosin staining excluded pathological changes in brain tissue such as diffuse edema, brain atrophy, degenerative, inflammatory, general hypoxic-ischemic changes, and verified possible microbleeds.

For immunohistochemical staining we have used: monoclonal antibodies to βAPP (βAPP - Monoclonal Mouse Anti-Human β-Amyloid, clone 6F/3D, dilution 1:50 DAKO, Glostrup, Denmark), labeling of the antigen-antibody complex with streptavidin-biotin method (DAKO, K 0690), and visualization of tissue sections by dropping diamino-benzidine (DAKO, code 3466). Immunohistochemical staining registered ßAPP positivity on the analyzed sections through the presence of axonal bulbs and/or varicosities, and it was semiquantitatively assessed by the Gentleman`s scale [11]:

- "0" no staining;
- "+" weak positivity (isolated, sporadic scattered axonal varicosities);
- "++" typical positivity (grouped axon bulbs and varicosities);
- "+++" strong positivity (diffusely spread, involving larger parts of the visual field or bundles of nerve fibers).

DAI grading was performed according to the Adams classification [10]. To check the efficacy of the selected βAPP immunohistochemical technique in cases of short survival, two groups were formed, adjusted by sex, age, and mechanism of injury: group I – 17 brains of deceased who died within two hours after the injury, and group II – 19 deceased who survived over two hours. The consent of the Ethics Committee of the University Clinical Center of the Republic of Srpska in Banja Luka was obtained for this research. Statistical analysis was performed with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Sex, age distribution, and mode of injury were presented numerically, as a percentage and medians. Nonparametric χ^2 and Fisher's exact test were used to compare categorical variables and to check the interconnection of individual variables. The Kruskal–Wallis and Mann–Whitney U tests were used to test the differences in the distribution of independent features with the ordinal scale of measurement. The Spearman's rank correlation coefficient was used to analyze the correlation of the two features with the ordinal scale of measurement. The statistical significance threshold was $p < 0.05$.

Table 1. Demographic characteristics, mechanism of casualties and associated craniocerebral injuries in observed groups and total

Variables	Total (36 cases)	≤ two hours (17 cases)	> two hours (19 cases)	p
Age (years): median (range)	51 (18–81)	45 (19–81)	54 (18–81)	0.302
Sex:				
male	30 (83.3)	15 (88.2)	15 (78.9)	0.472
female	6 (16.7)	2 (11.8)	4 (21.1)	
Mechanism of injury:				
driver/passenger	12 (33.3)	7 (41.2)	5 (26.3)	
pedestrian	10 (27.8)	4 (23.5)	6 (31.6)	0.372
motorcyclist/cyclist	8 (22.2)	4 (23.5)	4 (21.1)	
fall from height	4 (11.1)	2 (11.8)	2 (10.5)	
blow to the head	2 (5.6)	0 (0.0)	2 (10.5)	
Glasgow coma score:				
≤ 8	30 (83.3)	14 (82.4)	16 (84.2)	0.475
9–12	6 (16.7)	3 (17.6)	3 (15.8)	
AssCranioInjuries:				
Cranial fractures	28 (77.8)	13 (76.5)	15 (78.9)	0.323
HED	2 (5.6)	0 (0)	2 (10.5)	0.169
HSD	30 (83.3)	14 (82.4)	16 (84.2)	0.881
SAH	28 (77.8)	14 (82.4)	14 (73.7)	0.532
IVH	23 (63.9)	12 (70.6)	11 (57.9)	0.429
CCH	11 (30.6)	6 (35.3)	5 (26.3)	0.559
BSH	4 (11.1)	3 (17.6)	1 (5.3)	0.238
Contusions	18 (50.0)	4 (23.5)	14 (73.7)	**0.003 (0.000*)**
Pet. hemorrhage	11 (30.6)	5 (29.4)	6 (31.6)	0.888

Values are presented as numbers (%), median and range for years; AssCranioInjuries – associated craniocerebral injuries; HED – epidural hemorrhage; HSD – subdural hemorrhage; SAH – subarachnoid hemorrhage; IVH – intraventricular hemorrhage; CCH – corpus callosum hemorrhages; BSH – brainstem hemorrhages; Pet. hemorrhages – petechial hemorrhages; p value – χ^2 and Kruskal–Wallis* test; boldface type indicates statistical significance

Table 2. Distribution of βAPP immunoexpression in the observed brain regions (total)

βAPP	Front. (N)	CCG (N)	CCS (N)	Pons (N)
+	15	8	8	7
++	10	16	17	9
+++	2	2	3	12
0	9	10	8	8

βAPP – beta amyloid precursor protein; Front. – parasagittal white matter of the frontal lobe; CCG – genu of corpus callosum; CCS – splenium of corpus callosum; + – weak positivity; ++ – typical positivity; +++ – strong positivity; 0 – negative βAPP immunoexpression

RESULTS

Table 1. shows basic demographic data, mechanism of injury and associated craniocerebral injuries by group and in total. In our sample, male gender and traffic accidents as a mechanism of injury were dominant. The most common associated craniocerebral injuries were subdural hemorrhage (HSD), subarachnoid hemorrhage (SAH) and skull bone fracture. Statistical comparison of these variables between groups revealed a significant difference only in the presence of brain tissue contusions, which were significantly more often present (14 contusions) in the group that survived longer (χ^2 p = 0.003; Kruskal–Wallis p = 0.000, Mann–Whitney U p = 0.000).

Axonal injury

On a total sample of 36 brains, βAPP immunopositivity was demonstrated in 32 cases or 88.9%. According to the observed brain regions, in the parasagittal white matter, the axonal injury was confirmed in 75% of cases, in the genu of the corpus callosum 72.2%, in the splenium of the corpus callosum 77.8% and in the pons 77.8%. According to the Adams classification of the severity of the axonal injury, grade 1 of DAI was found in 20 (55.6%) cases, in nine cases grade 2, and grade 3 in three cases. βAPP immunostaining revealed a fairly even distribution of the axonal injury through the observed regions of brain tissue, with a noticeable shift in the expression intensity to the posterior structures of brain tissue and the highest frequency of strong immunopositivity in the pons region (Table 2, Figure 1). Spearman's correlation test confirmed a weak relationship between the presence and intensity of axonal injuries in the parasagittal white matter of the frontal lobe and the anterior parts of the corpus callosum (r = 0.395), and a moderately strong association between the posterior parts of the corpus callosum and the rostral region of the brainstem (r = 0.627).

In the group of deceased that survived up to two hours, βAPP immunostaining showed the presence of a DAI in 14 brains (82.3%), while in the group where the deceased survived more than two hours, βAPP immunopositivity was present in 18 of 19 cases (94.7%). The shortest survival period in which βAPP immunostaining confirmed an axonal injury was about 20–25 minutes, in three cases. The first case was a 30-year-old man, a cyclist, with a weak expression in the splenium of the corpus callosum (+) and a typical immunopositivity in the pons (++). Second case was a 35-year-old male, pedestrian, with weak immunopositivity in the white matter of the frontal lobe (+), typical through the corpus callosum (++) and very strong in the pons (+++). The third case was a 34-year-old man, a car driver, with a verified axonal injury in the frontal white matter (++), splenium of the corpus callosum (+), and pons (+++) (Figure 2). In the first

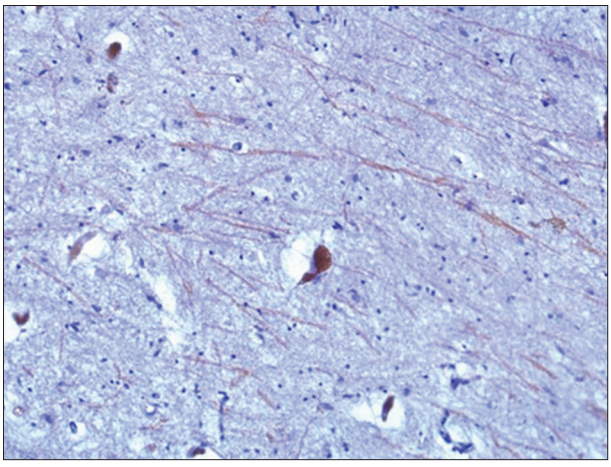

Figure 1. βAPP immunopositivity in the pons of a deceased who survived trauma for 50 hours; numerous, almost parallel-oriented, varicose thickened axons; a 54-year-old male, cyclist; βAPP immunostaining, 40 × magnification

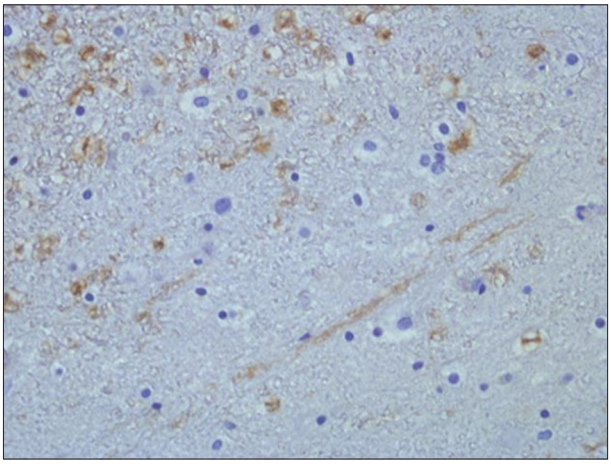

Figure 2. βAPP immunopositivity in the pons of deceased who survived trauma for 20–25 minutes; there are pronounced axonal bulbs and more discreet axonal varicosities that extend through the pons; a 34-year-old male, car driver; βAPP immunostaining, 40 × magnification

two cases, there were similar associated craniocerebral injuries: fracture of the roof and base of the skull, HSD, SAH, intraventricular hemorrhage and brainstem hemorrhage, with petechial hemorrhage in the white matter and corpus callosum in the first case. In the third case, in addition to the axonal injury, less pronounced HSD and SAH were found. When comparing the efficacy of βAPP immunostaining in the both groups, there was no statistically significant difference between observed regions (Table 3). The χ^2 test showed statistical significance between the presence of petechial hemorrhages in the parasagittal white matter of the frontal lobes and the axonal injuries in the anterior part (genu) of the corpus callosum ($p = 0.023$) but the association of petechial hemorrhages with the axonal injury in the white matter of the frontal lobes has not been confirmed ($p = 0.064$). The association of βAPP immunopositivity expression in the brainstem with diffusely disseminated and interhemispheric/perimesencephalic localized SAH was found ($p = 0.035$).

Table 3. Comparison of βAPP immunopositivity in the observed brain regions by groups

βAPP	Front. (N)		CCG (N)		CCS (N)		Pons (N)	
	≤ 2 h	> 2 h	≤ 2 h	> 2 h	≤ 2 h	> 2 h	≤ 2 h	> 2 h
+	8	7	2	6	2	6	2	5
++	5	5	7	9	11	6	5	4
+++	0	2	1	1	0	3	7	5
0	4	5	7	3	4	4	3	5
p	0.557 0.706*		0.290 0.051*		0.095 0.283*		0.547 0.394*	

βAPP – beta amyloid precursor protein; Front. – parasagittal white matter of the frontal lobe; CCG – genu of corpus callosum; CCS – splenium of corpus callosum; + – weak positivity; ++ – typical positivity; +++ – strong positivity; 0 – negative β APP immunostaining; p – χ^2 test, *Kruskal–Wallis test

In the control group (five males and five females, aged 21–77 years), six died of natural causes, three due to asphyxia and one of the consequences of stab wounds to the chest, without verified mechanical head injuries. Brain tissue immunostaining for βAPP was negative in all observed brain regions in all ten cases.

DISCUSSION

Over two-thirds of the victims with DAI are active workers. Men in traffic accidents are the most common casualties. This is in line with the results of other studies on DAI and craniocerebral trauma [12, 13]. The measured GCS values in the vast majority of casualties in this research indicated severe craniocerebral injury. The remaining, smaller number of cases with more favorable GCS (9–12) showed milder forms of DAI. Although not statistically significant, such distribution of GCS values is quite consistent with the fatal injury outcome and views that GCS is one of the indicators of severity of craniocerebral injury and recovery outcome predictor [14, 15]. In severe craniocerebral injuries with a fatal outcome, we most often saw a polymorphic pathomorphological image: skull fractures, various types of intracranial hemorrhage, and brain contusions with associated diffuse injuries of brain tissue, including axonal injuries. The available literature describes links between intraventricular hemorrhage and the presence of an axonal injury in the corpus callosum and pons [16]. In our study, such results were not confirmed and there was a significant association between DAI and most of the observed associated craniocerebral injuries. The connection between the expression of the axonal injury in the pons with SAH (interhemispheric and perimesencephalic), as well as diffusely spread SAH is distinguished. Similar results are described in recent studies [17, 18] which emphasizes the need for further research on whether this type of SAH can serve as a marker of severe DAI already in the initial phase of diagnosis (CT) and treatment of these patients. In forensic terms, this could indicate a very similar mechanism of injury by shearing forces of different layers of brain tissue and accompanying blood vessels. The association of petechial hemorrhage in the white matter of the frontal lobes and the axonal injury in the anterior segment of the corpus callosum, in this study, was not followed by the same relationship between petechial hemorrhage and axonal injury in the same localization (white matter of the parasagittal frontal region), although the significance level ($p = 0.064$) is at the very border of significance. These results do not contradict current views on the connection between microbleeds and axonal injuries [19, 20], but also do not confirm them with certainty. Reasons for this may be found in inconsistent research methodologies, the method of measuring the severity of certain types of injuries, etc. From the forensic point of view, cases where the axonal injury is a solitary craniocerebral injury are especially interesting. There were only two such cases in our study; the first is a 19-year-old young man, a car driver who survived for about 50 minutes, with a very strong immunopositivity in the brainstem and weak expression in the frontal white matter. The other was a 50-year-old man, a driver who survived six and a half hours, with typically pronounced immunopositivity in the corpus callosum and brainstem while a weak βAPP immune reaction was found in the frontal white matter. However, in both cases, there were associated injuries of other organ systems (contusions of lung tissue, fractures

of long bones, lacerations of the liver, etc.) which were not necessarily fatal but certainly contributed to the fatal outcome. We believe that the strong expression of the axonal injury in the brainstem in the first case, as well as the typical expression of the axonal injury through the brainstem and corpus callosum in the second case, can be considered as the immediate cause of death within the experienced polytrauma. No significant differences were found between the observed groups in terms of the prevalence of associated craniocerebral injuries, except in the case of brain contusions. Brain contusions were significantly more common in the group that survived longer. The probable explanation is that brain tissue contusions develop as a function of time and often progress in the posttraumatic period. In that way, the minor contusion injury, seen at the beginning, spreads over time and becomes easier to see visually. This expansion of the contusion focus was registered during the first 12 hours, sometimes the first 3–4 days after the injury, in which microvasculature lesions in the contused region and the release of transcription factor 1 and nuclear factor kappa B play an important role [21, 22, 23]. Expression of βAPP immunopositivity on a total sample close to 90% confirms the view of Gentleman et al. [11] that DAI is almost a regular finding in the case of a fatal blunt force head injury. In casualties that survived up to two hours, the immunopositivity is only slightly lower, but still over 80%, so we did not establish a significant difference between the observed groups. This study confirmed that βAPP immunostaining showed high sensitivity and efficacy in detecting axonal injuries in persons who died in a period much shorter than two hours. The shortest survival period with a positive βAPP immune reaction was about 20–25 minutes, confirmed in three cases. All three cases had been associated with various focal craniocerebral injuries. A similar short survival period in which βAPP immunostaining was successfully obtained is reported by Hortobágyi et al. [24].

The distribution of βAPP immunopositivity is fairly even throughout the observed regions of the brain, which corresponds to the notion of axonal injury as a diffuse cerebral injury. However, it is evident that the expression of βAPP immunopositivity increases from the anterior to the posterior structures of the brain (weak positivity is most common in the frontal white matter, typical through the corpus callosum and strong in the rostral parts of the brainstem). This finding is expected given the vital importance of brain regions such as the brainstem and corpus callosum. In the literature, the classification of the axonal injury severity according to Adams is generally accepted, which connects the most severe, grade 3 of axonal injury with the worst outcome [10], but this was not proven to be optimal in our research. There were only three cases (8.3%) of DAI grade 3, two cases of survival for half an hour and one case of survival for 17 hours. This is unusual in a sample of 36 severe craniocerebral injuries with a fatal outcome and deserves more careful analysis. Are macroscopically noticeable focal hemorrhages in the corpus callosum and brainstem really a relevant parameter or perhaps the presence of smaller, microscopically noticeable hemorrhages should be considered as valid criteria in this classification?

The limitations in this study were the relatively small total sample and the suboptimal number of different localizations from which brain tissue samples were taken for specific immunostaining. Data on the presence of axonal injuries in other predilection regions of the brain such as the thalamus, internal capsule, parahippocampal region, cerebellum, and lower parts of the brainstem would certainly be welcome and provide valuable information on the overall prevalence of this injury and possible association with other types of cerebral trauma. Finally, it was not possible to completely rule out the impact of serious injuries to other organ systems on the fatal outcome. In future research, it would be desirable to provide a larger sample without associated injuries to other body systems, in which multiple predilection cerebral regions for axonal injury development would be observed. Of particular forensic significance would be the study of an axonal injury as a solitary craniocerebral injury.

