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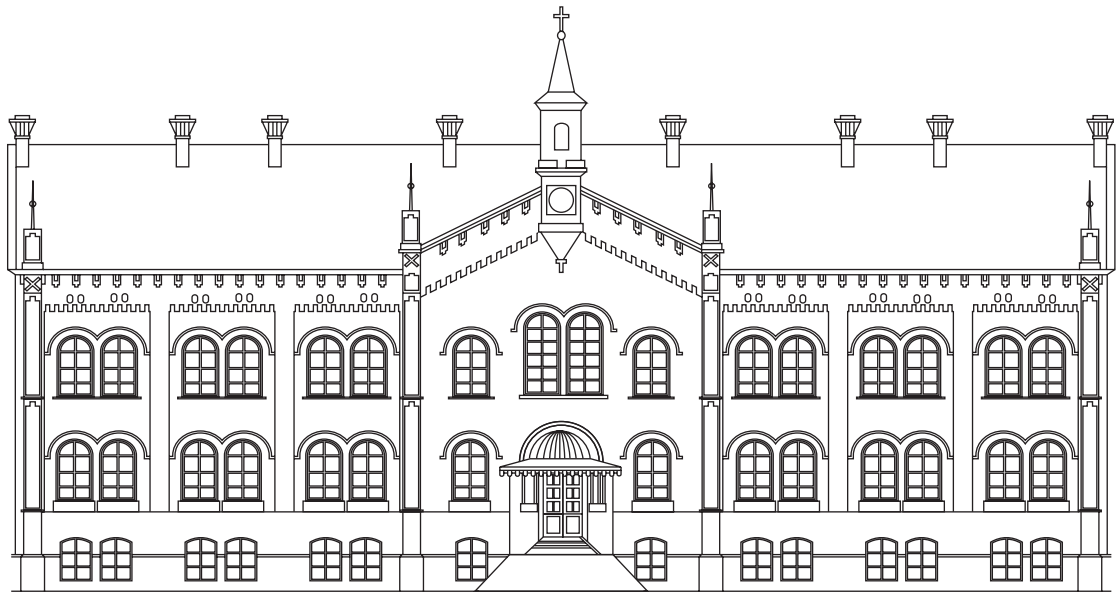

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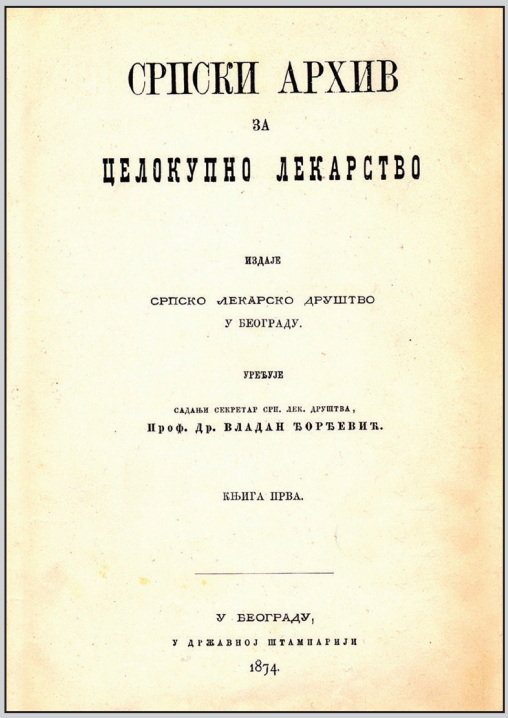

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

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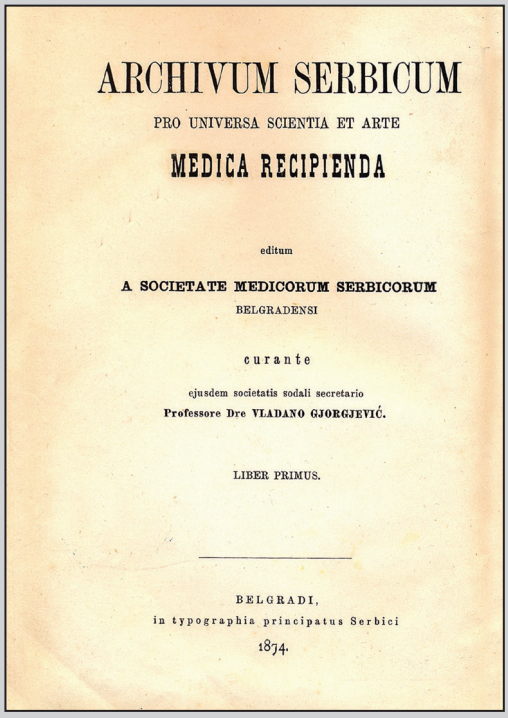
КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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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 151 ЈАНУАР–ФЕБРУАР 2023. СВЕСКА 1–2

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EDITORIAL / УВОДНИК

Physicians are humans and replaceable – the current millennium approach

Irrelevant of the challenges we face, at the beginning of each new year, we are confident it will be a better and more successful in every way. And it's simply human to feel that way. Standing on the shoulders of so many generations of healthcare workers in Serbia and worldwide and what their experience taught us over the past 150 years in Serbia [1, 2, 3], we embark on this year's adventures with nonetheless zeal. Since the beginning of the COVID-19 pandemic and its changing Serbia's health sector landscape since the early 2020, each new year brings new surprises [4, 5, 6].

First and foremost, we have to thank our reviewers for the time invested in our journal, despite their everyday clinical workload. These hours of unpaid work have been taken from their families, their loved ones and their own sleep (Table 1). This is the third year in a row that we try to rank them and familiarize you with their faces, for their investment that counts the most in the preservation of our journal. In 2022, the first place by number of reviews are shared between Professors Ljubomir Todorović (Figure 1) and Predrag Vučinić (Figure 2), the second Professors Dragoš Stojanović (Figure 3) and Jelena Milašin (Figure 4) and the third, Professors Nataša Rajković (Figure 5) and Nebojša Stojanović (Figure 6).

Both our doctors and our reviewers have shown remarkable dedication over the years, and the COVID-19 pandemic keeps draining them even more, as new strains of the virus appear [4]. In sign of support, our doctors used to receive applause each evening from the balconies since spring 2020, both in Belgrade and across Serbia. That standing ovation was a worldwide phenomenon. But then, we got tired, while they tirelessly – even when falling victims to COVID-19 themselves – kept standing up from their own sickbeds and kept fighting for the lives of their patients, until they were reinfected again. And that is what they do today. Not only our doctors, but our nurses and all allied healthcare professions that our healthcare system is based on. The merciless first wave of the COVID-19 pandemic took lives of so many of them in a devastating way [5, 7].