CONCLUSION

In postmortem proofing of DAI, βAPP immunohistochemical staining is a very powerful forensic diagnostic tool and shows efficacy in cases of survival less than half an hour. The observed link between interhemispheric/perimesencephalic SAH and axonal injury in the brainstem directs the focus of further research towards the interrelationship of these two types of craniocerebral injuries, which could, besides forensic, have an undeniable clinical significance in facilitating the diagnosis of more severe forms of axonal injuries in initial stages of treatment of these patients.

Conflict of interest: None declared.

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Експресивност и дистрибуција бета амилоидног прекурсорног протеинског имуномаркера у доказивању дифузне аксонске лезије

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САЖЕТАК

Увод/Циљ Дифузна аксонска лезија има важну улогу у савременој неуротрауми, у клиничком као и форензичком аспекту. Посебан изазов је њено доказивање у ситуацијама кратког надживљавања краниоцеребралне повреде, испод два сата.

Циљ овог рада је утврдити ефикасност имунохистохемијског бојења бета амилоидног прекурсорног протеина (*βAPP*) у постморталној дијагностици аксонске лезије код надживљавања повреде главе краћег од два сата, његову експресију и дистрибуцију кроз мождано ткиво смртно страдалих.

Методе Узорак од 36 смртно страдалих, одрасле доби, оба пола, у акцелерацијско-децелерацијским механизмима, подељен је у две групе: умрли до два сата и умрли после више од два сата од повређивања. Имунохистохемијским бојењем узорака мозданог ткива (фронтална парасагитална бела маса, гену и спленијум корпус клалозума, ростралнi део понса) регистрована је *βAPP* позитивност анализираних исечака. Добијени подаци обрађени су методама дескриптивне и инференцијалне непараметријске статистике, са нивоом статистичке значајности $p < 0,05$.

Резултати *βAPP* имунопозитивност потврђена је код 88,9% случајева (82,3% умрлих до два сата и 94,7% умрлих после више од два сата). *βAPP* имунопозитивност се појачава ка задњим структурама мозга. Најкраћи период надживљавања са детектованом *βAPP* имунопозитивношћу је 20–25 минута, у три случаја. Уочена је повезаност *βAPP* експресије у мождaном стаблу са интерхемисферично/перимезенцефаличном субарахноидалном хеморагијом ($p = 0,035$).

Закључак *βAPP* имунохистохемијско бојење показује ефикасност у доказивању дифузне аксонске лезије код надживљавања краћег од пола сата. Интерхемисферично/перимезенцефалично локализована субарахноидална хеморагија може указивати на теже форме дифузне аксонске лезије.

Кључне речи: краниоцеребрална траума; дифузна аксонска лезија; смртни исход; бета амилоидни пептид

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Splenic cyst following trauma, the intraoperative decision on definitive management

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SUMMARY

Introduction Posttraumatic splenic cysts are most commonly the result of blunt force trauma to the abdomen. They usually develop from subcapsular or intraparenchymal hematomas and are typically asymptomatic. Diagnostics includes clinical history and radiological imaging procedures. Surgical treatment is the only curative modality of treatment.

Case outline A 43-year-old female patient, without comorbidities, was admitted to the health institution for additional diagnostics and surgical treatment. Laboratory test results were within the reference ranges, while serological test results for hydatid disease were negative. An abdominal CT examination was subsequently performed confirming a splenic cyst positioned in the central part of the spleen. After a laparoscopic partial pericystectomy of the cyst, we identified another smaller cyst of the spleen. According to the estimation of the surgical team, the intraoperative appearance of the remaining tissue of the spleen was less than a third of the entire spleen. The decision was taken to perform a splenectomy in the best interests of the patient, bearing in mind the possibility of complications

Conclusion Accurate diagnosis of posttraumatic splenic cysts remains a challenge, despite state-of-the-art radiological imaging procedures that are applied. In addition to the well-known modalities of treatment, the laparoscopic surgical approach, i.e., minimally invasive treatment, should be the one of choice, if the situation allows it. The laparoscopic approach is a diagnostic and therapeutic method whose effect can especially be observed when the intraoperative finding differs from the preoperative radiological finding.

Keywords: spleen; splenic cyst; laparoscopy; partial pericystectomy

INTRODUCTION

Posttraumatic or secondary splenic cysts (SSC) are most commonly the result of blunt force trauma to the abdomen. According to the literature, the prevalence of SSC is 75–80% of all splenic cysts [1]. They usually develop from subcapsular or intraparenchymal hematomas. They are also called pseudocysts, as there are no epithelial cells in their outer wall, which is the main difference from true splenic cysts [1, 2].

These cysts are typically asymptomatic, although, in larger cysts, symptoms may occur in the form of abdominal discomfort especially in the left upper quadrant, occasional pain, nausea, and other nonspecific symptoms [2].

Diagnostics includes clinical history and abdominal ultrasonography; however, some more data can be obtained with computerized tomography (CT) and nuclear magnetic resonance imaging (NMRI) of the abdomen. Nevertheless, caution is advised, given that, as data available in current literature indicates, around 10% of splenic cysts are misdiagnosed. Differential diagnostics are most often considered: abscesses, hematomas, and primary cysts of the spleen [3, 4].

The only curative modality of treatment is surgical treatment, which can include less radical procedures through a minimally invasive approach, as well as splenectomy [1, 5].

The aim of this paper is to present an operating technique, with an emphasis on the importance of the intraoperative finding in reaching a decision on definitive treatment of posttraumatic splenic cysts.

CASE REPORT

This report presents a 43-year-old female patient, in good general health, without comorbidities, who was admitted to our department for additional diagnostics and management complaining of indeterminate abdominal symptoms. The clinical history showed that she had an allergy to iodine and contrast agents and that she had a skiing accident four years earlier. Several weeks after, a splenic cyst (4 × 3 cm) was diagnosed by abdominal ultrasound examination. Since then, throughout the regular check-ups, the cyst showed an increase in size compared to the previous examination. Prior to hospitalization, the patient felt symptoms in

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the form of discomfort and non-specific pain in the upper left quadrant of the abdomen, especially during physical activity.

Upon admission, an abdominal ultrasound was performed, which confirmed the finding of a cystic mass in the central part of the spleen. Laboratory test results were within the normal ranges. Serological test results for hydatid disease were also negative. An abdominal CT examination was subsequently performed confirming a splenic cyst which was 7 × 6 cm in size and predominantly positioned in the central part of the spleen, with the intracystic fluid density of 23 Hounsfield units (HU) (Figure 1).

After the diagnostic procedures were completed, a decision was made to carry out surgical treatment. Bearing in mind the experience of many years in minimally invasive splenic surgery, the strategy was to perform partial pericystectomy.

With the patient under general anesthesia, pneumoperitoneum was created with the use of the Veress needle. The patient was previously placed in the right lateral position in relation to the operating table. After the placement of working ports on the sites typical for this type of procedure, and after the introduction of the laparoscope, apart from a large splenic cyst with marked white discoloration of the capsule, as compared to the parenchyma. First, cyst puncture and the aspiration of the cyst content were performed. Next, partial pericystectomy with the removal of the cyst wall was carried out, up to the margin of healthy splenic tissue (Figure 2), using a harmonic scalpel (Ethicon Endo-Surgery, Inc, Cincinnati, OH, USA).

Then, to the surprise of the surgical team, a new cyst was identified laterally to the site of the previous cyst and towards the splenic hilum. This cyst was smaller, with the same characteristics as the larger one, and with no communication with the previous cyst (Figure 3).

The second cyst was treated in the same way as the first one. According to the estimation of the surgical team, the intraoperative appearance of the remaining tissue of the spleen was less than a third of the entire spleen. The decision of the team, at that moment, was to complete the procedure as planned preoperatively. However, a small amount of fresh blood appeared in the cavity of the larger cyst, and on the inner wall of the larger cyst. The decision was taken to perform a splenectomy in the best interests of the patient, bearing in mind the high possibility of bleeding. Laparoscopic splenectomy was performed in the standard manner, with the use of the aforementioned laparoscopic harmonic scalpel for dissecting the ligament system of the spleen and sealing the short gastric arteries. The vascular elements of the hilum were managed with an endovascular stapler (EndoGIA, Autosuture, Covidien, Mansfield, MA, USA). After complete mobilization and separation from the surrounding structures, the spleen was placed in a polyethylene bag for extraction (EndoCatch II, Autosuture, Covidien), within which the destruction and fragmentation of the remaining splenic tissue was performed, with the use of surgical instruments. The fluid aspirated from both cysts, parts of the cyst walls and the remaining splenic tissue were sent for pathohistological (PH) examination.

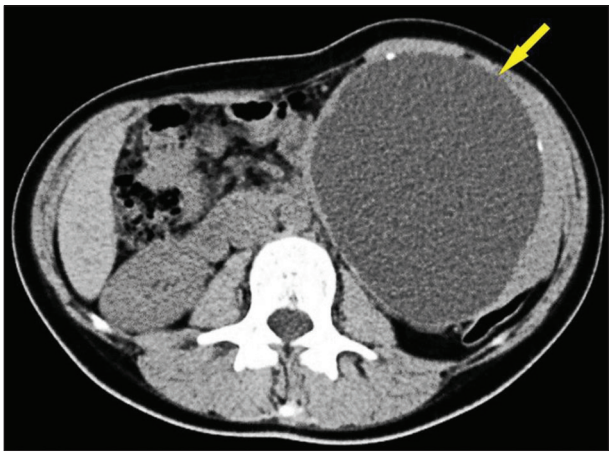

Figure 1. Preoperative abdominal computed tomography finding

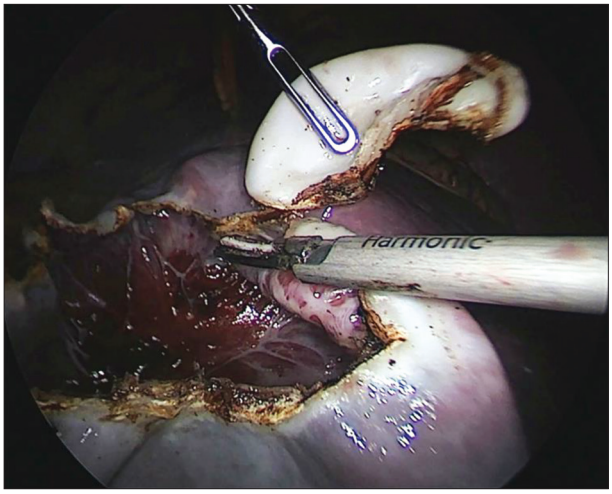

Figure 2. Image of partial pericystectomy and biopsy material sampling

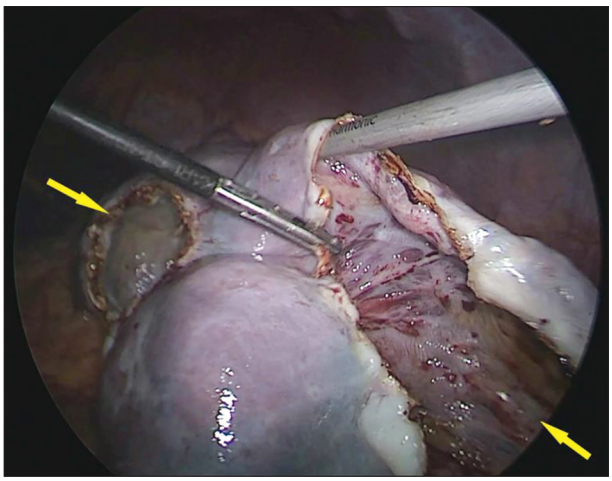

Figure 3. Intraoperative image of the spleen after the treatment of both cystic lesions

PH analysis confirmed a definitive finding of secondary pseudocysts of the spleen. The cyst walls were without epithelial tissue, and the cyst content without any special characteristics, while the remaining splenic tissue was also histomorphologically normal.

Postoperative recovery was uneventful. The patient was discharged from hospital on the third postoperative

day with prescribed prophylactic antibiotic treatment and postoperative immunization.

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

SSC comprise around 2/3 of all splenic cysts, and blunt abdominal trauma is considered to be the main cause of their formation. Most of them (80%) are unilocular; multilocular cysts are a rare finding [1, 6].

In our patient, the formation of the secondary cyst was linked to a previous abdominal trauma, similar as the most cases reported in literature. What makes this case characteristic is the fact that, despite the use of the modern imaging diagnostic, the second splenic cyst remained unnoticed.

SSC are not common and usually remain undetected, since they are mainly (30–60%) asymptomatic [7].

The diagnostics of these lesions should, in addition to the data obtained through anamnesis, include serological tests for hydatid infection, especially in endemic areas, as well as modern radiological imaging procedures, such as CT and NMRI [4, 8].

Surgical treatment presents the only safe and efficient treatment modality. Besides the complete splenectomy, there are also less radical surgical procedures, such as decapsularization, unroofing, partial pericystectomy, and, to a certain extent, partial splenectomy [1, 8, 9].

There are data in the literature on the effectiveness of sclerotization with alcohol as well as on percutaneous treatment known as PAIR (puncture, aspiration, injection, reaspiration) [10, 11]. The insight into the effectiveness of these techniques is limited to a small number of case reports, without larger series and without data on long-term follow-up of patients treated in this way.

Most authors suggests that caution is necessary in applying these procedures due to a high recurrence rate and the possibility that the cyst could be a diagnostically unrecognized parasitic cyst, which is why these methods should be reserved for patients in whom surgical treatment is contraindicated or who refuse surgery [1, 5, 7].

Accurate diagnosis of posttraumatic splenic cysts remains a challenge, despite widely available state-of-the-art radiological imaging procedures. In addition to the well-known treatment modalities, the laparoscopic surgical approach should be also considered. The minimally invasive approach is at the same time a diagnostic and a therapeutic method, and such an approach is particularly effective when the intraoperative finding differs from the preoperative radiological finding. Finally, minimally invasive surgery offers excellent cosmetic results compared to open surgery. The cosmetic effect should not be the most important deciding factor, but is an important detail, especially in younger people.

Conflict of interest: None declared.

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Посттрауматска циста слезине, интраоперативна одлука о дефинитивном третману

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САЖЕТАК

Увод Посттрауматске цисте слезине најчешће су последица тупе трауме абдомена. Обично настају из супкапсуларних и интрапаренхиматозних хематома и углавном су асимптоматске. Дијагностика обухвата историју болести и радиолошке процедуре снимања. Хируршки третман је једини потпуни модалитет лечења.

Приказ болесника Болесница стара 43 године, без коморбидитета, примљена је у здравствену установу због додатне дијагностике и хируршког лечења. Резултати лабораторијских испитивања били су у референтним вредностима, док су резултати серолошких тестова за хидатидну болест били негативни. Преглед абдомена компјутеризованом томографијом је потврдио цисту слезине која се налазила у централном делу слезине. Након лапароскопске парцијалне перицистектомије уочена је још једна мања циста слезине. Према интраоперативној процени хируршког тима, остатак ткива слезине је био мањи од једне трећине слезине. Донета је одлука да се учини спленектомија у најбољем интересу болесника, имајући у виду могуће компликације.

Закључак Прецизна дијагноза посттрауматских циста слезине остаје изазов, упркос примени најсавременијих радиолошких процедура. Поред добро познатих модалитета лечења, лапароскопски хируршки приступ, као минимално инвазивни третман, требало би да буде избор, ако ситуација то дозвољава. Лапароскопски приступ је дијагностичка и терапијска метода чији се ефекат посебно може уочити када се интраоперативни налаз разликује од преоперативног радиолошког налаза.

Кључне речи: слезина; циста слезине; лапароскопија; парцијална перицистектомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Risk factors and treatment approach for subarachnoid hemorrhage in a patient with nine intracranial aneurysms

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SUMMARY

Introduction In about one-third of the patients with aneurysmal subarachnoid bleeding, multiple intracranial aneurysms are confirmed. Risk factors such as female sex, smoking, hypertension, and age over 60 tend to be associated with multiple aneurysms. In this paper, we also discuss family predisposition and the treatment approach for multiple cerebral aneurysms.

Case outline Here, we present a case of a 64-year-old female patient, with spontaneous subarachnoid hemorrhage that had nine intracranial aneurysms. The patient was treated for hypertension for a long time, excessive smoker, and two of her nearest members of the family died from intracranial bleeding. The patient was fully conscious, without any neurological impairment. Subarachnoid bleeding was diffuse and neither brain-computer tomography finding or digital subtraction angiography could not suggest the source or location of bleeding among nine presented aneurisms. Magnetic resonance imaging had to be done, and the T1W fast spin-echo sequence showed a 9 mm large ruptured an aneurysm at the basilar tip, after contrast application, beside others. Three days after the insult, endovascular embolization was done and two basilar aneurysms were excluded from the circulation, including the one that bled.

Conclusion The patient had the majority of risk factors for multiple intracranial aneurysms. Knowledge of the family predisposition of multiple intracranial aneurysms allowed us to make a proper diagnostics of a patient's descendant and reveal a new patient.

Keywords: risk factors; subarachnoid hemorrhage; multiple intracranial aneurisms

INTRODUCTION

Even after a serious therapeutic breakthrough in recent decades, mortality and morbidity in patients with aneurismal spontaneous subarachnoid hemorrhage (SAH) remain unacceptably high. Overall case fatality is usually around half of the cases, although there are some novel studies that present in-hospital mortality much lower [1]. In more than one-third of the patients with SAH, multiple intracranial aneurysms are reviled [2] which usually makes their treatment difficult.