Historical sources claim doctors used to be revered as much as Gods [8], but how and when people substituted the admiration for aggression remains to be explained by historians, psychologist, philosophers and the unavoidable economists. The attacks on healthcare workers were already well-described long before the COVID-19 pandemic [9, 10], and it's making our colleagues across the Atlantic even teach new trainees not to carry their stethoscopes in the traditional around-the-neck fashion, not to facilitate a potential attack. The growing hospital violence is discouraging the healthcare sector, while the use of easily accessible social media is enabling the voicing dissatisfaction with the government.

The changes in the economy landscape due to COVID-19 pandemic is influencing the decision of pharmacoeconomists to further cut costs across healthcare field, as it is known as the notorious "big spender". Understaffing across all spectrums of jobs in healthcare has become the "new normal", where emergency departments (EDs) replace their doctors with nonphysician practitioners. Nevertheless, it physiologically creates the chain reaction that, for instance, creates "frequent fliers" – term already used for frequently hospitalized

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Figure 1. Professor Ljubomir Todorović (University of Belgrade, School of Dental Medicine, Belgrade, Serbia)

Figure 2. Professor Predrag Vučinić (University of Novi Sad, Faculty of Medicine, Department of Dentistry, Novi Sad, Serbia)

Figure 3. Professor Dragoš Stojanović (University of Belgrade, Faculty of Medicine, University Clinical Centre Zemun, Belgrade, Serbia)

Figure 4. Professor Jelena Milašin (University of Belgrade, School of Dental Medicine, Department of Human Genetics, Belgrade, Serbia)

Figure 5. Professor Nataša Rajković (University of Belgrade, Faculty of Medicine, University Clinical Centre of Serbia, Clinic for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia)

Figure 6. Professor Nebojša Stanković (Retired colonel, Military Medical Academy, Belgrade, Serbia)

end-stage heart failure patients necessitating repeated hospitalizations and imaging, thus increasing the costs in these very same EDs [11]. Even if we ignore the cost of this futile imaging, it is yet to be determined whether employment of nonphysician practitioner instead of medical doctors generates these requests for imaging, while the greater concern has to be for the patients and the radiation burden they increase, potentially aggravating their own long-term health.

The ongoing COVID-19 pandemic might mimic the turmoil the generations of our ancestors lived during the World War I and World War II, but, if so, when did we start to leave our sick and wounded on the battle field instead of carry them to safety and give them the time they need to heal and recover? In a society that promotes alienation, superficial virtual contact and borderline minimal education, where the children of today mandate how to be taught while their teachers should stay mum, it is on the healthcare sector to regroup and protect their own, for, apparently, no one else will.

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Table 1. The list of the Serbian Archives of Medicine reviewers in 2022

1. Aleksić Dubravka
2. Alempijević Đorđe
3. Antonijević Nebojša
4. Atanasijević Tatjana
5. Bančević Vladimir
6. Barać Aleksandra
7. Barišić Goran
8. Baskić Dejan
9. Bašić Dragoslav
10. Belojević Goran
11. Bila Jelena
12. Bila Jovan
13. Bilanović Dragoljub
14. Blagojević Duška
15. Bokun Jelena
16. Bošnjak Roman
17. Božić Marija
18. Brkić Zlata
19. Budić Ivana
20. Bumbaširević Uroš
21. Bumbaširević Vladimir
22. Cekovska Svetlana
23. Cerić Timur
24. Crnogorac Snežana
25. Cvetković Slobodan
26. Čolić Miodrag
27. Čolić Snježana
28. Čolović Radoje
29. Čupić Maja
30. Dačić Krnjaja Bojana
31. Damjanov Nemanja
32. Danilović Vesna
33. Dimitrijević Aleksandar
34. Dimitrijević Milovan
35. Doklestić Krstina
36. Dokmanović Lidija
37. Dubljanin Raspopović Emilija
38. Dučić Siniša
39. Dugonjić Sanja
40. Dulić Oliver
41. Džamić Zoran
42. Đanić Hadžibegović Ana
43. Đerić Dragoslava
44. Đikanović Bosiljka
45. Đukanović Ljubica
46. Glišić Branislav
47. Glišić Branislava
48. Gnjatić Mirjana
49. Gojnić Miroslava
50. Golubović Zoran
51. Golušin Zoran
52. Grujičić Danica
53. Habek Mario
54. Ilić Dragan
55. Ilijevski Nenad
56. Ille Tatjana
57. Ivanov Igor
58. Ivanović Mirjana
59. Janić Dragana
60. Janković Radmila
61. Janošević Predrag
62. Janjić Bojan
63. Javaid Kassim
64. Jeremić Jelena
65. Jotić Aleksandra
66. Jotić Ana
67. Jovanović Dragan
68. Jovanović Gordana
69. Jovanović Ivan
70. Jovanović Jovica
71. Jovanović Mladen
72. Jovanović Predrag
73. Jovanović Simić Jelena
74. Jovanović Tanja
75. Jović Rajko
76. Kadija Marko
77. Kalezić Nevena
78. Kalezić Tanja
79. Kinov Plamen
80. Klibanov Alexander
81. Knežević Aleksandra
82. Knežević Srbislav
83. Končar Igor
84. Kos Marina
85. Kovačević Igor
86. Kozić Duško
87. Kozomara Ružica
88. Kravić Stevović Tamara
89. Krejović Trivić Sanja
90. Krstić Miodrag
91. Krstić Zoran
92. Krstovski Nada
93. Kuzmanović Miloš
94. Lacroix Richard
95. Lalić Nensi
96. Lalošević Dušan
97. Lazović Milica
98. Lešić Aleksandar
99. Ležaić Višnja
100. Lovrenčić Huzjan Arijana
101. Lukić Ljiljana
102. Maksimović Nataša
103. Maliković Aleksandar
104. Mandić Stojmenović Gorana
105. Mandinić Zoran
106. Manojlović Radovan
107. Manojlović Slavko
108. Marić Dragana
109. Marić Nebojša
110. Marić Vesna
111. Marisavljević Dragomir
112. Marković Aleksa
113. Marković Evgenija
114. Marković Nikolić Nataša
115. Marković Roberta
116. Mayer Miroslav
117. Mazicioglu Mümtaz
118. Medić Deana
119. Micev Marjan
120. Mihailović Zoran
121. Mihalj Marija
122. Mijač Dragana
123. Mijović Romana
124. Mikić Aleksandar
125. Stojičić Milan
126. Milašin Jelena
127. Milašinović Marić Dijana
128. Milenković Branislava
129. Milenković Pavle
130. Miličić Miroslav
131. Milinčić Nemanja
132. Milisavljević Milan

133. Milošević Ivan
134. Milovanović Jovica
135. Milovanović Srđan
136. Misirlić Denčić Sonja
137. Mitković Marija
138. Mitković Milan
139. Mladenović Jasmina
140. Nakaš Enita
141. Nedeljković Ivana
142. Nedeljković Milan
143. Nedeljković Nenad
144. Nestorović Dragoslav
145. Nešković Vojislava
146. Nikitović Marina
147. Nikolić Dimitrije
148. Nikolić Đurović Marina
149. Nikolić Igor
150. Nikolić Ivan
151. Nikolić Jakoba Nataša
152. Nikolić Jelena
153. Nikolić Predrag
154. Nikolić Živorad
155. Obradović Đuričić Kosovka
156. Obrenović Kirćanski Biljana
157. Odalović Božidar
158. Opačić Galić Vanja
159. Ostojić Predrag
160. Ostojić Slavica
161. Palibrk Ivan
162. Pantić Igor
163. Parapid Biljana
164. Parezanović Vojislav
165. Pavićević Polina
166. Pavlović Milorad
167. Pavlović Zorana
168. Peco Antić Amira
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175. Petrović Bojan
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184. Poskurica Mileta
185. Potić Jelena
186. Prcić Sonja
187. Predojević Jelica
188. Prijić Sergej
189. Pugliatti Maura
190. Radević Tatjana
191. Radlović Nedeljko
192. Radlović Vladimir
193. Radojičić Aleksandra
194. Radosavljević Aleksandra
195. Radosavljević Davorin
196. Radovanović Drakče
197. Radovanović Zoran
198. Radulović Danilo
199. Radunović Goran
200. Rajković Nataša
201. Rasulić Lukas
202. Reddy Krishna
203. Resan Mirko
204. Risimić Dijana
205. Ristić Aleksandar
206. Ristić Arsen
207. Ristić Gorica
208. Roganović Jelena
209. Romić Predrag
210. Rožman Primož
211. Sahinli Hayriye
212. Saibene Alberto
213. Santrač Nada
214. Sarajlija Adrijan
215. Savić Aleksandar
216. Savić Đorđe
217. Savić Slobodan
218. Sekulić Ana
219. Sengul Ilker
220. Sgourida Maria
221. Siller Matula Jolanta
222. Simić Tatjana
223. Sinđić Antunović Sanja
224. Spiroski Igor
225. Srdić Galić Biljana
226. Srećković Sunčica
227. Staletović Danijela
228. Stamenković Bojana
229. Stamenković Dragoslav
230. Stamenković Miroslav
231. Stamenković Željka
232. Stanković Nebojša
233. Stanojlović Svetlana
234. Stefanović Neda
235. Stević Marija
236. Stojanović Dragoš
237. Stojanović Ivan
238. Stojanović Miroslav
239. Stojanović Roksanda
240. Stojčev Stajčić Ljiljana
241. Stojimirović Biljana
242. Stojković Filipović Jelena
243. Stojšić Milosavljević Anastazija
244. Stošović Rajica
245. Svetel Marina
246. Šarenac Tatjana
247. Šćepan Ivana
248. Šijački Ana
249. Škiljević Dušan
250. Škodrić Trifunović Vesna
251. Šušak Stamenko
252. Šušnjar Snežana
253. Teofilovski Parapid Gordana
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257. Todorović Milena
258. Tomašević Todorović Snežana
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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison of Delta and Omicron variant of COVID-19 infection cases in Montenegro

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SUMMARY

Introduction/Objective At the end of 2021, Omicron wave (B.1.1.529) SARS-CoV-2 variant superseded the Delta variant (B.1.617). The main goal of the research is to provide a detailed and comprehensive presentation of data related to people infected with the coronavirus in Montenegro. The specific goal of the research is to determine whether virus mutations influenced the course of the epidemic during its two-year duration.

Methods This is a retrospective study. We used data from the Institute of Public Health of Montenegro. Our sample consisted of 127,134 people who tested positive for Delta or Omicron infection aged 0–100 years, who had a positive PCR test for COVID-19 between August 17, 2021 and April 17, 2022.

Results The respondents aged 40–49 years were taken as a reference group for age. The results showed that the age group from 20 to 29 years old was affected 1.03 times more than the reference group – persons belonging to the age group of 30–39 years were affected 1.07 times more than the reference group. The Central region was taken as the reference group for the region. The results showed that people who live in southern region got sick 1.14 times more often and people who live in northern region got sick 1.20 times less than people from the central region.

Conclusion The biggest predictor that a person would get sick is the age group. Also, the predictor is the region, and in our research, it was southern region.

Keywords: COVID-19; SARS-CoV-2; age; Delta and Omicron strain; Montenegro

INTRODUCTION

At the end of 2020 the Delta variant (B.1.617.2) was discovered in India for the first time. In June 2021, the World Health Organization stated that the Delta would become the most prevalent strain in the world [1]. The SARS-CoV-2 Delta VOC is 40–60% more transmissible than the Alpha (B.1.1.7) [2].

The World Health Organization marked the variant B.1.1.529, commonly known as Omicron, on November 26, 2021 as variant of interest. The Omicron variant is the most divergent strain seen in significant numbers so far during the pandemic, raising concerns that it may be linked to greater transmissibility, lower vaccine efficiency, and an increased risk of reinfection. The Omicron SARS-CoV-2 variation is more transmissible than the Delta variant [3].

The World Health Organization also reported that the Omicron variant has a growth advantage with a doubling time of 2–3 days compared with the Delta variant, which may provide evidence that transmission capacity of the Omicron variant was stronger than Delta [4]. According to the available evidence, the incubation period is shorter for Omicron variant [5].

METHODS

We used data from the Institute of Public Health of Montenegro from the beginning of pandemic until April 17, 2022. Our sample consisted of 127,134 persons who tested positive for Delta or Omicron infection aged 0–100 years who had a positive PCR test for COVID-19 between August 17 and April 17 2022. Period of Delta variant was August 17 until December 21, 2021 and period for Omicron variant was December 22, 2021 until April 17, 2022. In accordance with the Emergency Care Data Set and World Health Organization protocols, a genome sequencing method was performed on samples that were suspicious for the presence of the Delta and Omicron variant, which confirmed the presence of each strain. In relation to the share of positive cases in the number of tested persons, an approximation was made of share of strains. Observation period was between August 17, and April 17, 2022.

In this paper, we used the χ^2 test of independence and logistic regression. The χ^2 test of independence provides information not only on the significance of observed differences, but also on detailed information on exactly which categories account for any differences found. Logistic regression was conducted to assess the impact of multiple factors on the likelihood of contracting COVID-19. The model contains

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three independent categorical variables sex, region, and age. The whole model with all predictors was statistically significant $p < 0.00$. A given set of variables explains 12–17% of the variance. The model correctly classifies 58.7% of all cases. Montenegro is divided into three regions: southern, central, and northern region. For purpose of this paper, we have chosen the central region as reference region. Also, the age group from 40 to 49 years was used as the reference age for the purposes of the paper.