It is believed that the risk factor for the formation of multiple aneurysms is identical to a single intracranial aneurysm. It seems that both external factors and genetic are of significance with the insult. Magnetic resonance imaging (MRI) plays an important role in the diagnostic workup of SAH patients with multiple aneurysms, while endovascular embolization could be the therapeutic option in the majority of the cases. Other adverse events can complicate SAH, like electrocardiographic (ECG) changes caused by electrolyte disbalance [3]. Abnormal ECG changes in patients with acute SAH are as high as 65%, and if fluctuate from one abnormal change to another are usually associated with a poor outcome [4].

CASE REPORT

A 63-year-old woman was admitted with a severe headache, vomiting, and stiff neck. She was fully conscious, without any neurological deficit. Initial computer tomography (CT) brain scan revealed diffuse SAH, Fisher grade III (Figure 1).

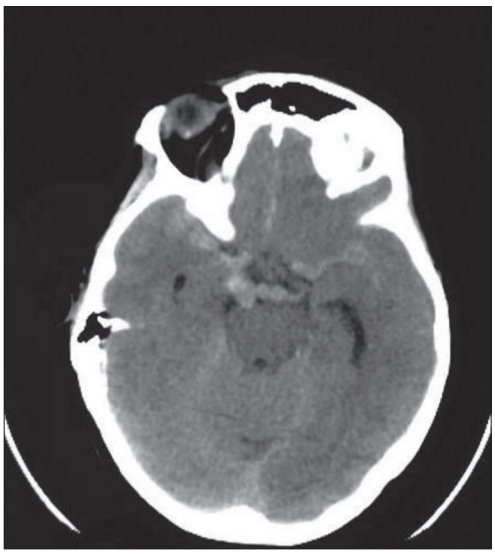

Figure 1. Initial brain computed tomography scan demonstrates diffuse subarachnoid hemorrhage and aneurism-like formation in front of the pons

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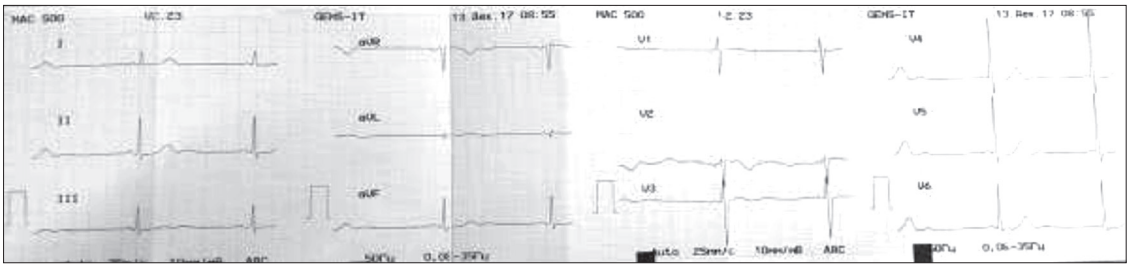

Figure 2. Echocardiography of the patient presented with bradycardia 35 per minute and prolonged QT interval

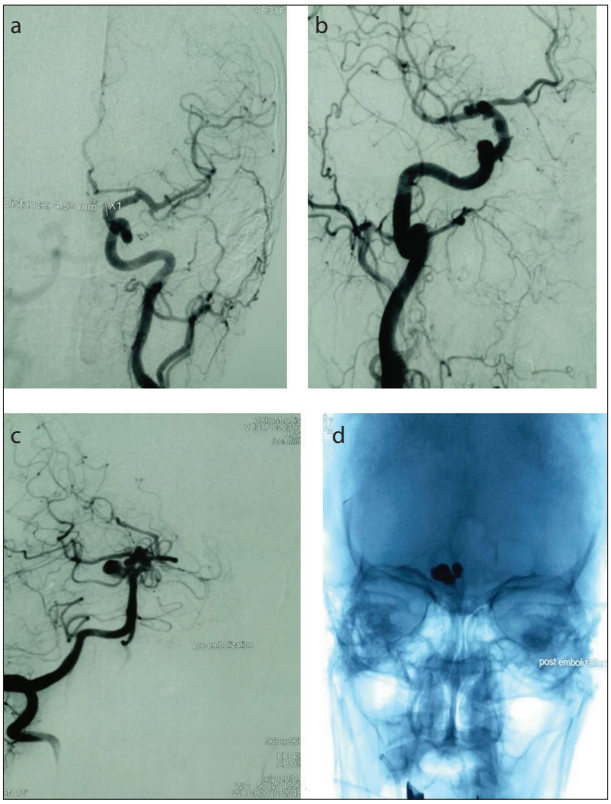

Figure 3. Digital subtraction angiography of the magistral cerebral vessels and postembolization finding; A – digital subtraction angiography of the branches of the left internal carotid artery; two aneurysms are revealed: one on supraclinoid segment of the internal carotid artery at the ostium of the anterior communicant arteria, and the second smaller at the bifurcation of the middle cerebral artery with the dimension of the 2.5 mm; B – digital subtraction angiography of the branches of the right internal carotid artery; three aneurysms are revealed: one on supraclinoid segment of the internal carotid artery, the biggest one at the bifurcation of the internal carotid artery with the dimension of the 6 mm; C – multiple aneurysms, four of them, on the tip of the basilar artery; D – postembolization finding – complete embolization of the ruptured and nearby aneurisms

The patient has been treated for arterial hypertension for 20 years, smoking 15 cigarettes a day for four decades, with intracranial bleeding in family history. The patients' father and brother died from massive intracranial bleeding. MRI of the patient's son revealed an aneurysm, also.

ECG findings revealed sinus bradycardia and prolonged QT interval (Figure 2). The serum finding showed low values of potassium throw all the periods of hospitalization ranging from 2.6 to 3.4, while serum finding showed a normal level of sodium. Therefore, getting sufficient potassium was imperative during therapy, and we managed it through Ringer lactate solution administration, 2000 ml, and an ampule of potassium chloride once a day.

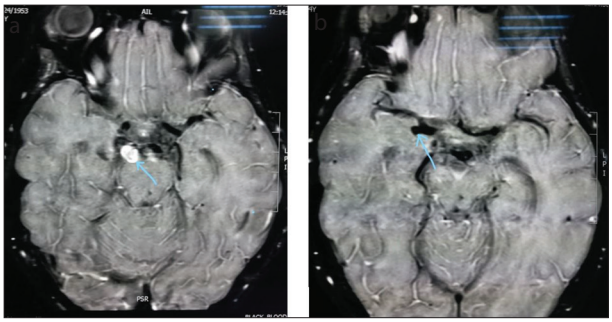

Figure 4. Magnetic resonance angiography of the brain T1W FSE sequence reveals an exact aneurysm that has ruptured (A) compared to unruptured (B)

The patient was treated with Mannitol solution 125 ml every six hours during and corticosteroids Lemod Solu 40 mg every eight hours for three days, analgesics, and antihypertensive therapy as amlodipine and ramipril in a dose of 5 mg in the morning.

Digital subtraction angiography (DSA) revealed nine aneurysms (Figure 3), four on the tip of the basilar artery (BA) (Figure 3 A), three on the right internal carotid artery(ICA) (Figure 3B), one on the left ICA and one on the bifurcation of the left middle cerebral artery (Figure 3C). Neither the deposit of blood clot in the brain CT, nor the shape or size of an aneurysm displayed on the DSA, and could not point out an exact aneurysm that had ruptured.

Therefore, we have examined the patient using the Philips Ingenia 1.5-T magnet resonance scanner (Phillips, Amsterdam, The Netherlands). Sequence Bleck blood T1-weighted 3D VWi was obtained using a flow-sensitized 3D fast spin-echo technique (T1W FSE) and it showed a 9 mm ruptured aneurysm at the basilar tip, after contrast application (Figure 4) where the intramural high signal and intimal flap were observed. Next to it, also at the basilar tip, two more unruptured aneurysms were located. Three days after the insult, endovascular embolization was done and two of the basilar aneurysms were excluded from the circulation (Figure 3D). After the intervention, the patient was fine, without any neurological deficit nor complications. An antiplatelet therapy – acetilsalil acid was administrated in a dose of 100 mg a day after the intervention.

She was released from the hospital 10 days after hemorrhage. The other six aneurysms are to be treated several months later after the patient had fully recoved.

The patient gave her informed consent about this publication.

DISCUSSION

Newly published studies present a typical patient with SAH and multiple aneurysms as a female, with a history of hypertension [5] and smoking [2].

In any of reviewed studies [6, 7], dealing with multiple aneurysms there were no more than five or six saccular cerebral aneurysms in one patient, but highly significant association between the presence of multiple aneurysms and hypertension, cigarette smoking, family history of cerebrovascular disease, female sex, and postmenopausal state in female patients was found. Nine aneurysms in one patient is a number unique for our case report. In these large studies, the authors did not consider family predisposition. We managed to link the deaths caused by intracranial bleeding of two closest relatives of the patient (father and brother) to actual hemorrhage and recommended a MRI to the patient's son that also revealed an aneurism.

Each of the factors that correlate with SAH in multiple cerebral aneurysms has either unexplained or unsatisfying explained role in the pathogenesis of the cerebral aneurysms or their rupture. Nowadays we accept the etiology of it as multifactorial, with environmental factors as a major, but also genetic one as important.

The possible role of smoking in the pathogenesis of the aneurysm formation or SAH could be explained by serum elastase/α_1-antitrypsin imbalance or increased elastase activity of cigarette smokers [8]. These can not be taken aside from the role of inflammatory and cell adhesion molecules, enzymes and hormones, and other cerebral proteins that affect cerebral vessels and damage it, which is crucial for the formation and rupture of aneurysms [9]. Smoking is connected to a transient increase of the blood pressure for a few hours and it could play an important role in the rupture of an aneurysm. A bimodal pattern of SAH occurs in the morning and the evening [10] when cigarette smoking and alcohol use usually displays its peaks.

Solid majority of the SAH patients are hypertonic [11]. One interesting hypothesis tries to find a connection between chronic arterial hypertension (HTA) and the formation of the aneurisms. Initiation of the effecting HTA is injuring the endothelium, occlusion of the vasa vasorum, and disruption of the synthesis of elastin and collagen. Subsequently, intima thickens, tunica media displays foci of necrosis and internal elastic lamina degenerates. These structural changes in the arterial wall cause a focal weakening in the arterial wall with resultant bulging. In an unselected series of 737 aneurysm patients, authors confirmed that hypertension and female sex are positive risk factors for multiple cerebral aneurysms [12].

Female sex is also of significance in multiple intracranial aneurism etiology, as the large study shows: women exhibited higher rates of bilateral (6.8% *vs.* 2.6%, respectively, $p < 0.05$) and multiple (11.5% *vs.* 5.2%, respectively, $p < 0.05$) aneurism comparing to man [13].

Family predisposition for multiple intracranial aneurysms was not debated widely in the literature. Nevertheless, by reviewing the literature we managed to find a few papers dealing with this issue. In a huge study group of 8680 asymptomatic patients, results showed that aneurysms were found in the general population of 6.8% rising to 10.5% in those with a family history of SAH [14]. Multiple aneurysms were more common in the familial group than in the sporadic group, in one recent study that compared a group of patients with two first-degree relatives with SAH and a group of patients without it. Interestingly, the age at the time of rupture was similar between relatives usually in the fifth or sixth decade [15].

The specific genes involved have not yet been identified. A good trace to this breakthrough could be its certain association with some genetic disorders that exhibit some syndromes or diseases. Some of them are more often associated with multiple intracranial aneurysms like Marfan syndrome, polycystic renal disease, Rendu–Osler–Weber syndrome, pseudoxanthoma elasticum, Klippel–Trenaunay–Weber syndrome, type III collagen deficiency, and fibromuscular dysplasia.

In multiple intracranial aneurismal cases where SAH occurs, it is impossible to always determine which aneurysm has bled, a fact of essential importance in further therapy. Brain CT and DSA usually present well known radiological signs and by following them and using a simple algorithm that is based on aneurysm location it is possible to identify the site of aneurysm rupture in 97.5% of cases [16]. Also, some morphologic and hemodynamic parameters can identify the ruptured intracranial aneurysm in patients with multiple intracranial aneurysms [17, 18]. Nevertheless, new radiologic techniques have found their purpose in dealing with this particular issue. One of the MR pulse sequences, spin-echo plays a major role in determining a ruptured aneurysm. T1W images are also required for assessing the degree of contrast enhancement on postcontrast scans [19]. On the other hand, conventional postcontrast 3D T1-weighted TSE sequences are more adequate in detecting unruptured cerebral aneurism [20]. So, in our case report, the T1WFSE sequence enables us the adequate treatment of the patient.

Hyponatremia is the most common electrolyte abnormality seen in patients with aneurysmal SAH, presented in more than a half of the patients, and it is usually present owing to syndrome of inappropriate antidiuretic hormone secretion [21]. Its impact on patients outcomes remains questionable [22].

In this case levels of sodium were normal, while hypokalemia was noticed. Its cause may be complex, involving both potassium losses from the body and intracellular shifts of potassium. SAH often causes a prolongation of the corrected QT (QTc) interval during the acute phase.

Our therapy of the patient consisted, among other medications, of application of potassium chloride inside a solution of Ringer. The embolization of an aneurysm is the first treatment option for the multiple intracranial aneurysms, especially if the bleeding spot is at the posterior part of the circle of Willis. Skillful and experienced neuroradiologist, besides technical precondition, is a must.

Conflict of interest: None declared.

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Фактори ризика и терапијски приступ болеснику са субарахноидалном хеморагијом и са девет интракранијалних анеуризми

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САЖЕТАК

Увод У трећини случајева болесника са анеуризмалним субарахноидним крварењем потврђено је присуство вишеструких интракранијалних анеуризми. Код мултиплих церебралних анеуризми фактори ризика су женски пол, пушење, хипертензија и старост преко 60 година. У овом раду смо дискутовали о факторима ризика, породичној предиспозицији и приступу лечењу вишеструких церебралних анеуризми.

Приказ болесника Представљамо случај болеснице старе 64 године, са спонтаним субарахноидним крварењем, која је имала девет интракранијалних анеуризми. Она се дуже време лечила од хипертензије, прекомерно је пушила, а два најближа члана породице умрла су од интракранијалног крварења. На пријему је болесница била потпуно свесна, без икаквих неуролошких оштећења. Субарахноидно крварење је било дифузно и нити налаз компјутерске томографије мозга, нити дигитална суптракциона ангиографија нису указивали на место крварења, тј. на то која је од девет анеуризми крварећа. Дакле, требало је урадити снимање магнетном резонанцом, а *T1W* брзи спин-ехо низ показао је да је руптурирала анеуризма дијаметра 9 *mm* на врху базиларне артерије. Три дана након крварења урађена је ендоваскуларна емболизација и две базиларне анеуризме су искључене из циркулације, крварећа и некрварећа.

Закључак Овај ретки случај био је праћен већином фактора ризика за развој вишеструких интракранијалних анеуризми. Знање о породичној предиспозицији настанка вишеструких интракранијалних анеуризми усмерило нас је откривању новог пацијента – болесничиног сина.

Кључне речи: фактори ризика; субарахноидно крварење; вишеструке интракранијалне анеуризме

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Customized polymethylmethacrylate cranioplasty using a low-cost 3-dimensional printed mold

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SUMMARY

Introduction Significant cranial defects result from a decompressive craniectomy following head trauma, malignant brain edema, intracranial hemorrhage, or resection of tumor affected bone. Unrepaired cranial defects are not just a tremendous esthetic problem. The underlying brain is unprotected, prone to injury, and this state can lead to the so-called "syndrome of the trephined" with mood instability, headaches, and even a neurological deficit. Currently, there is no widely accepted uniform technique of cranial vault shape restoration. Combining 3D technology with the use of polymethylmethacrylate is a challenging field that can bring good functional and aesthetic results and, in the case of smart design, become efficient, low-cost technology. We offer a possible solution to a problem that would be acceptable in neurosurgical practice.

Case outline We present a 37-year-old male patient with a massive hemicranial defect as a consequence of previous decompressive craniectomy following severe craniocerebral injury the previous year. Together with engineers from the appropriate 3D modeling studio, we have designed a two-part mold by laser printing technology using biocompatible advanced polyamide. We made a customized polymethylmethacrylate graft intraoperatively using this mold and achieved good aesthetic results.

Conclusion Reports of 3D printing assisted cranioplasties are growing, describing different techniques and cost- estimation. We hope to introduce a low-cost and simple method for repairing a skull defect.

Keywords: craniectomy; cranioplasty; skull defect; polymethylmethacrylate; 3D printing

INTRODUCTION

With first records dating over 3000 years B.C., cranioplasty is one of the oldest neurosurgical procedures aimed at restoring cranial vault integrity. The benefit to the patients is unquestionable since results concern not only esthetics and mechanical protection of intracranial structures but affect a considerable amount of subjective disturbance and even lead to regression of neurological deficit. Although as old as the first attempts of neurosurgery, there is no widely accepted uniform technique performing a cranioplasty. Materials currently used differ and can be autografts or more commonly used in modern neurosurgery – allografts.