The objective of the research is to determine, on the basis of the available data, how the epidemic in Montenegro moved in relation to virus mutations. In addition, specific goal is to determine how the epidemic moved in relation to Delta and Omicron mutations, which are disease rates in the population with special emphasis on age.

All data are properly named and paper represents analysis of datasets. We had approval from the Institute of Public Health of Montenegro to use the database. Also, corresponding author is a member of the Database team in Institute of Public Health of Montenegro. Thus, the research has been done in accord with the ethical standards of the institution.

RESULTS

During the period in which the Delta strain was dominant, a total of 373,813 cases were tested in Montenegro, with 54,402 new cases and 14.55% share of positive tests. In the period when the Omicron strain was dominant, the number of new cases was 72,732 (25.56%) out of total 284,540 tested (Table 1).

Table 1. Distribution of new cases, number of tested and percentage of positive cases in tested for the Delta/Omicron period

Period	New cases	Tested	%
Delta	54,402	373,813	14.55
Omicron	72,732	284,540	25.56

As shown in Tables 2 and 3, the highest number of new cases was found in the age category 30–39 in Delta strain 9746 (17.91%) as well as in Omicron strain 14,931 (20.53%).

As presented in Table 4, the χ^2 test of independence showed a link between sex and the incidence of Delta strain and Omicron strain. Men were statistically significantly more affected by the Delta strain, and women statistically significantly more by the Omicron strain, C^2 (n = 127,726) = 0.07, p = 0.01 p < 005; fi = 0.07 with a very small influence of sex on getting sick from Delta and Omicron strains.

Table 5 presents an analysis of the association of disease by Delta and Omicron strains by region in Montenegro. The χ^2 test of independence determined relation between regions and incidence of Delta and Omicron strains. The northern region was statistically significantly more affected by Delta strain compared to the central and southern regions, while the southern region was statistically significantly more affected by Omicron strain compared to the central and northern regions, with a very large influence of the region on the incidence of Delta and Omicron strains C^2 (n = 127,726) = 0.51; p = 0.00 p < 0.05, fi = 0.51 (Table 5).

Relation between the age groups and incidence of Delta and Omicron strains was also determined by the χ^2 test of independence. The age group from 20 to 29 years was statistically significantly more affected by the Delta strain compared to the other age groups, while the age group from 30 to 39 years was statistically significantly more

Table 2. Distribution of new cases in relation to age and sex – Delta

Age	Male	%	Female	%	Total	%
0–9	1250	4.98	1193	4.07	2443	4.49
10–19	3258	12.99	3286	11.21	6544	12.03
20–29	3296	13.14	3867	13.19	7163	13.17
30–39	4336	17.29	5410	18.45	9746	17.91
40–49	4033	16.08	5009	17.08	9042	16.62
50–59	3355	13.38	4030	13.74	7385	13.57
60–69	3059	12.20	3450	11.77	6509	11.96
70–79	1693	6.75	2023	6.90	3716	6.83
80–89	641	2.56	893	3.05	1534	2.82
90+	158	0.63	162	0.55	320	0.59
Total	25,079	100%	29,323	100%	54,402	100%

Table 3. Distribution of new cases in relation to age and sex – Omicron

Age	Male	%	Female	%	Total	%
0–9	1515	4.58	1248	3.15	2763	3.80%
10–19	2369	7.17	2427	6.12	4796	6.59%
20–29	4726	14.3	5791	14.6	10,517	14.46%
30–39	6629	20.05	8302	20.93	14,931	20.53%
40–49	5708	17.27	7135	17.98	12,843	17.66%
50–59	4594	13.9	5823	14.68	10,417	14.32%
60–69	4261	12.89	4973	12.53	9234	12.70%
70–79	2193	6.63	2545	6.41	4738	6.51%
80–89	814	2.46	1126	2.84	1940	2.67%
90+	251	0.76	305	0.77	556	0.76%
Total	33,060	100	39,675	100	72,735	100

Table 4. Characteristics of the Delta cohort and Omicron cohort among male and female patients; sex has been associated with both infection risk and severe outcomes of SARS-CoV-2 infections; men were statistically significantly more affected by the Delta strain, and women statistically significantly more by the Omicron strain with a very small influence of sex on getting sick from Delta and Omicron strains

Sex	Delta	Omicron	Total
Male	25,209 (46.14%)	33,235 (45.47%)	58,444 (45.76%)
Female	29,426 (53.86%)	39,856 (54.53%)	69,282 (54.24%)
Total	54,635 (42.77%)	73,091 (57.23%)	127,726 (100%)

p = 0.01, p < 0.05

Table 5. Characteristics of the Delta cohort and Omicron cohort among south, central and north region; the northern region was statistically significantly more affected by Delta strain compared to the central and southern regions, while the southern region was statistically significantly more affected by Omicron strain compared to the central and northern regions, with a very large influence of the region on the incidence of Delta and Omicron strains

Region	Delta	Omicron	Total
South	14,486 (26.71%)	22,123 (30.26%)	36,609 (28.76%)
Central	29,338 (54.11%)	39,345 (53.84%)	68,683 (53.95%)
North	10,395 (19.18%)	11,623 (15.90%)	22,018 (17.29%)
Total	54,219 (42.59%)	73,091 (57.41%)	127,310 (100%)

p = 0.01, p < 0.05

Table 6. Characteristics of the Delta cohort and Omicron cohort among age groups; the age group from 20 to 29 years was statistically significantly more affected by the Delta strain compared to other age groups, while the age group from 30 to 39 years was statistically significantly more affected by the Omicron strain, with a very small influence of sex on the incidence of Delta and Omicron strains Distribution of strains by age

Age	Delta	Omicron	Total
0–9	2437 (4.47%)	2777 (3.82%)	5214 (4.09%)
10–19	6580 (12.08%)	4829 (6.64%)	11,409 (8.97%)
20–29	7228 (13.27%)	10,588 (14.55%)	17,816 (14%)
30–39	9795 (17.98%)	14,992 (20.61%)	24,787 (19.49%)
40–49	9062 (16.63%)	12,971 (17.82%)	21,975 (17.28%)
50–59	7405 (13.6%)	10,464 (14.37%)	17,869 (14.04%)
60–69	6546 (12.01%)	9263 (12.73%)	15,809 (12.44%)
70–79	3721 (6.83%)	4751 (6.52%)	8472 (6.66%)
80–89	1542 (2.83%)	1943 (2.67%)	3485 (2.74%)
90+	167 (0.3%)	200 (0.27%)	367 (0.29%)
Total	54,483 (42.83%)	72,724 (57.17%)	127,203 (100%)

$p = 0.01$, $p < 0.05$

affected by the Omicron strain, with a very small influence of sex on the incidence of Delta and Omicron strains C^2 (n = 127,726) = 0.10; p = 0.00 p < 0.05 f = 0.10 (Table 6).

As a reference group for age, the age of respondents from 40 to 49 years was taken. The results showed that:

- people belonging to the age group from 0 to 9 years old got sick 1.25 times less compared to the reference group,
- people between the ages of 10 and 19 were 1.94 times less likely to get sick compared to the reference group,
- persons belonging to the age group from 20 to 29 years old got sick 1.03 times more than the reference group,
- people who belong to the age group of 30 to 39 years got sick 1.07 times more than the reference group,
- persons belonging to the age group of 60 to 69 years got sick 1.09 times less compared to the reference group,
- persons belonging to the age group of 70 to 79 years got sick 1.10 times less compared to the reference group.

The Central region was taken as the reference group for the region. The results showed that people who belong to the southern region got sick 1.14 times more often than people who belong to the reference group, i.e., the central region and people who belong to the northern region got sick 1.20 times less than people who belong to the reference group, i.e., central region.

Only two independent variables made a unique statistically significant contribution to the model (age and region). Sex did not contribute significantly to the model (Table 7).

Table 7. Logistic regression models parameters of set of predictor variables for COVID-19; only two independent variables made a unique statistically significant contribution to the model (age and region); sex did not contribute significantly to the model

Category	B value	Standard error	Wald test	Degrees of freedom	Significant p	Exp (B)
Sex (1)	0.009	0.011	0.636	1	0.425	1.009
40–49			1221.867	9	0.000	
0–9	-0.223	0.031	51.634	1	0.000	0.800
10–19	-0.663	0.023	798.024	1	0.000	0.515
20–29	0.028	0.021	1.859	1	0.173	1.028
30–39	0.066	0.019	12.236	1	0.000	1.069
50–59	-0.001	0.021	0.004	1	0.947	0.999
60–69	0.009	0.021	0.160	1	0.689	1.009
70–79	-0.088	0.026	11.547	1	0.001	0.916
80–89	-0.101	0.037	7.431	1	0.006	0.904
90+	-0.165	0.106	20.413	1	0.120	0.848
Central region			330.840	2	0.000	
South region	0.133	0.013	100.909	1	0.000	1.143
North region	-0.182	0.016	134.295	1	0.000	0.833
Constant	0.347	0.016	470.842	1	0.000	1.415

Exp (B) – odds ratio

DISCUSION

In a study with 55,269 cases of COVID-19 in Sweden, the results of the logistic regression analysis indicated that the risk of severe disease remained high among unvaccinated, first-time infected patients of both sexes during the Omicron period in the age group 65+, as well as among men in the age group 40–64 years with two or more comorbidities [6]. Our research has shown that women were statistically significantly more affected by the Omicron strain. In retrospective cohort study in England, with laboratory-confirmed SARS-CoV-2 infection conducted between November and January 2022, showed that the adjusted hazard ratio estimates varied with age for all endpoints examined. The adjusted hazard ratio for hospital admission was 1.10 in those younger than 10 years, decreasing to 0.25 in 60–69-year-olds, and then increasing to 0.47 in those aged at least 80 years. From December 15, 2021 to January 17, 2022 outpatient-diagnosed cases with Omicron variant infection (n = 222,688) were concentrated among adults aged 20–39 years and had lower odds of being either very young or very old in comparison to contemporaneously identified individuals with Delta variant infection (n = 23,305). From February 3 to March 17, 2022, among individuals tested as outpatients, BA.2 Omicron sub variant cases (n = 1,905) did not differ from BA.1* sub variant cases (n = 12,756) in demographic or clinical attributes, with the exception that BA.1* detection was more concentrated among individuals aged 20–49 years than BA.2, which was comparatively more common among both children and older adults [7]. Individual-level data on laboratory-confirmed COVID-19 cases resident in England between November 29, 2021, and January 9, 2022, has shown that risk of severe outcomes is substantially lower for Omicron than for Delta, with higher reductions for more severe endpoints and significant variation with age [8]. Retrospective chart reviews with 13 adult emergency departments in academic hospitals in Paris area from November 29,

2021 to January 10, 2022 showed that the median age of analyzed population was 58 years and 49% were women. The proportion of Omicron increased from 1.3% in the first week of inclusion to 86% in the last week. Compared to the Delta variant, patients infected with Omicron were younger and more often female [9]. The observational cohort study in Denmark, with RT-PCR-confirmed cases of SARS-CoV-2 from November 21 and December 19, 2021 has shown significantly lower risk of hospitalization with Omicron infection compared with Delta infection among both vaccinated and unvaccinated individuals, suggesting an inherent reduced severity of Omicron [10]. In the general population infection, the rates are similar between males and females. In previous pandemics, male sex has been associated with worse clinical outcomes [11] In Montenegro, the age group from 20 to 29 years was statistically significantly more affected by the Delta strain compared to other age groups, while the age group from 30 to 39 years was statistically significantly more affected by the Omicron strain, with a very small impact of sex on the incidence of Delta and Omicron strains.

CONCLUSION

During the Delta strain period, the largest number of new cases was in the age category of 40 to 49 years for both sexes, followed by 30 to 39 years of age. In the period of the Omicron strain, the situation is similar, and the largest number of new cases was found in the age category from 30 to 39 followed by the age group 40 to 49 years for both sexes.

Men were statistically significantly more affected by the Delta strain, and women statistically significantly more by the Omicron strain, with a very small influence of sex on the incidence of the Delta and Omicron strains. The northern region was statistically significantly more affected by the Delta strain compared to the central and southern regions, while the southern region was statistically significantly more affected by the Omicron strain compared to the central and northern regions, with a very large influence of the region on the incidence of the Delta and Omicron strains. The age group from 20 to 29 years old was statistically significantly more affected by the Delta strain compared to other age groups, while the age group from 30 to 39 years old was statistically significantly more affected by the Omicron strain, with a very small influence of sex on the incidence of Delta and Omicron strains. The biggest predictor that a person will get sick is the age group. Research showed that related to reference group (40 to 49) age group from 20 to 29 and 30 to 39 years is predictor. Additionally, the predictor is region, and in our research, it was southern region.

Note: The paper is a part of a master thesis of the corresponding author.

Conflict of interest: None declared.

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Поређење случајева заразе сојевима делта и омикрон ковида 19 у Црној Гори

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САЖЕТАК

Увод/Циљ Крајем 2021. варијанта таласа омикрон (*B*.1.1.529) *SARS-CoV*-2 заменила је варијанту делта (*B*.1.617).
Основни циљ истраживања је да пружи детаљан и свеобухватан приказ података који се односе на особе заражене вирусом корона у Црној Гори. Специфични циљ истраживања је да се утврди да ли су мутације вируса утицале на ток епидемије током њеног двогодишњег трајања.

Методе Ово је ретроспективна студија. Користили смо податке Института за јавно здравље Црне Горе. Наш узорак се састојао од 127.134 особе старости од 0 до 100 година које су биле позитивне на делта или омикрон инфекцију и које су имале позитиван *PCR* тест на ковид 19 између 17. августа 2021. и 17. априла 2022.