CASE REPORT

We present a 37 years-old-male, who was admitted to our clinic for an elective cranioplasty procedure, 13 months following surgery after a traffic accident. Initial surgery included the evacuation of acute subdural hematoma and decompressive craniectomy due to malignant brain edema. Neurological status on admission revealed mild right-sided hemiparesis, and the patient-reported occasional headaches and light dizziness. Local status included clearly manifested massive bone defect, deformity of the skull contour, without active skin infection or any skin efflorescence (Figure 1).

Routine non-enhanced computed tomography (CT) scan of the head was performed, using a bone window to build a 3D model, and data were further used for modeling by digital sculpting relying on symmetry and geometry present on the other half of the skull. Preoperative design, planning and modeling are conducted in selected studio for 3D modeling (Voxellab D.O.O.©, Belgrade, Serbia). The model was furbished using ZBrush 2021® (Pixologic©, Los Angeles, CA, USA). Based on the implant model, a 3D model of two-sided mold was created using Rhinoceros 6® software (McNeel©, Seattle, WA, USA). Finally, manufacturing of a two-part mold by selective laser sintering (3D printing) technology was conducted, using biocompatible PA2200 material (advanced polyamide 12) on Formiga P110 Velocis® (EOS©, Krailling, Germany) device with a resolution of 0.1 mm per layer, on 170°C ensuring high-level precision of construction and surface quality. The manufacturing process and material are certified for use in the medical and food industries. The material is biocompatible according to EN ISO 10993-1. The manufactured parts are isotropic and temperature-stable up to 163°C. Post-production of molds included sandblasting with glass and ceramic beads for maximum removal of unsintered

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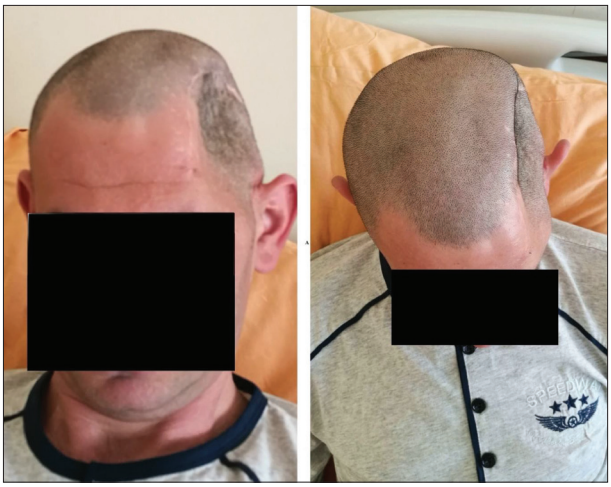

Figure 1. Patient with large hemicranial defect before surgery

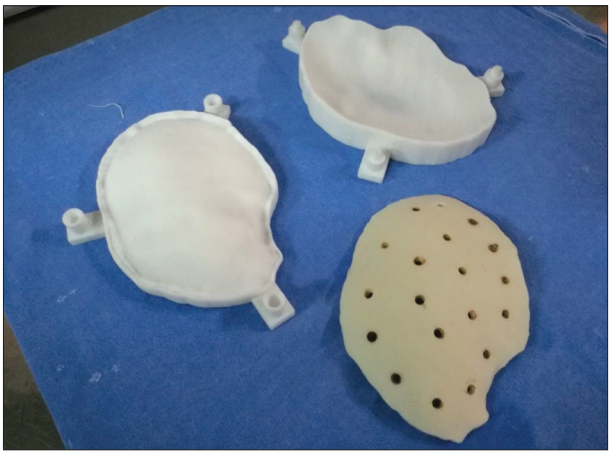

Figure 2. Two-part polyamide mold made by laser printing technology and the resulting polymethylmethacrylate graft shaped by manual mold compression

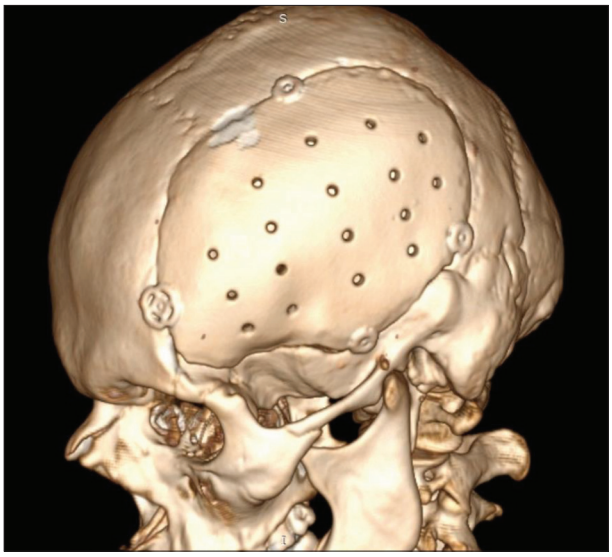

Figure 3. 3D computed tomography cranial reconstruction after the operation

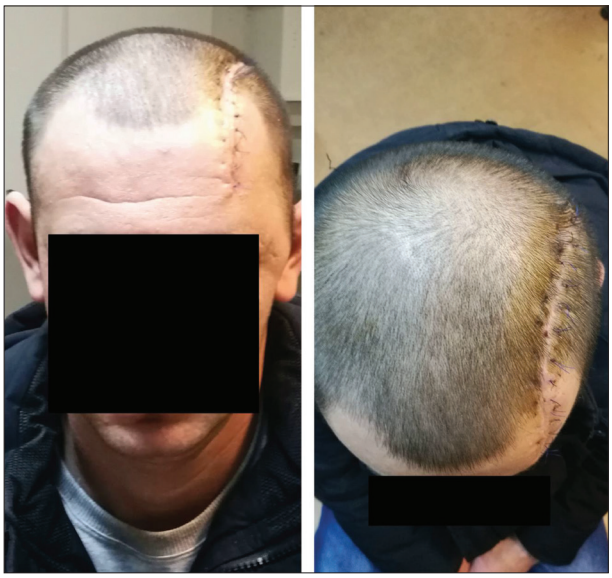

Figure 4. Restoration of head shape after the operation – pleasurable esthetic result

powder and raising the quality of surfaces, and additional polishing of the inner surfaces of the mold for easier separation of the mold and the implant. Time consumed for 3D modeling, printing, and post-production processing was eight days, and the estimated cost per patient was €550–€600.

Since preoperative check-up revealed no absolute contraindications for operation, such as hydrocephalus, brain swelling, or infection, using 3D printed prefabricated molds, polymethylmethacrylate cranioplasty was performed. The same skin incision was used, and soft tissue dissection from the dura was carefully conducted. An evident impression of the left hemisphere and tissues above were noted. Significant adhesions of the inner dura to the arachnoid were found, and since it was the dominant hemisphere, no further dissection was performed, and no central tenting sutures could have been placed. The mold was sterilized prior to surgery in a standard autoclave at 134°C for 20 minutes and unpacked during the operation following all sterile procedures. Two packings of Poly(methyl methacrylate) (PMMA) (Gentafix, Teknimed s.a.s. Vic-en-Bigorre, France) were used for molding an implant intraoperatively. Molds were soaked with sterile saline and used for fabricating a final prosthesis. After the initial phase and achieving enough hardness not to deform, the mold was opened, allowing final stages of polymerization to occur outside of the mold and thus avoiding deformation of mold or implant. Multiple punctuations were made in the implant, allowing evacuation of potential fluid collection, as well as soft tissue ingrowth, obliterating potential epidural space (Figure 2). Fixation was achieved using CranioFix® (Aesculap©, Center Valley, PA, USA), epicranial drain was left in place, and soft tissue reconstruction was performed in anatomical layers. There were no intraoperative complications during prosthesis molding or implantation. After surgery, aesthetic results were obvious, and the patient was without new neurological deterioration (Figures 3 and 4). Initial CT scan demonstrated epidural effusion of non-blood liquid, without compressive effect (Figure 5). Local punction was performed, and fluid, which was a mixture of blood and saline, was drained. Following punction, an immediate control scan

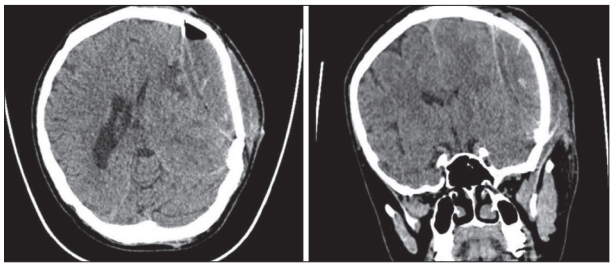

Figure 5. Epidural effusion following surgery

was performed, which showed no liquid or blood remnants in epidural space. No further complications were noted. During the 12 months follow-up, hemiparesis regression was confirmed. The patient also reported the withdrawal of subjective complaints.

Written consent was obtained from the patient to publish all the shown material. This study was conducted according to the institutional standards on ethics.

DISCUSSION

Attempts to restore the integrity of the skull are as old as neurosurgery. According to literature, defects larger than 6–10 cm^2 subject to reconstruction, and those larger than 50 cm^2, or more than 12 cm in axis should subject to custom-made cranioplasty [1–4]. Indications for reconstructive surgery concern not only aesthetic and social expectations of the patient but also the improvement of cerebral protection. Relieving cortex of soft tissue compression and restoration of normal cerebrospinal fluid circulation and venous blood return leads to neurologic improvement and diminishing of a group of symptoms counted in the syndrome of the trephined [5]. So far, many techniques and materials were tested, but still, no uniform procedure is established.

Many conditions result in cranial defects. The most common reasons are decompressive craniectomies due to intracranial hematoma, malignant edema or hemispheric ischemic lesions, comminution fractures or resection of tumor affected bone. Plenty of reports regarding this operation emphasize the use of bone graft preserved subcutaneously or in the bone bank. Still, this only provides a solution in cases of unfractured bone, excluding patients with wounds over bone flap, making them especially prone to infection and possibility of implantation under abdominal skin, since numerous cranial trauma cases also require general surgery operation. It is also an important fact that many, especially the third world and developing countries, have no bone banks. Despite all fulfilled conditions, there are still risks of bone graft resorption, especially in children, or infection and the consequent need for new operation. Younger age, bone flaps larger than 75 cm^2, and shunt dependency are recognized risk factors for bone resorption [6]. Even in the absence of resorption, initial damage or intraoperative drilling can cause a skull-graft mismatch, creating a significant esthetic defect. In the end, the exact discrepancy can be seen only intraoperatively.

Since World War II, the use of artificial materials is becoming more frequent. Characteristics expected to meet are biocompatibility, inertness, radiolucency, rigidity, but the material should also be light, non-magnetic, simple for handling and placement, and with low thermal conductivity [7, 8, 9]. Currently, most used alloplastic materials encompass metals, acrylic materials, plastics, and hydroxyapatite as representative of bioceramics [4]. A number of papers concerning allograft cranioplasty grows, but large studies comparing different materials with official recommendations are lacking. Data describing hydroxyapatite use, show good bio integration, demonstrating osteoconductive capabilities, making it particularly interesting for the pediatric population, but also showing a higher chance of prosthesis fracture and dislocation, as well as significantly higher price per piece [4]. Usage of titanium in cranioplasty offers good quality and persistence but is not flawless. Its fabrication is more complicated [7]. Patient's complaints of thermal conductions are well noted, with some series even reporting a higher incidence of infection in these patients compared to those operated using PMMA [4]. It is also important to emphasize that titanium offers minimal potential for an intraoperative correction [7]. Still, the main concern for health systems is a relatively high price, ranging $3000–$5000 [1, 4, 8, 10, 11].

Reports of 3D printing assisted cranioplasties are growing, describing different techniques and cost-estimation. Using PMMA offers many advantages over other materials. Significantly lower cost comparing to titanium makes it affordable to most health systems. Simplicity in use, low thermal conductivity, and the possibility for intraoperative modification make it especially helpful in reconstructive surgery. Still, it requires additional use of fixation hardware and develops high temperatures during polymerization, carrying a risk of thermal damage to surrounding tissues.

Methods described in literature differ significantly in every step of fabrication and implantation of the graft. Some authors propose the utilization of previously prepared and sterilized prosthetics, stating the lower price, shorter operation time, reduction of blood loss, and lower infection rate [7, 12]. One must consider that using premade PMMA grafts requires plasma or ethylene-dioxide sterilization, which is not widely available, increasing price, but more importantly diminishing the possibility of intraoperative correction [7, 13]. Further differences concern the method of obtaining the final prosthesis. Although printing a prosthesis model, followed by making a plaster cast and additional molding of PMMA final graft is possible, we find it unnecessary and too complicated since it can result in significant mold and prosthesis deformation [7, 9]. Using one-side mold achieves precise curvature but makes it almost impossible to achieve the exact volume of the graft, fill the trephine holes, and bares risks of uneven and bumpy inner side of the graft [1, 10]. Direct printing of two-sided mold allows immediately obtaining not only correct contour and shape, but also thickness of the bone and therefore better fixation and durability. Despite some studies stating the possibility of mold deformation during sterilization, we did not encounter such problems

[1]. Screw-assisted molds and those designed in such a manner that so that PMMA can be poured into them complicate opening of the mold and allowing final stages of polymerization of the PMMA to occur outside of the mold, avoiding sticking and deforming of both mold and prosthesis [10, 11].

Precise recommendations regarding the timing of the operation are still to be established. Current studies attribute a higher rate of hydrocephalus in early cranioplasty (< 90 days) following trauma, but also find a higher incidence of extra-axial effusion in delayed procedures [4]. We address epidural effusion seen in our case to inability to place central tack-up sutures due to dura-arachnoid scaring, arising from the late-term of the operation.

Although technically undemanding, skull reconstruction still carries risks of early and late postoperative complications [4, 5, 9, 10]. The overall rate of complication differs, usually raging 5–25% [9, 13]. We would like to emphasize, in particular early postoperative care, including mandatory CT scan. As seen in our case, brain hemisphere atrophy presents a risk for fluid collection and extra-axial hematoma, without evident neurological deterioration, further endangering the patient. Even in good result months following the surgery and esthetically satisfying appearance, with reduction of subjective complaints and social disturbances, late complications described in the literature suggest the need to periodical check-ups.

Beside excellent esthetic outcome, shorter operation time, reduced blood loss and infection rate, without donor site morbidity, using printed customized molds offers the possibility of intraoperative correction and remanufacturing of the graft in case of infection or prosthesis fracture [9, 14, 15].

The total price of graft manufacturing is under 600$, making it lower than the prices stated in the literature, ranging $600–$5000. By using our proposed method, we hope to overcome two major concerns regarding cranioplasty – price and time consumed in planning and manufacturing of the prosthetics. Still, temporalis muscle atrophy, commonly seen following decompressive craniectomies, still remains an esthetical problem, with the best method of augmentation yet to be found. We hope that our fast, precise, efficient, and low-cost method of customized cranioplasty assisted by 3D printing technology will be accepted and funded by the Serbian National Health Insurance Fund.

Conflict of interest: None declared.

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Краниопластика полиметилметакрилатом коришћењем индивидуализованог калупа начињеног тродимензионалним штампачем

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САЖЕТАК

Увод Дефекти лобање често настају као последица декомпресивних краниектомија након краниоцеребралне повреде, малигног едема мозга, интракранијалног крварења или ресекције кости захваћене тумором. Кранијални дефекти који нису репарирани нису само значајан естетски проблем. Мождано ткиво је у таквим случајевима незаштићено, подложно повредама и ови болесници понекад испољавају карактеристичан синдром који се одликује нестабилношћу расположења, главобољама, па чак и неуролошким дефицитом. Тренутно не постоји широко прихваћена и стандардизована техника пластике лобање. Комбиновање технологије тродимензионалне штампе са употребом полиметилметакрилата представља алтернативу, са значајним потенцијалом за добре естетске и функционалне резултате, са смањеним трошковима израде. Овде приказујемо једно од решења које би могло бити прихватљиво у неурохируршкој пракси.

Приказ болесника Представљамо 37-годишњег мушкарца са масивним хемикранијалним дефектом после декомпресивне краниектомије учињене због тешке краниоцеребралне повреде годину дана раније. Заједно са инжењерима из студија за тродимензионално моделирање, дизајнирали смо дводелни калуп технологијом ласерског штампања користећи биокомпатибилни напредни полиамид. Током саме операције смо затим направили индивидуализовани полиметилметакрилатни графт према овом калупу и постигли добре естетске резултате.

Закључак Краниопластике начињене уз помоћ технологије тродимензионалног штампања су све више у употреби и већ су описане различите технике, мада још увек нису бројне. Надамо се да ћемо описаним начином увести релативно јефтин и једноставан, али ефективан метод за репарацију дефекта лобање.

Кључне речи: краниектомија; краниопластика; дефект лобање; полиметилметакрилат; тродимензионално штампање

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Internal carotid artery "donut" aneurysm treated using DERIVO flow-diverting stent

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SUMMARY

Introduction Intracranial aneurysms with a radiological sign of a donut are a medical priority and have been described in a small number of cases. This radiological sign occurs in aneurysms in which there is partial thrombosis inside aneurismal sac and circular laminar flow between the aneurismal wall and the thrombus in its center. Consequently, there is a central contrast-filling defect of the aneurysm sac observed on different angiographic imaging methods.

Case outline We present a 35-year-old female patient admitted for examination due to frequent headaches, visual disturbances on the left and loss of sight on the right eye. Digital subtraction angiography (DSA) showed an aneurysm on the right internal carotid artery measuring 25.6 × 25 mm, while neck measured 11 mm and included part of the C6 and C7 segments. Treatment decision was made that placing a flow-diverting stent across the aneurysm neck would be most beneficial in this case. After the procedure, the patient was discharged in the same general condition as she was before admission to the hospital. Seven months after the intervention, she reported for her first DSA control examination. Normal position of the left A1 segment was demonstrated, suggesting shrinkage of the aneurysm sac. An improvement of vision on both eyes was stated.

Conclusion: We present a patient with a "donut" aneurysm on the internal carotid artery, successfully treated with a flow-diverting stent.

Keywords: "donut" aneurysm; DERIVO stent; digital subtraction angiography (DSA)

INTRODUCTION

Intracranial aneurysms with a radiological sign of a donut are a rarity and have been described in several cases. In this paper, we present a case of a "donut" aneurysm on an internal carotid artery treated with a DERIVO embolization device (DED).

CASE REPORT

A 35-year-old female patient was admitted for examination due to frequent headaches, visual disturbances on the left and loss of sight on the right eye. On non-enhanced computed tomography and computed tomography angiography examinations, giant aneurysm with centrally positioned thrombus was diagnosed (donut shape aneurysm) on the right internal carotid artery (ICA). Digital subtraction angiography (DSA) showed an aneurysm on the right ICA measuring 25.6 × 25 mm, while the neck measured 11 mm and included part of the C6 and C7 segments. The aneurysm sac was directed upwards and medially, dislocating supraclinoid segments of the left ICA. The right ICA itself was narrow in diameter throughout its whole course, especially around the neck of the aneurysm. Proximal diameter of the ICA was 3.2 mm, with pre-aneurysmatic narrowing with radius drop to 1.9 mm, while the distal part measured 2 mm. The right A1 segment was aplastic and the right anterior cerebral artery (ACA) was filling from the left A1 segment, which was elevated due to the compressive effect of the aneurysm.

Treatment decision was made that the positioning of a flow-diverting (FD) stent across the aneurysm neck would be most beneficial in this case. The patient was prescribed with a loading dose of dual antiplatelet therapy four days prior to intervention, consisting of clopidogrel (Plavix, Sanofi Winthrop Industrie, Paris, France) 75 mg twice per day, and acetylsalicylic acid (Aspirin, Bayer, Leverkusen, Germany) 100 mg per day. Under conditions of general anesthesia, a DED (Acandis, Pforzheim, Germany) measuring 4.5 × 30 mm was then placed using both push and pull techniques, in order to maximize the radial force of the stent. Flow-diverting effect was demonstrated immediately on post-procedural angiograms (B3 degree of occlusion by O'Kelly–Marotta classification [1]). The patient was discharged in the same general condition she was before admission to the hospital.

Seven months after the intervention, the patient reported for her first DSA control examination. An improvement of vision on both

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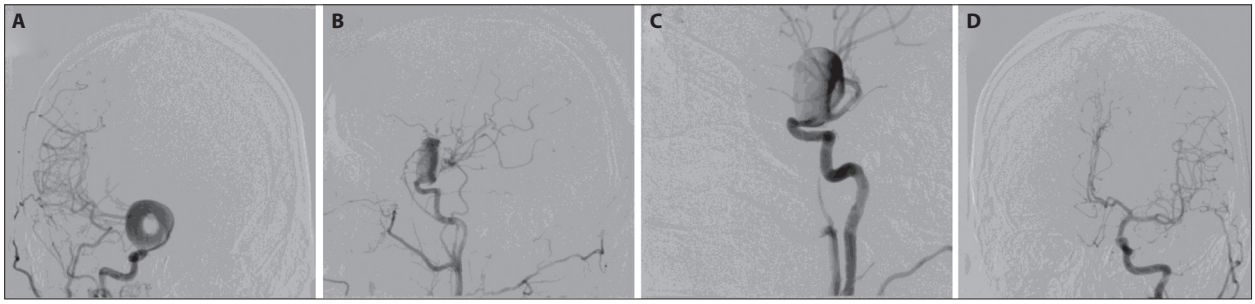

Figure 1. Pre-treatment angiograms; (A) antero-posterior, (B) lateral view of the right internal carotid artery (ICA) show a giant aneurysm with a "donut sign" on the right ICA, which appears narrow, with pre-aneurysmatic stenosis and very thin post-aneurysmatic diameter of the vessel; the right A1 is not showing; (C) antero-posterior view of the left ICA demonstrates both anterior cerebral arteries filling from the left A1, which is elevated due to compressive effect of the aneurysm

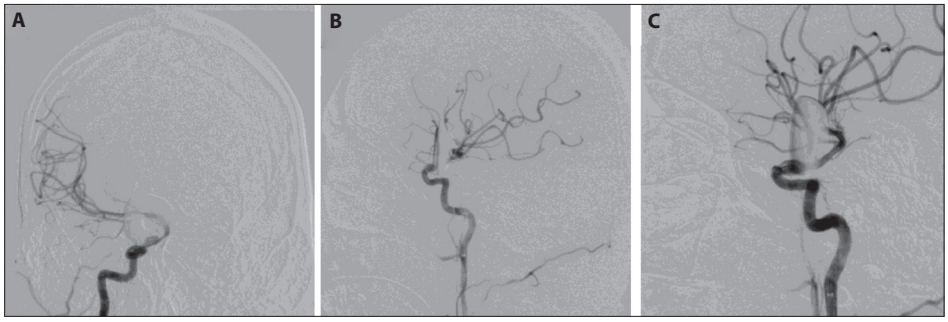

Figure 2. Post-procedural angiograms; (A) antero-posterior and (B) lateral position view of the right internal carotid artery show stasis of the contrast inside the aneurysm sac (grade B3 by O'Kelly–Marotta classification)

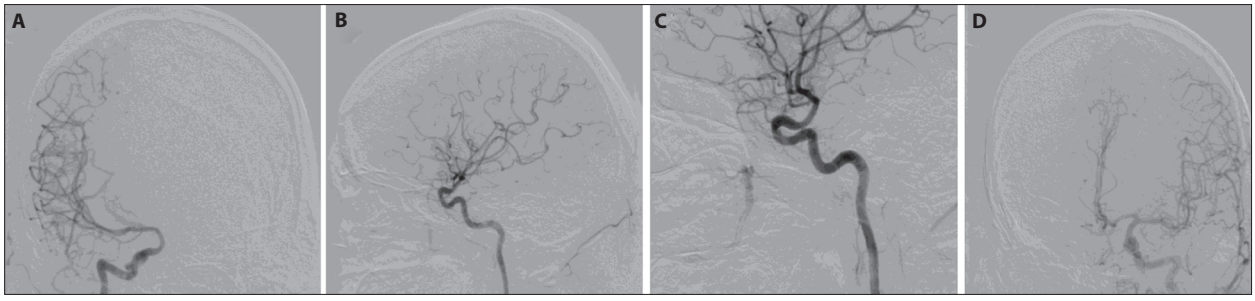

Figure 3. Control digital subtraction angiography after seven months; (A) antero-posterior, (B) lateral position view of the right internal carotid artery; there is no aneurysm filling; (C) antero-posterior view of the left ICA; A1 segment is falling to its normal state due to aneurysm shrinkage

eyes was stated. Control DSA was performed under conditions of local anesthesia on Monoplane Axiom Artis AX (Siemens, Munich, Germany). Selective right internal carodit artery catheterization was performed with 5F SIM2 diagnostic catheter (Terumo, Tokyo, Japan) and 7 ml of Omnipaque350 (GE healthcare, Chicago, IL, USA) contrast agent was administrated for angiograms, while for the 3D in space sequence we applied 12 ml of contrast in two-second intervals. Angiogram of the left carotid artery was taken from the common carotid artery (CCA) with 10 ml of contrast and with the application of digital compression on the right CCA. No communication between the sides and normal position of the left A1 segment was demonstrated, suggesting shrinkage of the aneurysm sac. Both ACAs were filled exclusively from the left ICA. Angiograms of the right carotid circulation performed selectively from the ICA showed that the aneurysm was completely excluded from the circulation with preserved patency of the parent blood vessel. Supraclinoidally, the right ICA still remained dislocated closer to the mediosagittal line, but was gradually falling back to its normal position.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

Phenomenon of "donut sign" or "donut aneurysm" was first described in 2014 by Van Rooij et al. [2]. This radiological sign occurs in aneurysms in which there is partial thrombosis inside the aneurismal sac and circular laminar flow between the aneurismal wall and the thrombus in its center. Consequently, there is a central contrast-filling defect of the aneurysm sac on different angiographical imaging methods. So far, this rarity has been described in

only several cases of ruptured and unruptured aneurysms, both in carotid and posterior circulation [3–6].

Several methods have been used in treating this type of aneurysm: microvascular dissection and clipping, or endovascular treatment (stent-assisted coiling, combined treatment with Woven EndoBridge and coils and FD stent in two cases) [2, 3, 4, 6]. Microsurgical direct-clipping giant aneurysms needs adequate craniotomy and visualization of the parent artery and its branches with minimal parenchymal retraction and minimal manipulation of the adjacent neurovascular structures. Giant aneurysms which have wide neck and complex anatomy of the surrounding vessels cannot be clipped directly. In these cases, it is necessary to use other therapeutical methods [7]. Proximal trapping (Hunterian ligation / proximal occlusion) is a relatively simple and well-established procedure technique that has been used in an attempt to divert flow away from the aneurysm and to induce thrombosis. Proximal trapping could be used only after the patient is able to successfully tolerate a balloon test occlusion (BTO). The risk of ischemic complications exists even in patients with negative BTO, and its rate is as high as 33% [8, 9]. Bypass after complete trapping can be done as a low-flow bypass (50 ml/min) or a high-flow bypass (> 50 ml/min), double-barrel bypass, and in situ bypass with grafts derived either from the radial artery or the saphenous vein. Hemorrhagic complications occur in 7.5% of patients treated with bypass (2.5% of patients treated with FD), and postoperative ischemic complications occur in 15% of FD and bypass groups respectively (5% of patients treated with FD). The rate of complete aneurysm occlusion at six months was 42.5% in the FD group and 95% in the surgical group ($p < 0.0001$), and early bypass thrombosis occurred in 15% [8]. Kiselov et al. [8] evaluated patients postoperatively with diffusion-weighted images to detect clinically silent ischemia. Brasiliense et al [10] reported silent ischemia after procedure in 62.7% cases.

Based on up-to-date experience, endovascular treatment has been proven to be the method of choice. In cases with large aneurysms with mass effect and partial thrombosis, recanalization after coiling is expected in a high percentage of cases [2, 11]. FD stents have proven to be an effective alternative to coiling, but they had several important disadvantages, such as a lack of immediate effect, need for antiplatelet therapy, and relatively long latency for aneurysm exclusion to take place [12, 13].

Evolution of the FD devices over the years have diminished the complication rates in recent years [13]. DED is a second-generation FD stent composed of 24 wires made of nitinol and radio-opaque platinum core that are folded back at the distal end, thus providing a network consisting of a total of 48 wires. At the proximal and distal end, there are three markers of iridium and platinum, for better visualization [14, 15]. These technical features result in improved radio-opacity and occlusion rate, as well as reduced incidence of adverse events [13, 16].

In our case, immediately after the implantation of the DED, the flow in the aneurysm sac was reduced, leading to graduate progressive aneurysm exclusion from the circulation and reduction of its compressive effect on nearby anatomical structures.

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Анеуризма унутрашње каротидне артерије по типу „крофне" третирана помоћу стента ДЕРИВО за преусмеравање протока

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САЖЕТАК

Увод Интракранијалне анеуризме са радиолошким знаком крофне су медицински приоритет и досад су описане у малом броју случајева. Овај радиолошки знак се јавља у анеуризмама где постоје парцијална тромбоза унутар анеуризматске вреће и циркуларни ламинарни ток крви између зида анеуризме и централног тромба. Последично, на различитим ангиографским визуелизационим методама постоји централни дефект у пуњењу анеуризме контрастним средством.

Приказ болесника Представљамо болесницу стару 35 година, примљену на испитивање због честих главобоља, визуелних сметњи на левом оку и губитка вида на десном оку. Дигитална суптракциона ангиографија приказала је анеуризму на десној унутрашњој каротидној артерији, која је мерила 25,6 × 25 *mm*, док је промер врата био 11 *mm* и обухватао је део сегмената Ц6 и Ц7. Одлучено је да би позиционирање стента са ефектом преусмеравања протока преко врата анеуризме било најбоље у овом случају. Након процедуре болесница је отпуштена непромењеног општег стања. Седам месеци након интервенције болесница је примљена на прву контролну дигиталну суптракциону ангиографију. Леви сегмент А1 се вратио у нормалну позицију, сугеришући смежуравање анеуризматске вреће. Такође, болесница је навела побољшање вида на оба ока.

Закључак Приказујемо болесницу са анеуризмом облика крофне на десној унутрашњој каротидној артерији, успешно третираној стентом за преусмеравање протока.

Кључне речи: анеуризма по типу „крофне"; стент ДЕРИВО; дигитална суптракциона ангиографија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Idiopathic granulomatous mastitis – new approach in operative treatment

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SUMMARY

Introduction Idiopathic granulomatous mastitis (GM) is described as a very rare, non-lactating, chronic mastitis that occurs primarily in women of childbearing age. Significant clinical problem related to GM is the diagnostic differentiation from breast cancer. Less advanced forms of GM can be successfully treated with limited surgical excisions and radical treatment is recommended only for the most extensive forms.

Case report First examination of the patient, by the surgeon at Oncology Institute of Vojvodina was in December 2018, when initial suspicion of breast cancer was set up. Core needle biopsy was performed and after histopathological (HP) analysis, confirmation of GM was obtained. The patient was initially offered Prednisone and Methotrexate therapy, which she refused and accepted only surgical treatment. Surgical treatment was performed few weeks after needle biopsy and consisted of performing a nipple spearing mastectomy with excision of the orifices of all fistulous ducts and their primary sutures. The HP findings of the operative specimen confirmed the diagnosis of GM. While there were no signs of dieses relapse, patient was suggested secondary reconstruction of the left breast. Twelve months after the primary operation, secondary breast reconstruction was performed with the interposition of a contoured silicone implant into a muscle pocket in a standard manner.

Conclusion Nipple spearing mastectomy with secondary breast reconstruction is esthetically satisfactory treatment for patients with locally advanced GM.

Keywords: idiopathic granulomatous mastitis; nipple spearing mastectomy; secondary breast reconstruction

INTRODUCTION

Idiopathic granulomatous mastitis (GM) is described as non-lactating chronic mastitis that occurs primarily in women of childbearing age, but could be found in patients many years after breast feeding. Kessler and Wolloch [1] have first described this rare illness in 1972. Since the clinical and radiological imaging of GM are similar to the breast cancer, it could lead to misdiagnosis before the final histopathological (HP) diagnosis.

Many authors suggested that possible etiology of GM include breast infection with microbes, autoimmune disorders and hyperprolactinemia [2]. Uncertain etiology leads to non-optimal treatment for GM and included watch and wait strategy, antibiotics, steroids and surgery as earliest and most widely used therapy [3–8]. In cases when GM is localized in only one part of the breast, local excision is used and radical treatment is recommended only for the most extensive forms, when all breast tissue is involved [2, 3, 5].

CASE REPORT

First examination of the patient by the surgeon at the Oncology Institute of Vojvodina was in December 2018, when initial suspicion of breast cancer was set up. In history patient has one child (nine years old), non-smoker, with a negative family history of breast cancer. Ultrasound findings supplied by the patient are characterized as BI RADS 5 in the left breast. On clinical examination in the left breast, at the border of the lateral quadrants a tumor mass, about 5 cm in diameter, was palpated, which protrudes and deforms the skin of the breast with three active fistulous ducts (two located in upper medial quadrant and one periareolar in the same quadrant) from which the clear secretion is conducted (Figure 1).

Core needle biopsy was performed a few days after the clinical examination and HP analysis of the specimen, which confirmed the GM diagnosis (Figure 2).

After a core needle biopsy, a few more fistulas were produced (in upper lateral quadrant), with pronounced inflammation of the left breast skin, and *per os* antibiotics (augmentin + metronidazole) were included in the therapy. The inflammatory process affected almost all of the breast tissue except the area just below the nipple areola complex.