Резултати Као референтну групу за узраст узети су испитаници старости од 40 до 49 година. Резултати су показали да је старосна група од 20 до 29 година оболела 1,03 пута више од референтне групе – особе које припадају старосној групи од 30 до 39 година оболеле су 1,07 пута више од референтне групе. Централни регион је узет као референтна група за регион. Резултати су показали да су људи који живе у јужном региону оболевали 1,14 пута чешће, а људи који живе у северном региону 1,20 пута мање него људи из централног региона.

Закључак Највећи предиктор да ће се човек разболети је старосна група. Такође, предиктор је регион, а у нашем истраживању јужни регион.

Кључне речи: ковид 19; *SARS-CoV*-2; старост; сојеви делта и омикрон; Црна Гора

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

STAT3 gene expression in ameloblastomas and odontogenic keratocysts

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SUMMARY

Introduction/Objective *STAT3* (signal transducers and activators of transcription) is involved in different physiological processes, including cell proliferation and survival. High expression of this protein is observed in various types of cancer.

This study aimed to investigate the gene and protein expression of *STAT3* in a series of odontogenic cysts and tumors to provide more information about their biological profile.

Methods The *STAT3* gene expression at mRNA was quantified by real-time quantitative polymerase chain reaction (RT-qPCR) in 23 odontogenic keratocysts (OKCs) and seven ameloblastomas (AMs), and compared to the non-neoplastic oral mucosa. We also assessed the expression of *STAT3* gene at protein levels, using immunohistochemistry, in 43 OKCs and 47 AMs.

Results *STAT3* transcripts were found in 96.6% of the tumors studied; however, the gene was downregulated in OKC and AM compared to the non-neoplastic oral mucosa. The *STAT3* gene expression at mRNA level was higher in sporadic OKC than in syndromic OKC ($p = 0.04$). There was no difference in *STAT3* gene expression at mRNA level between OKCs and AMs ($p = 0.88$). Immunostaining of STAT3 revealed no significant difference between sporadic and syndrome OKC ($p > 0.05$), nor between conventional and unicystic AMs ($p > 0.05$). Ameloblastomas exhibited significantly higher STAT3 immunostaining than OKCs ($p = 0.03$). In OKC and AM, STAT3 immunostaining was predominantly cytoplasmic and no difference in the cellular localization of STAT3 was observed between these lesions ($p = 0.58$).

Conclusion Our findings showed low expression of *STAT3* gene in OKCs and AMs in relation to non-neoplastic oral mucosa. However, higher *STAT3* immunostaining was observed in AMs compared to OKCs.

Keywords: odontogenic cysts; ameloblastoma; STAT3 transcription factor; gene expression; immunohistochemistry

INTRODUCTION

Odontogenic keratocysts (OKCs) and ameloblastomas (AM) are benign heterogeneous lesions of the jaws that arise from disturbances in tooth formation and are characterized by locally aggressive growth and recurrent rates [1]. The fifth edition of the World Health Organization (WHO) Classification of Head and Neck Tumours has considered the advanced molecular investigation, a fact that may cause a clinical impact [2]. As observed during odontogenesis, the development of the two lesions is related to the interaction between the odontogenic epithelium and ectomesenchyme. This process is mediated by signaling pathways forming a complex network [3]. One such pathway is the STAT3 signaling pathway, that has been suggested to be involved in the pathogenesis, progression, and recurrence of odontogenic tumors [4, 5].

STAT3 acts as signal transducers and transcription activators that play key physiological roles, including proliferation, survival, differentiation, and apoptosis [6, 7]. In addition to its participation in developing tooth germs and their disorders, the STAT3 signaling pathway is hyperactivated in most human cancers [8, 9]. It is generally associated with poor clinical prognosis [10, 11].

The STAT3 pathway has been the focus of studies on head and neck tumors. An *in vitro* study concluded that STAT3 is involved in the motility, metastasis, and progression of oral squamous cell carcinoma [12]. Recently, it has been suggested that the phosphorylation of STAT3 by IL-22 is essential for the increased invasion capacity of oral squamous cell carcinoma cell lines [13]. Furthermore, STAT3 activation is associated with the regulation of immunomodulatory proteins in head and neck tumors, and may therefore be a promising target for therapeutic intervention [12, 13].

Few studies have investigated the expression of STAT3 in odontogenic cysts and tumors [4, 5]. STAT3 and other related pathways participate in the epithelial-mesenchymal transition

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of AM via IL-6 and the acquisition of epithelial stem cell-like properties by this tumor [5]. However, it was not found STAT3 immunostaining in OKCs, only during their malignant transformation to primary intraosseous squamous cell carcinoma [4]. Therefore, the present study aimed to evaluate the STAT3 gene expression (mRNA and protein) in a series of odontogenic cysts and tumors, including sporadic OKC, OKC associated with nevoid basal cell carcinoma syndrome (NBCCS), conventional AM, and unicystic AM, to provide more information about the biological profile of this group of lesions.

METHODS

All procedures performed in studies involving human participants have been 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. The study has been approved by the Ethics Committee of the School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil (Protocol Nº 646.051). After approval, 47 cases of AM (30 conventional and 17 unicystic) and 43 cases of OKC (35 sporadic and eight associated with NBCCS) were obtained from the archives of the Surgical Pathology Service of the School of Dentistry, Federal University of Bahia, and University of São Paulo. The histopathological diagnosis was based on the World Health Organization Classification [2].

The samples were submitted to immunohistochemistry using STAT3 antibody. The odontogenic cysts and tumors selected comprised cases collected between 2002 and 2014. Thirty samples including seven AMs (five conventional and two unicystic) and 23 OKCs (14 sporadic and nine associated with NBCCS) were selected for gene expression analysis. Three samples corresponding to non-neoplastic oral mucosa from healthy individuals undergoing surgical excision of third molars, for orthodontic reasons, were included.

The samples were collected between 2005 and 2013 and stored in RNAlater solution (Ambiom®) at -80ºC until the experiments were performed.

RNA extraction and reverse transcription

Total RNA was extracted from 25–30 mg of frozen OKCs and AMs according to manufacturer specifications (RNeasy Mini Kit, Qiagen, Hilden, Germany). Genomic DNA was eliminated with DNase I (DNase I Amplification Grade Kit, Invitrogen, Carlsbad, CA, USA). RNA purity was evaluated by spectrophotometry (NanoDrop, Thermo Scientific, Wilmington, DE, USA) and values of 1.9–2.05 (A260/280) were considered satisfactory. The quantity of total RNA was determined by fluorimetry (QuBit™, Life Technologies, Camarillo, CA, EUA). The integrity of total RNA was confirmed by agarose gel electrophoresis (containing 1% formaldehyde). The cDNA was synthesized from 2 µg total RNA using oligo (dT) primers and the SuperScript II Reverse Transcriptase Kit (Invitrogen) in a reaction volume of 20 µL according to the protocol of the manufacturer. The reaction mixtures were incubated at 42°C for 2 minutes, followed by 65°C for 50 minutes, 42°C for 55 minutes, 70°C for 15 minutes, 37°C for 20 minutes, and 4°C for 5 minutes. The efficiency of reverse transcription was evaluated by amplifying the *GAPDH* and *B2M* reference genes.