After the inflammatory process calming down and spontaneous closing of the fistulous ducts in upper lateral quadrant, the patient performed tests for tuberculosis, sarcoidosis and Wegener's granulomatosis, and all of them were negative. Smears taken from fistulous ducts

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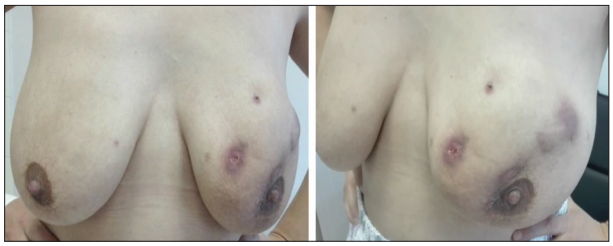

Figure 1. Clinical findings one day before the operation; three active fistulous ducts; palpable tumor masses in lateral quadrants of the left breast (approximately 5 cm in diameter)

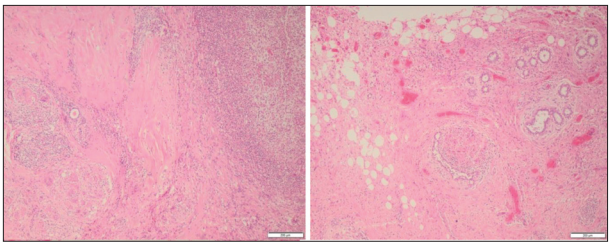

Figure 2. Histopathological findings of granulomatous mastitis; A – preserved lobular architecture of the mammary gland (lower left) and an abscess rimmed by granulomatous inflammatory infiltrate (upper right); hematoxylin and eosin, 20 ×; B – a granuloma containing numerous giant cells protruding into a duct; hematoxylin and eosin, 20 ×

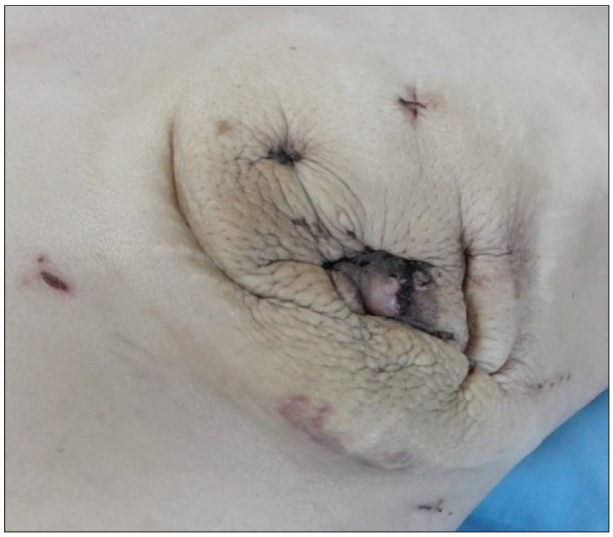

Figure 3. Clinical findings after the surgery and removing the drains; stitches on the sites from excided fistulous canals; two incisions on the border of the breast and abdominal skin are from drains; vital skin flap, after performing nipple spearing mastectomy with nipple areola complex preservation

were negative on bacteria and fungi. The patient was initially offered Prednisone and Methotrexate therapy, with the presentation of all possible positive and negative effects of the proposed therapy, which she refused and accepted only surgical treatment.

Surgical treatment was performed few weeks after the HP conformation of the diagnosis and consisted of performing a nipple areola complex spearing mastectomy with excision of the orifices of all fistulous ducts and their primary closing with adsorptive stitches (Vicryl 5-0, Ethicon, Ethicon Inc., Raritan, NJ, USA). Intraoperative, many clear collections of 2–5 centimeters in diameter were found within the breast tissue, with a pronounced inflammatory component, which blended with the surrounding healthy breast tissue. The operative wound was drained with two 21 Gauge drains. Operative wound (radial incision on the border of lateral quadrants) is closed with adsorptive stitches (Vicryl 5-0).

Antibiotic was not used in postoperative treatment while there were no signs of operative wound infection.

After reducing the secretion on the drain bags, and removing the drains, the patient was discharged from hospital for further home treatment. Local findings after the operation are shown on Figure 3. Definitive HP analysis of operative specimen confirmed GM (Figure 2).

After the operation, the patient was regularly monitored by the surgeon on a monthly basis.

Since there were no signs of disease relapse (12 months after primary operation), the patient was suggested secondary reconstruction of the left breast with silicone breast implants. Postoperative breast ultrasound finding was without signs of disease in the left breast.

Secondary breast reconstruction was performed with the interposition of a contoured 530 cc silicone breast implant (Mentor Medical Systems B.V, Leiden, Netherlands) into a muscle pocket made of a large pectoralis mayor and serratus anterior muscle in a standard manner with liberation of breast skin from muscles.

One year after the secondary operation there are no signs of local relapses and patient is very satisfied with esthetical results (Figure 4).

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

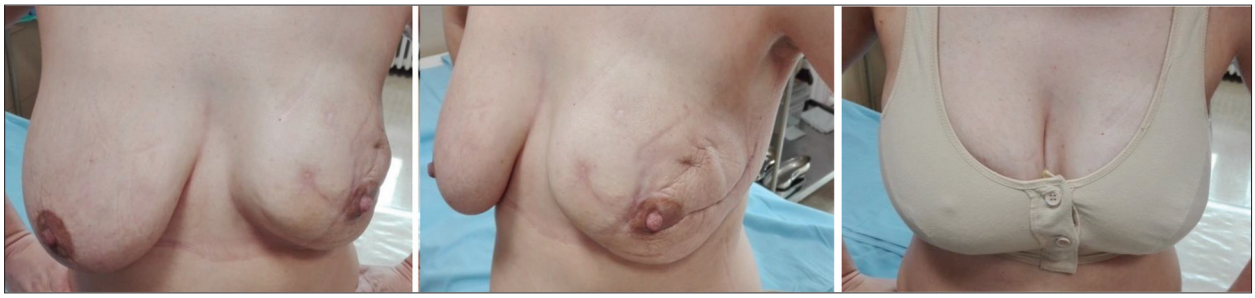

Figure 4. Clinical findings after the secondary reconstruction using silicone breast implants; postoperative clinical finding one month after the operation; reconstructed breast is smaller than the healthy one; in this operative treatment, we have not made contralateral breast reduction since the patient has shown no interest in performing that procedure

DISCUSSION

According to our findings, this was the first case of secondary breast reconstruction using contoured silicone implants in GM treatment.

Both surgical and nonsurgical (antibiotic therapy, oral steroids, observation) treatments have been advocated as the first-line treatments of GM [3–8]. Because the clinical and imaging features of GM are very similar to those of breast carcinoma, tissue biopsy remains the gold standard to confirm the diagnosis [9].

Li [10] believes that GM is self-limiting disease with good prognosis and suggests that conservative management with close surveillance would be the best treatment modality. In his study, 50% of the patients had spontaneous complete resolution of disease after 14.5 months and did not relapse. When compared to other therapeutically modalities, observation therapy has the longest recovery time causing physical and emotional pain [8, 10].

GM is mostly represented like breast infection with large abscesses and antibiotics (a combination of amoxicillin and metronidazole) are usually used, but many studies have shown no benefit of this therapeutic model [6, 9, 11]. Many authors have found that GM is in some cases related to anaerobic Corynebacterium infection [12. 13, 14].

Some researchers believe that the pathogenesis of GM may be an autoimmune response to the secretion of mammary ductal proteins, so they use steroids to treat GM and some positive results have been achieved [10, 15]. Complete clinical and radiological regression was observed in 63% of the patients when using methylprednisolone (0.5 mg/kg/day for four weeks) but with high recurrence rate (31%) and longer recovery time [15]. Steroid therapy also has a notable problem that it may have side effects at high doses. Immunosuppressive therapy is recommended for patients who have relapsed after steroid therapy and have steroid resistance or unbearable side effects, but the therapeutic effect of methotrexate remains unclear [6]. Otherwise, best recovery time is shown after the use of methylprednisolone and surgery (one month) versus using only steroid therapy (six months) ($p = 0.001$) [16, 17].

In terms of recurrence and post-treatment recovery, surgery has been one of the main treatments since GM was first reported, and studies including surgical resection as a first-line treatment showed significantly superior results compared with steroid therapy alone [3, 5, 6, 16, 17]. Possible surgical treatments include breast conserving surgery and mastectomy (depending on the size of involved breast tissue with GM [3, 5, 6, 16, 17]. Like nonsurgical treatment options, surgery, as well, has some disadvantages, primarily bad postoperative aesthetic effect and loss of breastfeeding. After the breast conservative surgery, recurrence rate of GM is 10%, and after the mastectomy 0% [6].

Performing nipple spearing mastectomy with secondary breast reconstruction using silicone breast implants is aesthetically satisfactory treatment for patients with locally advanced GM. Further studies are needed to confirm this hypothesis.

Conflict of interest: None declared.

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Идиопатски грануломатозни маститис – нов приступ хируршком лечењу

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САЖЕТАК

Увод Идиопатски грануломатозни маститис (ГМ) изузетно је ретка врста хроничне упале дојке која се обично јавља код жена након порођаја. Значајан диференцијално дијагностички проблем код ГМ је то што клинички имитира карцином дојке. Мање форме ГМ успешно се могу третирати ограниченим ексцизијама, а радикални третман препоручен је само у случајевима екстензивних форми које захватају већи део ткива дојке.

Приказ болесника Први преглед болеснице од стране хирурга на Институту за онкологију Војводине био је у децембру 2018. године, када је постављена иницијална сумња на постојање карцинома леве дојке, с обзиром на клинички налаз. Урађена је иглена биопсија туморске масе, а патохистолошки налаз говорио је у прилог ГМ. Болесници је иницијално понуђена терапија преднизоном и метотрексатом, коју је она одбила и прихватила једино оперативни третман. Операција је учињена неколико недеља након иглене биопсије, када је изведена супкутана мастектомија са презервацијом комплекса ареоле и мамиле уз ексцизију свих отвора фистулозних канала и њихову примарну сутуру. Патохистолошки налаз оперативног материјала потврдио је дијагнозу ГМ. С обзиром на то да није дошло до релапса болести након 12 месеци од примарне операције, болесници је предложено да се изведе секундарна реконструкција леве дојке. Изведена је контурираним силиконским имплантом који је пласиран у мишићни џеп на стандардан начин.

Закључак Супкутана мастектомија уз секундарну реконструкцију дојке естетски је прихватљив третман код болесница са локално узнапредовалим обликом ГМ.

Кључне речи: идиопатски грануломатозни маститис; супкутана мастектомија; секундарна реконструкција дојке

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Implementation of accelerated partial breast irradiation at the Oncology Institute of Vojvodina

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SUMMARY

Introduction Early breast cancer is usually treated with breast conserving surgery followed by radiation treatment. Whole breast irradiation is standard of care so far, but currently there is an increasement in accelerated partial breast irradiation for selected patients which showed many advantages. The aim of this paper is to present the implementation of the accelerated partial breast irradiation in Oncology Institute of Vojvodina.

Case outline A 54-year-old woman was referred to radiotherapy after breast conserving surgery. After she met all of the inclusion criteria, she underwent accelerated partial breast irradiation with 38.5 Gy in 10 fractions. Active breathing control device was used during the treatment and cone beam computed tomography was performed before each fraction for the purpose of target position control. She terminated therapy in good health condition with only adverse effect of mild radiation dermatitis of irradiated area. On the first follow up, she was without any symptom or sign of disease or complication.

Conclusion Accelerated partial breast irradiation is safe and effective. Radiation oncologist should be encouraged to implement this technique.

Keywords: breast cancer; radiation therapy; accelerated partial breast irradiation

INTRODUCTION

In EU countries incidence of breast cancer is 109.8/100,000 per year and mortality rate is 38.4/100,000. Serbia has incidence rate of 60.8/100,000, and each year there are 4000–4600 new cases diagnosed [1]. Early breast cancer is usually treated with breast conserving surgery (BCS) followed by radiotherapy. Whole breast irradiation (WBI) is commonly given in 5–6 weeks and 45–50 Gy is delivered. Boost dose (10–16 Gy) is given to the tumor bed in most patients, after many studies have confirmed its benefit [2]. Although it is well established that radiation therapy (RT) after BCS decreases local recurrence and improves overall survival, in practice we are faced with the fact that patients are discouraged from long treatment duration and there are many logistical issues: distance from RT facility, lack of beds in RT units, lack of transportation, social care issues, etc. [3]. For all mentioned, an interest to shorten treatment duration was born.

Accelerated partial breast irradiation (APBI) is a type of RT when radiation fractions are given more than once per day and it's based on the fact that the most of tumor recurrences are at or near the tumor bed. Patient selection should be strict: histology of invasive ductal carcinoma, size $\leq$ 2 cm (T1), over 50 years old, negative surgical margins $\geq$ 2 mm, no lymphovascular invasion, positive hormonal receptor status and BReast CAncer gene negative [4, 5, 6].

The main goal of this paper is to present external beam RT technique of APBI through the presentation of the first case of this kind performed at the Oncology Institute of Vojvodina.

CASE REPORT

A 54-year-old woman was referred to a radiologist for regular annual breast examination at the Oncology Institute of Vojvodina. In the low-medial quadrant of the left breast ultrasound examination revealed an impalpable BI RADS 4 lesion and CORE biopsy was performed. Histology showed invasive ductal carcinoma, no other specification type, grade 3 and, after the tumor board review, the patient underwent BCS. Definitive histology was invasive ductal carcinoma, grade 2, pT1bN0 without lymphovascular invasion, hormone receptor positive and HER 2 negative, Ki-67 was 30%. five clips were placed in tumor bed. The patient decided to decline adjuvant chemotherapy and continue with anastrozole and RT as the tumor board recommended. She fulfilled all of the criteria for APBI and after signing written consent she started with preparation for external beam RT. For the reason that tumor was left-sided, medially located to be more precise, it was decided to use Active Breathing Control device (ABC, Elekta Crawley, Crawley, UK) during the treatment (Figure 1).

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Figure 1. Active breathing control device (ABC, manufacturer Elekta Crawley, Crawley, UK)

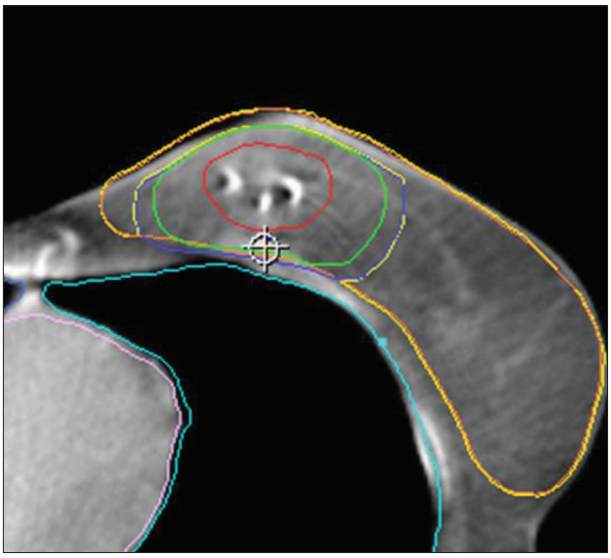

Figure 2. Target structures for accelerated partial breast irradiation: surgical cavity (red), clinical target volume (green), planning target volume (blue)

She was scanned in supination with breast immobilizing device (Wing-board, Civco Medical Instruments Co Inc., Orange City, IO, USA). Eighty percent of maximum inhale volume was used as the reference line for further radiotherapy daily treatment fractions. First step in delineation was to define surgical cavity which includes surgical clips and change in surrounding tissues. Clinical target volume (CTV) was created as expansion of 15 mm around the surgical cavity and planning target volume was created by adding a 5 mm margin to the CTV (Figure 2).

Skin, lungs, heart, and contralateral breast were contoured as organs at risk. Dose prescription was 38.5 Gy in five days (two daily fractions of 3.85 Gy) [3]. Treatment planning (volumetric arc therapy) was performed by treatment planning system Monaco v 5.11 (Elekta Crawley) by two medical physicist and dose-volume histograms was analyzed together with two radiation oncologists. All of the organs at risk received radiation doses within tolerance limits [4] (Figure 3).

Treatment was image-guided, cone beam computed tomography (CT) was performed before each fraction to ensure that target position was correct. Matching was done for bone structures and for soft tissue separately for every fraction. Up to 5 mm shift was allowed for target position and correction was automatically performed by the software XVI (Elekta Crawley), installed on the accelerator. During the treatment, radiation dermatitis grade I occurred at the third fraction in the irradiated area and afterwards the patient treated the affected skin with emulsions. Erythema persisted until the end of the treatment. Performance status of the patient was ECOG 1 (Eastern Cooperative Oncology Group) during entire treatment, and she did not experience any other complication. Two weeks after the end of therapy, irradiated breast skin was completely healed, the patient was feeling well without any

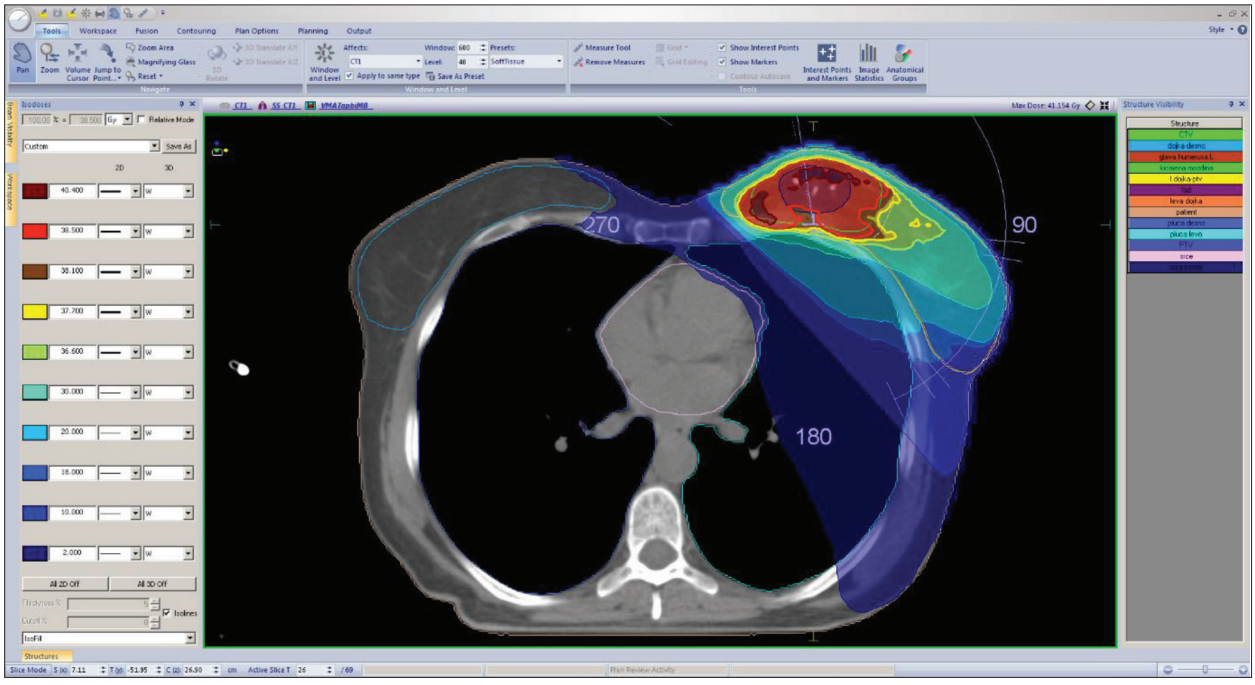

Figure 3. Dose distribution for breast and surrounding structures

health disturbances. At first follow-up, two months after radiotherapy termination, she was feeling well, without any signs of disease or complications.

DISCUSSION

During the past decades, breast cancer radiotherapy moved towards reducing treatment duration. First, START A and START B studies have showed that post-lumpectomy RT in duration of three weeks is safe and effective [7, 8]. Second, good cosmetic results were evident. These findings enabled other radiation treatment schemes. Interstitial brachytherapy was the first developed APBI technique [9]. After positive results of long term follow up studies, this technique was accepted in experienced centers as comparable to WBI in terms of efficacy and toxicity [9, 10]. Furthermore, there is a novel approach to delivery of APBI – image guided breast brachytherapy that maintains a high level of precision by using breast immobilization via breast compression and image guidance [11]. Irradiation of tumor bed immediately after surgical procedure was investigated in TARGIT A and ELIOT study [13, 14]. Due to the controversies of some aspects of these studies, intraoperative RT is not currently widely accepted.

Baglan was the first to initially describe external beam radiotherapy based APBI [14]. It can be performed as "simple" 3D RT, intensity modulated radiotherapy, volumetric arc therapy, with photons, electrons or as proton therapy. External beam APBI technique starts with identification of tumor localization before BCS inside the breast and translating this information into current imaging data set [15]. Total safety margin from tumor in all six directions should be at least 2 cm. For tumor delineation, first step is to define surgical cavity on CT scans, which includes surgical clips and change in surrounding tissues or tumor cavity according to ultrasound or magnetic resonance imaging, and second step is delineation [16, 17]. In the presented case, surgical cavity was defined using visible five clipses on CT scans.

The main advantage of external beam RT APBI is that it is non-invasive, the treatment does not depend on manual skills of the staff that performs therapy and quality assurance issues are simpler compared to brachytherapy. Dose homogeneity is better compared to brachytherapy and balloon catheter techniques. On the other hand, defining surgical cavity is a potential problem and substantial inter-observer variability of CTV delineation have been observed [18]. Surgical clips and tissue density should be main guides for delineation and to avoid inappropriate contouring. Indications for APBI changed over time, ASTRO and GEC-ESTRO recommendation were adopted in most countries, although different oncological associations come up with different selection criteria [5, 6]. Multicentric cancer makes the patient unsuitable for APBI and defining the risk for multicentric disease is essential to avoid patient selection bias. For elderly with early breast cancer APBI is a very attractive treatment option, considering the complexity of patient transport, associated comorbidities, etc. [18, 19].

Long-term outcomes of APBI were investigated by recent OCOG-RAPID and NSABP B-39/RTOG0413 trials [20, 21, 22]. Both trials demonstrated non-inferiority of APBI compared with WBI in terms of ipsilateral breast tumor recurrence rate [20, 21, 22]. These results were confirmed in Florence III trial as well [23]. In OCOG-RAPID trial acute toxicity was reduced in APBI group but late toxicity and breast cosmesis were worse. Results of other conducted trials showed that the cosmetic outcome is better in the APBI group as compared to the WBI group [25]. On the other hand, telangiectasia and mild breast fibrosis are significantly higher in the APBI group although the fibrosis related to APBI is low grade and limited to the tumor bed and does not significantly affect overall cosmetics. Actually, published randomized controlled trials have shown inconsistent outcomes [24]. Recent meta-analysis has shown that APBI compared to WBI has similar toxicity side effects and cosmetic effects [25]. Further studies are needed to confirm these findings.

Pandemic of COVID-19 virus also forced radiotherapy centers worldwide to implement shorter treatment schedules with the goal to minimize exposure for both patients and health care providers. In the light of that, APBI is a desirable option for selected patients, without inferiority in overall survival and local control of breast cancer patients [26].

In conclusion, it needs to be emphasized that APBI is a cost-effective technique. Treatment costs are reduced and the patient gets back to their normal activities sooner. Radiation oncologist should be encouraged to implement this technique, especially in low- and middle-income countries with limited resources.

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Имплементација акцелерисане парцијалне ирадијације дојке у Институту за онкологију Војводине

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САЖЕТАК

Увод Поштедна операција дојке уз радиотерапију је стандардни приступ у лечењу раног карцинома дојке. До сада је најчешће била примењивана ирадијација целе дојке, међутим, све више је у употреби акцелерисана парцијална ирадијација, која је показала бројне предности код селектованих пацијената.

Циљ овог рада је приказ имплементација акцелерисане парцијалне ирадијације дојке у Институту за онкологију Војводине.

Приказ болесника Код 54-годишње жене је индикована радиотерапија дојке након поштедне операције. С обзиром на то да је испунила све критеријуме, одлучено је да се спроведе акцелерисана парцијална ирадијација дојке са дозом 38,5 греја у 10 фракција. У току радиотерапије свакодневно је коришћена активна контрола дисања помоћу уређаја и компјутеризована томографија купастим пољем ради контроле позиције мете. Болесница је завршила терапију у добром општем стању, са јединим нежељеним ефектом у виду благог радијационог дерматитиса ирадиране регије. На првој контроли није имала ниједан симптом или знак болести, нити компликацију.

Закључак Акцелерисана парцијална ирадијација дојке је безбедна и ефикасна. Радијационим онколозима се препоручује имплементација ове технике.

Кључне речи: карцином дојке; радиотерапија; акцелерисана парцијална ирадијација дојке

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Gluten-related disorders

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SUMMARY
Gluten-related disorders are a heterogeneous group of clinical entities caused by intolerance to wheat, rye, and barley flour components. They occur in 3–5% of genetically predisposed persons and, based on pathogenic and clinical features, are classified into celiac disease, non-celiac gluten sensitivity, and wheat allergy. There are also specific entities such as dermatitis herpetiformis or gluten ataxia, which can occur either within the celiac disease or independently. This article based on the current knowledge shows the basic details of the pathogenesis, clinical expression, diagnosis, and treatment of these disorders.
Keywords: celiac disease; wheat allergy; non-celiac gluten sensitivity; gluten ataxia; dermatitis herpetiformis

INTRODUCTION

Gluten-related disorders (GRDs) cover a group of heterogeneous immune-mediated clinical conditions triggered by the ingestion of wheat, rye, and barley flour [1, 2, 3]. GRDs are in second place among the most frequent food intolerances. They affect about 3–5% of the genetically predisposed human population [4–7]. Classification of GRDs based on variations in pathogenesis and clinical expression identifies celiac disease, non-celiac gluten sensitivity, and wheat allergy [1–4, 8]. As a specific manifestation of the celiac disease or specific clinical entities within the GRDs, gluten ataxia and dermatitis herpetiformis are separated [1, 8–13]. Underlying the pathogenesis of the celiac disease, dermatitis herpetiformis, and gluten ataxia is the immune system's response to gluten intake, IgE-mediated and/or non-IgE-mediated immune response in wheat allergy, and stimulation of the innate immunity with direct cytotoxic effects of gluten and some non-immunological mechanisms in non-celiac gluten sensitivity [1–4, 8, 14]. The basis of the treatment of GRDs is a gluten-free diet [1–4, 8, 15].

CELIAC DISEASE

Celiac disease (CD) is a lifelong systemic autoimmune disorder induced by gliadin and related prolamins of wheat, rye, and barley [1, 16, 17]. Prevalence is about 1% of the general population of European, North African, Indian, and Middle Eastern origin with appropriate genetic foundation [18]. The frequency of CD is more common among close relatives of the diseased, especially those of the first line (~10%) and in patients with other autoimmune diseases (3–10%), such as diabetes mellitus type I, autoimmune thyroiditis, Sjögren's syndrome, Addison's disease, autoimmune liver diseases, juvenile idiopathic arthritis, myasthenia gravis, systemic lupus erythematosus, psoriasis, dilated cardiomyopathy, and others [4, 19]. Multiple-major prevalence of the CD is also recorded in the IgA selective deficit, as well as in Down, Turner, and Williams syndrome [20, 21].

Gluten-sensitive enteropathy is one of the most common findings in patients with CD. This type of enteropathy is nonspecific, affects the small intestine and recedes after switching to a gluten-free diet. Enteropathy in patients with CD can be symptomatic and asymptomatic, and various extraintestinal manifestations and complications are possible [16, 17, 22].

There is no doubt that there is a significant influence of genetic factors in the appearance of CD and its hereditary nature. HLA class II genes have the central role, but it is almost certain that there is influence of other gene loci as well [1, 23]. HLA DQ2 haplotype is identified in about 90% and HLA DQ8 haplotype in about 10% of patients with CD [23, 24, 25]. However, the disease expression, besides

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genetic predisposition and exposure to gluten, also requires the influence of other external factors [1, 23, 26]. Gliadin peptide hydrolysate binds to the HLA class II glycoproteins on antigen-presenting cells (APC) inducing the activation of intestinal CD4+ T-lymphocytes which, by secreting pro-inflammatory cytokines, lead to inflammation of the small intestinal mucosa [23]. This process is preceded by tissue transglutaminase-mediated deamination of the glutamine residue of gliadin hydrolysate, which increases the affinity of their bonding with HLA DQ2 and DQ8 molecules [23]. Humoral immunity also plays a significant role in the pathogenesis of CD, which is confirmed by the presence of autoantibodies to reticulin, endomysium, tissue transglutaminase, and other body structures [22, 23]. The duodenum and the proximal part of the jejunum are most often affected by changes [23]. According to the modified Marsh criteria, inflammation of the small intestinal mucosa is classified into infiltrative, infiltrative-hyperplastic, and destructive [27]. Destructive enteropathy is additionally classified into partial, subtotal, and total.

The symptomatic form of the disease has two modes of clinical presentation (classical and non-classical) and is less frequent compared to the asymptomatic form [1]. In the classical clinical presentation, chronic diarrhea, malabsorption, and secondary malnutrition are most often observed, while in the non-classical form, extraintestinal manifestations such as isolated hypertransaminasemia, constipation, iron deficiency anemia, chronic fatigue, abdominal pain, aphthous stomatitis, short stature and delayed puberty, infertility, enamel hypoplasia, decreased bone density (osteopenia or osteoporosis), polyneuropathy, alopecia, epilepsy, depression, anxiety, and others are most common [1, 17, 23, 28, 29]. Newborns and young children usually have the classic clinical form of the disease, unlike older children and adults. [23]. In CD diagnosed too late or treated inadequately, very serious complications are possible, such as "cell crisis" in children of the youngest age, i.e., total gastrointestinal insufficiency followed by severe hydro-electrolytic and nutritive disbalance, or T-cell small intestinal lymphoma, intestinal adenocarcinoma, ulcerative jejunoileitis and refractory sprue in adults [23, 30].

The main way to diagnose CD is enterobiopsy with subsequent pathohistological analysis of the small intestinal mucosa [31]. According to the latest recommendations of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) defined in 2010, this procedure is necessary not only in patients with symptoms and/or signs corresponding to CD, but also with present IgA antibodies to tissue transglutaminase (AtTG) above 100 U/ml, positive anti-endomysial antibodies and "celiac HLA" (DQ2 and/or DQ8) [32, 33]. The diagnosis of CD could be verified by the clinical recovery and AtTG disappearance after introducing a gluten-free diet [16]. Such attitude in the CD diagnostics is based not only on high sensitivity and specificity of IgA AtTG as a serological marker of the disease (> 95%) but also on a highly significant correlation of their titer with the degree of small intestinal mucosa damage, as well as on the almost unavoidable (> 98%) presence of HLA DQ2 and/or DQ8 [16]. Serological indicators of the disease, such as autoantibodies to endomysium and tissue transglutaminase, and antibodies to deaminated gliadin peptides, have high sensitivity and specificity, but not an absolute diagnostic validity [16]. Therefore, they are primarily used in the detection of asymptomatic and atypical forms of the disease, as well as in the assessment of the consistency of elimination diet in cases with the already verified disease [32]. The latest ESPGHAN criteria recommend gluten provocation test and subsequentional patohistological analysis for confirmation or exclusion of CD in cases where gluten-free diet was introduced before enterobiopsy, in cases where patohistological findings were not typical, and in cases where samples for patohistological analysis were too small or inadequate for final decision. This procedure should not be conducted before the sixth year of life and during puberty because of the negative influence on permanent teeth and growth [16, 31].

Because CD is a lifelong disorder, the gluten-free diet is the foundation of successful treatment [1, 16, 33]. Some additional treatment such as supplementation of iron and folates as well as other vitamins and microelements could be required especially during the initial phase of treatment. Restriction of lactose intake may also be required in some patients [34]. Semi-elementary and/or additional parenteral nutrition, edema removal, and stabilization of the water–salt balance could be required in patients with severe forms of the disease. Sometimes, short-term glucocorticoid therapy is used [35].

GLUTEN ATAXIA

Gluten ataxia (GA) is one of the most frequent and serious gluten-induced autoimmune neurological diseases with clinical presentation mainly in the middle and late adult age [1, 9, 10]. It occurs as a result of damage to the cerebellum as one, and sometimes the only manifestation of the CD [8, 36]. Gluten-induced ataxia also occurs in the absence of CD [37, 38]. Antibody cross-reactivity may be one of the mechanisms involved in GA pathogenesis because of the similarity between gluten proteins and antigenic epitopes on Purkinje cells [36]. Diagnostic delay leads to an irreversible loss of Purkinje cells, followed by permanent neurological damage [8, 39]. GA is most often manifested with dysarthria, pyramidal dysfunction, gait problems, limb ataxia, pyramidal dysfunction, altered eye motion, progressive loss of stability, and inability to stand straight [8, 9]. Less than 10% of GA patients have gastrointestinal symptoms and about one-half have small intestinal histology compatible with CD [39]. Immunoassays of patients with GA show positive IgA and/or IgG antigliadin antibodies, presence of antibodies to TTG2 (from the gut), and TTG6 (from brain tissue) [8]. In most patients at the time of diagnosis, brain magnetic resonance revealed cerebellar atrophy [8, 9]. GA therapy involves a rigorous gluten-free diet throughout life, which reduces disability and prevents further progression of the disease [8, 9, 39]. When the diet does not give satisfactory results, immunosuppressive drugs can be used [37].

DERMATITIS HERPETIFORMIS

Dermatitis herpetiformis (DH) is an autoimmune skin disease that usually occurs within CD and manifests with blistering rash and cutaneous IgA deposits. It may present at any age, but it predominantly presents in middle-aged persons [1, 13, 25]. It was found to be more frequent in men than in women, contrary to other autoimmune diseases, but some recent studies point out that sex imbalance may reduce with incising age, and may not be so profound [40]. Also, an Italian large study revealed interesting findings of the high prevalence of DH within the pediatric population, which is usually underreported [13]. DH is characterized by cutaneous lesions that are polymorphic in nature, relapsing and itching, localized on the face, shoulders, knees, elbows, buttocks, and sacral region. Typical CD HLA haplotypes (DQ2 and DQ8) are usual for patients with DH. Although the gastrointestinal symptoms in DH are rare and mild, a higher number of intraepithelial lymphocytes and celiac-type intestinal atrophy are present in almost 70% of patients with apparently normal biopsy findings. Tissue transglutaminase (TTG2) specific autoantibodies could be found in the small bowel mucosa and serum of patients with DH and CD, but skin biopsy in DH shows typical TTG3-(epidermal transglutaminase) targeted IgA antibodies [13]. Strict and timely introduced lifelong gluten-free diet alleviates cutaneous symptoms, improves enteropathy symptoms, and minimizes the risk for complications, especially small intestinal B-cell lymphoma [17]. For the same patients, Dapson helps ease itching and controls the development of cutaneous lesions [17, 41].

WHEAT ALLERGY

Wheat allergy (WA) is IgE-mediated and/or non-IgE-mediated allergic adverse reaction to wheat proteins. According to clinical manifestation, WA can be divided into (1) classical form of food allergy with the involvement of gastrointestinal tract, skin, and possibly respiratory tract, (2) inhalant allergy (Baker's asthma and rhinitis), (3) wheat-dependent, exercise-induced anaphylaxis (WDEIA), and (4) contact urticaria. In Europe, the reported prevalence of WA is 3.6% for all ages [8].