Real-time quantitative polymerase chain reaction (RT-qPCR) and analysis of gene expression

The RT-qPCR assays were carried out in duplicate using inventoried TaqMan Gene Expression Assays™ for the *STAT3* gene (Hs00374280_m1), as well as for the *GAPDH* (Hs02758991_g1) and *B2M* (Hs00984230_m1) reference genes. The reactions were run on the ViiA™ 7 Real-Time PCR System (Applied Biosystems™, Foster City, CA, USA) using 96-well plates, in a total volume of 20 µL. Each well contained 2.5 ng/µL cDNA of the sample (8 µL), 1 µL of the assay, 10 µL TaqMan PCR Master Mix (Applied Biosystems™), and 5 µL RNAse-free water. The amplification program consisted of an initial cycle at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The calibrator sample (control) of the reactions consisted of a pool of samples of non-neoplastic oral mucosa. After the amplification runs and dissociation, the relative quantification (RQ) values were calculated using the Expression Suite v.1.0.3 (Applied Biosystems™).

Immunohistochemistry

For immunohistochemistry, 4-µm paraffin-embedded tissue sections were deparaffinized and rehydrated using routine methods. The STAT3 antigen epitopes were exposed by immersing the sections in citrate buffer, pH 6.0, in moist heat for 45 min, followed by the blockade of endogenous peroxidase (Peroxidase Blocking Solution™, Dako Corporation, Carpinteria, CA, USA) for 10 minutes protected from light and of tissue proteins (Protein Blocking Solution™, Dako Corporation) for 10 minutes. The sections were incubated with the primary STAT3 antibody (clone F-2, Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted at 1:100, overnight at 4°C. Next, the HRP Link and HRP Enzyme reagents (Advance™, Dako Corporation) were applied to the histological sections for 20 minutes each. The reactions were developed with 3,3-diaminobenzidine (Dako Corporation) for 5 minutes in a dark chamber, and the slides were counterstained with Harris hematoxylin. Lung squamous cell carcinoma sections were used as a positive control of the reactions. Phosphate-buffered saline was used as negative control in all reactions.

Immunohistochemical analysis

A previously trained examiner performed the immunohistochemical analysis under a light microscope coupled to a digital camera system (Axiocam ICC3; Zeiss, Göttingen, Germany, 2008) using the Axio Vision 4.8 software (Zeiss).

Brown-stained cells were defined as immunopositive and the intensity of staining was classified as follows: 0 = no staining, 1 = mild staining, 2 = moderate staining, and 3 = intense staining. The proportion of positive cells was scored 0–3, where 0 = up to 5% of positive cells, 1 = 6–25% of positive cells, 2 = 26–75% of positive cells, and 3 = > 75% of positive cells. Multiplication of the intensity score (0–3) by the proportion of stained cells (0 to > 75%) resulted in the following final scores: when the product of the two scores was 0, the case was classified as negative; when the product of the two scores ranged 1–3, the case was classified as low immunohistochemical expression; when the product was ≥ 4, the case was classified as high immunohistochemical expression [14]. The distribution of proteins was evaluated particularly in the basal, intermediate, and superficial layers of sporadic and syndromic (NBCCS) OKCs, as well as in the tumor islands, cystic epithelial lining, and areas of squamous metaplasia of AMs.

Statistical analysis

The sample data did not show a normal distribution according to the Gauss curve. Differences between groups were evaluated using the Mann–Whitney and Fisher's exact tests. All statistical calculations and graphics were performed with the GraphPad Prism 5.01 program (San Diego, CA, USA). A p value < 0.05 was considered statistically significant.

RESULTS

Gene expression profile of *STAT3* in odontogenic keratocyst and ameloblastoma

Expression of the *STAT3* gene was found in 29 (96.66%) of the 30 odontogenic cysts and tumors studied. *STAT3* transcripts were detected in all cases of OKC, with RQ values ranging 0.095–19.39 (median = 0.402, SD = 1.807). This gene was downregulated compared to the non-neoplastic oral mucosa (control) (Figure 1). It should be noted that the RQ values deviated from the values of the other samples in two OKC cases (9.021 and 19.394). These cases were sporadic/recurrent OKCs. Sporadic/recurrent OKCs exhibited higher RQ values than syndromic OKCs (p = 0.04; Mann–Whitney test) (Figure 2). In AMs, no *STAT3* transcripts were detected in one case, with RQ values ranging 0–1.315 (median = 0.325, SD = 1.975). The gene was downregulated compared to the to the non-neoplastic oral mucosa (control) (Figure 1). No difference in *STAT3* gene expression was observed between OKCs and AMs (p = 0.88; Mann–Whitney test; Figure 3).

STAT3 protein in odontogenic keratocyst and ameloblastoma

We evaluated STAT3 immunostaining in 90 odontogenic tumors, including 43 OKCs and 47 AMs. The distribution of scores for the proportion of stained cells and staining intensity, the product of scores, and staining pattern are summarized in Table 1.

Immunostaining in OKC was evident mainly in the basal and intermediate layers of the epithelium lining the fibrous cystic wall (Figure 4a). There was no significant difference in the proportion of positive cells, staining intensity, or final STAT3 score between sporadic OKCs and OKCs associated with NBCCS (p > 0.05; Fisher's exact test). Similarly, no difference was observed in nuclear staining of STAT3 between

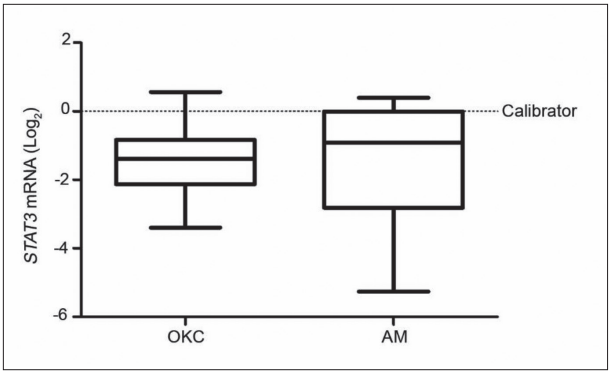

Figure 1. Relative expression (Log2) of the *STAT3* mRNA in odontogenic keratocysts (OKC) and ameloblastomas (AM) compared to the calibrator (control) sample