WA is characterized by the following symptoms: skin rash, wheezing, itching, and swelling in the mouth, nose, eyes, and throat (typical IgE-mediated allergy symptoms), gastrointestinal symptoms like cramps, bloating, diarrhea, but WA can sometimes manifest itself with anaphylactic shock. Allergens that most commonly trigger WDEIA are alfa-amylase inhibitors and a subtype of grain protein, ω-5 gliadin [8, 42].

In establishing WA on wheat proteins can be measured by specific IgE antibodies and skin reactions. An oral provocation test is usually required to confirm the diagnosis since the level of specific IgE antibodies and skin reactions exhibit low specificity and sensitivity. A double-blind placebo-controlled trial is considered a gold standard for the diagnosis of food allergy but, in clinical practice, the reduction in symptoms associated with a wheat-free diet indicates that WA is present. In those who have exhibited wheat-induced anaphylaxis, oral provocation should never be done [8, 14].

Usually, patients with WA are not allergic to other cereal prolamines in rye, barley, and oats, and their diet is less restrictive than that in CD. Elimination of wheat products is the basis of treatment of WA. In children with predominant gastrointestinal manifestations, as with other food allergies, 75% of wheat tolerance develops in adolescence [14, 17].

NON-CELIAC GLUTEN SENSITIVITY

In the past decade, non-celiac gluten sensitivity (NCGS) has received growing attention. Studies report that prevalence ranges 0.63–6% due to the challenging diagnosis [4, 42, 43]. The diagnosis of NCGS is established when CD and WA are excluded, and the same improvement in extraintestinal and gastrointestinal symptoms is observed after the introduction of a gluten-free diet [1]. Also, symptoms display significant overlap with irritable bowel syndrome. Although CD and NSGS may have similar symptoms, a careful retrospective investigation revealed that there are some differences in the clinical presentation between NCGS patients and CD patients. nutrient deficiency, malabsorptive symptoms, and autoimmune diseases are rarer in NCGS patients than in CD patients. Abdominal discomfort, bloating, meteorism, diarrhea, 'foggy mind,' fatigue, headache, and joint pain are most commonly reported as symptoms in patients with NCGS [4]. The pathogenesis of the disease is not clear, but some explanations include the activation of the innate immune response and mediation of the immunological system [44]. Regarding genetic susceptibility, HLA-DQ2 and HLA-DQ8 haplotypes could be found in half of the patients with NCGS, which is lower than that in CD patients, but not as low as in the general population [45]. NCGS frequently occurs in parents, siblings, and children of patients with CD, predominantly in females [46]. Serological tests show that more than half of the patients with NCGS have anti-gliadin antibodies (AGA) IgG antibodies in circulation, which disappear after gluten withdrawal. In clinical practice, physicians have to be aware that AGA IgG is not a reliable serologic marker for NSGS because it can be detected in various autoimmune diseases, and among healthy people as well [47]. Although gluten and other protein components of grain cereals (α-amylase trypsin inhibitor) can be responsible, they are not the only ones that could cause symptoms. Some grains and cereals are rich in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), which can cause gastrointestinal symptoms as well. Intake of food with low levels of FODMAPs can lead to a significant reduction in symptoms even if gluten has not been eliminated from the diet. Although groceries without gluten often have low levels of FODMAPs, patients recognized as NCGS suffer from irritable bowel syndrome with symptoms resulting from consuming FODMAPs rather than gluten [17].

For now, the diagnosis is still based only on the exclusion of CD and WA in addition to the double-blind placebo-controlled study, which is ideal but not always possible in clinical practice [17]. Starting a gluten-free diet establishes improvement in most patients, but the diet does not need to be strict, because inadvertently introduced traces of gluten usually do not cause symptoms [4, 8]. It is still unknown whether NCGS is a permanent condition in all patients [48, 49, 50].

CONCLUSION

GRDs make a heterogeneous group of clinical entities caused by genetically determined intolerance to wheat, rye, and barley flour components. After an adult form of lactose intolerance, GRDs are the most common food-related disorder. The pathogenesis of CD, GA, and DH is based on the gluten-activated autoimmune process, of WA on IgE- and/or non-IgE-mediated reaction to gluten and other proteins of said cereals, while NCGS is the result of stimulation of the innate immunity and some non-immunological mechanisms. The basis of treatment of GRDs is gluten-free diet. The condition is lifelong for the autoimmune forms of the disorders; WA, especially if it occurs in the youngest age, is mostly transitory. For now, it is not clear whether all patients with NCGS must be on lifelong gluten-free diet.

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Поремећаји везани за глутен

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САЖЕТАК

Поремећаји везани за глутен су хетерогена група клинички ентитета који настају услед неподношења компонената пшеничног, јечменог и раженог брашна и испољавају се обично код генетски предиспонираних особа. На основу патогенетских и клиничких карактеристика класификују се на целијачну болест, алергију на пшенично брашно и нецелијачну осетљивост на глутен. Као специфични ентитети, присутни у оквиру целијачне болести или без ње, издвајају се глутенска атаксија и херпетиформни дерматитис. У овом чланку, базираном на савременим сазнањима, приказани су основни детаљи везани за патогенезу, клиничку експресију, дијагностику и терапију ових поремећаја.

Кључне речи: целијачна болест; глутенска атаксија; херпетиформни дерматитис; алергија на пшенично брашно; нецелијачна глутенска сензитивност

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CURRENT TOPIC • АКТУЕЛНА ТЕМА

Predictors of response to BCG therapy in non-muscle invasive bladder cancer

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SUMMARY
Intravesical BCG (*Bacillus Calmette–Guerin*) therapy represents the therapy of choice for intermediary- and high-risk non-muscle invasive bladder cancers after transurethral resection. However, up to 40% of these patients do not show adequate response to the therapy (BCG failure) and 15% of them experience the progression of the disease to muscle-invasive bladder cancer. In such cases, radical cystectomy is indicated. Studies suggest that early radical cystectomy in patients with BCG failure is followed by better survival compared to delayed radical cystectomy. The prediction of response to BCG therapy could enable early identification of patients on which this therapy would have no effect and who should undergo early radical cystectomy.
Keywords: bladder cancer; intravesical BCG therapy; BCG failure; radical cystectomy

INTRODUCTION

Non-muscle invasive bladder cancers (NMIBC) are malignant urothelial bladder cancers that do not invade the detrusor. NMIBC are divided into pathological subcategories – Ta and Tis (which are limited to urothelium) and T1 (which invades lamina propria) [1]. After transurethral resection of bladder tumor (TURBT), T1 and Tis tumors are much more likely to cause recurrence or disease progression.

In order to assess the individual risk for progression of the disease, several scoring systems and tables of risk have been developed [2]. The choice of the adequate modality of treatment is made on the basis of risk groups. For the low-risk tumors, it is recommended to use one dose of intravesical chemotherapy immediately after TURBT. For the intermediate-and high-risk tumors, it is recommended to use adjuvant BCG intravesical therapy after TURBT, during the period of 1–3 years, depending on the level of risk [3].

Up to 40% of patients with intermediate- and high-risk NMIBC do not show adequate response (absence of refractory disease after the first or the second induction cycle of BCG) to the therapy (BCG failure) and 15% of patients experience the progression of the disease to muscle-invasive bladder cancer [4]. Early radical cystectomy is indicated for patients who did not show the adequate response to BCG therapy. Ninety-two percent of patients with BCG failure reach two-year survival if radical cystectomy is performed in a period shorter than two years since the beginning of BCG therapy. If radical cystectomy is performed later than two years since the beginning of BCG treatment, two-year survival rate falls down to only 56% [5]. Since the postponement of radical cystectomy is accompanied by significantly lower survival rate, the proper identification of patients who have a high risk of BCG failure is crucially important.

UNSUCCESSFUL RESPONSE TO INTRAVESICAL BCG THERAPY – BCG FAILURE

BCG failure is defined as recurrence or persistence of urinary bladder carcinoma of high-grade during or after BCG therapy [3]. The appearance of low-grade relapses during or after BCG therapy does not represent a BCG failure. Having in mind the heterogeneity of bladder tumors that show BCG failure, they are further classified into three specific types: BCG-refractory, BCG-relapse, and BCG-intolerant.

BCG-refractory condition is defined as the impossibility of achieving disease-free state or as persistence of high-grade urinary bladder carcinoma six months after the initiation of the adequate BCG treatment [6].

BCG-relapse tumors represent high-grade tumors which reappear after the disease-free status is attained, upon the completion of BCG therapy. BCG-relapse failure may be sub-classified as early (during the first 12 months from the initiation of BCG therapy), intermediary (12–24 months from the initiation of therapy), and late (after more than 24 months) [3].

BCG-intolerant state represents the persistence of the disease when adequate BCG therapy cannot be applied because of its side effects and complications [6].

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BCG-unresponsive tumors encompass all BCG-refractory and some BCG-relapse tumors (TaT1/HG tumors which reappear in the period of six months from the completion of BCG therapy or the appearance of *carcinoma in situ* (CIS) in the period of 12 months from the completion of BCG therapy). BCG-unresponsive patients are a sub-group of patients with the highest risk of recidivism and disease progression, so the continued administration of BCG therapy would have no effect on them [6].

PREDICTORS OF RESPONSE TO BCG IMMUNOTHERAPY

Predictors of response can be divided into clinical-pathological, molecular, and genetic. Studies of interrelation between clinical-pathological factors and disease progression/recurrence risks enabled the creation of a scoring system for disease progression and recurrence risk assessment. European Association of Urology (EAU) NMIBC 2021 scoring model enables risk assessment only in relation to disease progression, but not in relation to recurrence [2]. On the other hand, Club Urológico Español de Tratamiento Oncológico (CUETO) prediction model enables both progression and recurrence prediction after 12 intravesical BCG instillations, administered in the period of five to six months after TURBT. This model incorporates seven clinical-pathological parameters: T stage (Ta, T1), grade (G1, G2, G3), number of tumors (< 3, > 3), concurrent carcinoma *in situ*, presence of recurrent tumors, age (< 60, 60–70, > 70), sex [7]. In comparison to the European Organisation for Research and Treatment of Cancer (EORTC) model, recurrence risk assessed on the basis of 2006 CUETO model (based on six clinical-pathological factors) is lower for all risk groups, while the risk of progression is lower only for high-risk patients. This points to the protective effect of BCG therapy. Additional studies confirmed that CUETO model is more accurate, although it has been noted that both models overestimate the progression and recurrence risk for high-risk patients [7, 8]. Seeing that EORTC prediction model is based upon the results of the trials where the patients were predominantly treated with intravesical chemotherapy, its reliability for the prediction of BCG-failure is limited. Conversely, the shortcoming of the CUETO model is the fact that it is based upon the results of the trials where the patients were predominantly treated with adjuvant BCG therapy over the period of one year. For that reason, this model does not allow for a clear prediction of response to BCG therapy in high-risk patients, since they should receive BCG therapy during three years [9].

Many authors studied immunomodulatory effects of sex hormones and their influence on the effect of BCG therapy. The studies upon which the CUETO model is based have shown that female sex represents an important recurrence predictor [8].

A trial from 2007, which included 805 patients with high-grade Ta, T1, or CIS treated with intravesical BCG therapy, showed gradual attenuation of therapy response connected with age [10]. Despite this, BCG shows higher efficacy than intravesical chemotherapy for patients older than 70 years with TaT1 tumors of intermediary and high risk [11].

Ferro et al. [12] detected that obesity is significantly connected with an increased risk of recurrence (hazard ratio, HR: 5.33) and progression (HR: 2.52) of disease in patients with T1G3 tumors.

Smoking has a proven immunosuppressive effect, due to which it can represent one of the additional factors suspected of having an impact on the effect of intravesical BCG therapy [13].

In a study from 2020, De Jong et al. [14] examined the connection between sub-staging T1 and the occurrence of BCG failure. In relation to the degree of invasion of lamina propria, T1 tumors are divided into two groups – microinvasive and extensively invasive. Extensive invasion of lamina propria was statistically significantly related to the occurrence of BCG failure ($p = 0.002$) [14]. In a study by Herr et al. [15], residual T1 tumors were detected in 26% of patients after a second-look TURBT (indicated for high-risk tumors four to six weeks from the initial resection). Despite the administration of BCG therapy, disease progression to muscle invasive bladder cancer was detected in 82% of patients from this group. These data imply that for a certain number of patients with high-grade T1 tumors and residual T1 tumors after a second-look TURB an early cystectomy is a better therapeutic strategy than BCG therapy and other bladder-sparing strategies [16].

Inflammatory response triggered by the cellular immune system is the basis of anti-tumor effect of BCG immunotherapy [16]. Several authors assumed that the determination of a patient's capability to generate an adequate immune response would be an important predictor of response to BCG therapy. Multivariate analysis showed that interleukin-2 (IL-2) level in urine represents an independent prognostic factor for the occurrence of response to BCG therapy [17]. In a study authored by Saint et al. [18], patients with urinary IL-2 concentration below 27 pg/µmol of creatinine after the induction cycle of BCG therapy had a statistically significantly higher risk of recurrence in comparison to the patients with higher values ($p = 0.0009$). Kaempfer et al. [19] analyzed the expression of genes for IL-2 in mononuclear cells of peripheral blood during BCG therapy, by analyzing the appearance of IL-2 messenger RNA (mRNA). By comparing the patients with remission and with relapse of the disease, they noticed a statistically significantly higher level of IL-2 mRNA induction in patients with remission ($p = 0.0001$). Multivariate analysis showed that IL-2 mRNA induction has also been associated with the prolonged disease-free period ($p = 0.0001$) [19]. IL-8 is also a potential biomarker of response to BCG therapy. A study from 2017 showed with statistical significance that recurrence-free survival (RFS) was shorter in patients with higher levels of IL-8 in comparison with patients who had lower levels of this cytokine (14 months *vs.* > 78.4 months, $p = 0.004$) [20]. Programmed death-ligand 1 (PD-L1) is a transmembrane protein which can be expressed on the surface of a tumor and tumor-infiltrating immune

cells. Patients with CIS treated with adjuvant BCG therapy display an increased expression of PD-L1 in the group of BCG non-responders in comparison to BCG responders (p = 0.035; OR: 0.1204; CI 95%: 0.0147–1.023) [21]. The proteins of the cell cycle (TP53, retinoblastoma protein, and Ki-67) have also been analyzed as potential immunohistochemical predictors of response to BCG therapy. A meta-analyses from 2016 and 2018 showed the connection of the TP53 and Ki-67 hyperexpression with the disease progression in NMIBC patients [22].

Genetic polymorphisms may influence the response on intravesical BCG therapy. Polymorphisms of nucleotide excision repair genes, characterized by the appearance of variant alleles *XPA*, *XPC, ERCC6, XRCC1*, and *ERCC2*, are associated with the decreased RFS in patients who underwent BCG therapy [23]. A systemic review from 2016 points out that polymorphisms of IL-6 and IL-4 genes are associated with an increased risk of recurrence during BCG therapy, while polymorphisms of IL-8 and tumor necrosis factor-α genes are statistically associated with the decreased risk of recurrence [24]. Decobert et al. [25] point out that the appearance of two variant alleles of the natural resistance-associated macrophage protein gene (*NRAMP-1*) is statistically significantly associated with the decreased RFS in patients who received BCG therapy. Although the detection of genetic polymorphisms has significant potential for predicting response to BCG therapy, these results should be further confirmed.

CONCLUSION

The prediction of response to BCG therapy could enable a better selection of patients for this type of therapy, as well as the early identification of patients on which this therapy would have no effect and who should undergo early radical cystectomy. Clinical practice showed that, most commonly used, clinical-pathological parameters are often insufficient in relation to the prediction of response to BCG therapy. Therefore, a large number of studies are now focusing on molecular and genetic predictors. Cytokines, among which IL-2 in particular, and also the studies with immunohistochemical markers (in particular PD-L1/PD-1) show promising results. Further research and inclusion of new clinical-pathological predictors, as well as larger studies on molecular and genetic markers, are necessary in the future.

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Предиктори одговора на *BCG* терапију код карцинома мокраћне бешике без захватања мишићног слоја

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САЖЕТАК

Интравезикална *BCG* (*Bacillus Calmette–Guerin*) имунотерапија представља терапију избора код карцинома мокраћне бешике без захватања мишићног слоја умереног и високог ризика после трансуретралне ресекције. Међутим, код чак 40% ових болесника изостаје одговор на *BCG* терапију (енгл. *BCG failure*), а код 15% њих долази до прогресије болести у карцином мокраћне бешике са захватањем мишићног слоја. Код болесника који нису одговорили на *BCG* терапију индикована је радикална цистектомија. Примена ране радикалне цистектомије код ових болесника праћена је бољим преживљавањем у односу на одложену радикалну цистектомију. Предикција одговора на *BCG* терапију би омогућила рану идентификацију болесника код којих она не би имала ефекта и који би захтевали примену ране радикалне цистектомије.

Кључне речи: карцином мокраћне бешике; интравезикална *BCG* терапија; изостанак одговора на *BCG*; радикална цистектомија

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови**,** наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

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

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

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

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

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

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

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

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

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

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

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

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

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

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

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

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

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

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

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

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

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

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

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

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