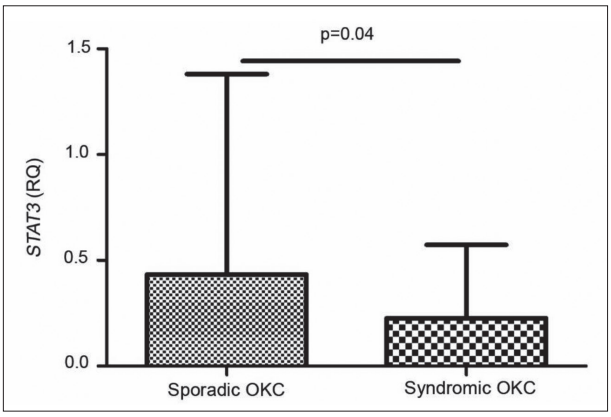

Figure 2. Comparison of *STAT3* mRNA relative quantification (RQ) between sporadic odontogenic keratocysts (OKC) and syndromic OKCs by Mann–Whitney test

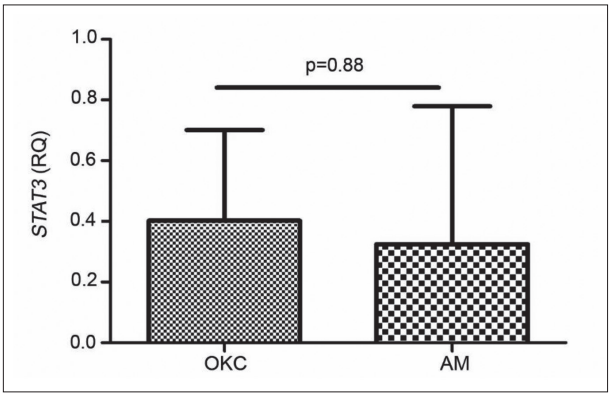

Figure 3. Comparison of *STAT3* mRNA relative quantification (RQ) between odontogenic keratocysts (OKC) and ameloblastomas (AM) by Mann–Whitney test

Table 1. Distribution of scores regarding *STAT3* in odontogenic keratocysts and ameloblastomas

Criteria evaluated	Sporadic OKC (n = 35)	Syndromic OKC (n = 8)	Conventional AM (n = 30)	Unicystic AM (n = 17)
Stained cells				
Score 0	13	1	3	7
Score 1	10	2	8	3
Score 2	4	5	5	2
Score 3	8	0	14	5
Intensity				
Absent	13	1	3	7
Mild	10	6	9	4
Moderate	7	1	7	4
Intense	5	0	11	2
Product of scores				
Negative	13	1	3	7
Low expression	13	6	12	4
High expression	9	1	15	6
Cell staining				
Absent	13	1	3	7
Only cytoplasmic	13	4	17	7
Cytoplasmic and nuclear	9	3	10	2
Only nuclear	0	0	0	1

OKC – odontogenic keratocysts; AM – ameloblastomas

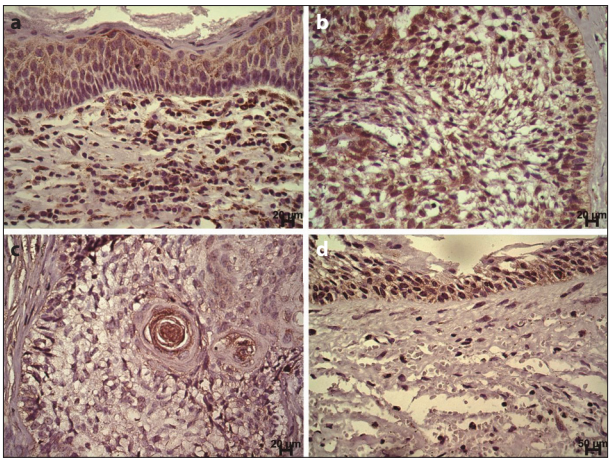

Figure 4. STAT3 immunostaining; (a) cytoplasmic staining in cells of the intermediate layer of the cystic epithelial lining in odontogenic keratocysts; (b) predominantly cytoplasmic and possible nuclear staining in central and peripheral areas of tumor islands in conventional ameloblastoma; (c) immunostained area of squamous metaplasia in conventional ameloblastoma; (d) cytoplasmic staining in all layers of the cystic lining of unicystic ameloblastoma

sporadic and syndromic OKCs (p = 0.66; Fisher's exact test). Immunostaining in AMs was more evident in the tumor parenchyma of epithelial islands and in all layers of the cystic lining. Furthermore, staining was more common in peripheral than in central cells of the tumor islands. STAT3 immunoreactivity was also observed in areas of squamous metaplasia (Figure 4b–d). No significant difference was found in the proportion of positive cells, staining intensity, or final STAT3 score between conventional and unicystic AMs (p > 0.05; Fisher's exact test). There was also no difference in nuclear staining of STAT3 between conventional and unicystic AMs (p = 0.32; Fisher's exact test).

A significant difference in STAT3 immunostaining occurred between OKCs and AMs, with a higher proportion of positive cells in the latter (p = 0.03; Fisher's exact test). However, there was no difference in nuclear staining of STAT3 between OKCs and AMs (p = 0.58; Fisher's exact test).

DISCUSSION

Advances in understanding STAT3 signaling and its role in tumor progression and aggressiveness have rendered this transcription factor a potential target in different studies on head and neck pathologies [4, 5, 12, 13]. Our study aimed to evaluate the *STAT3* gene expression at mRNA and protein levels in OKC and AMs, common lesions with variable degrees of aggressive behavior and different recurrence rates, to gain insight into the molecular profile of this group of odontogenic lesions.

STAT3 transcript in OKC and AM was downregulated compared to the to the non-neoplastic oral mucosa (control). Interestingly, 67.44% of the OKC cases and 78.72% of the AM cases exhibited low or high immunolabelling of STAT3. Thus, *STAT3* gene expression was more evident at protein than at mRNA level in the cases studied. In malignant head and neck tumors, expression of STAT3 protein is related to cell migration, and proliferation and tumor progression [4, 15]. STAT3 exists in two isoforms, α and β; STAT3β is a less abundant isoform that reduces the transcriptional function of α [16]. The primer used in the present study was specific for the α and β isoforms and we can therefore rule out the possibility that the downregulation of the gene had occurred in a specific isoform. We hypothesized that a limited number of available samples for qPCR and standard deviation could explain our results. This aspect represented a limitation of this study. Perhaps a larger number of cases and a laser-microdissection-based analysis could clarify this matter.

In the present study, STAT3 immunostaining in OKC and AM was predominantly cytoplasmic, while nuclear staining was less common. STAT3 is present in the cytoplasm under basal and inactive conditions. The nuclear translocation after activation is fundamental for the function of this protein as a transcription factor and regulator of specific genes [17]. The predominantly cytoplasmic immunostaining of the protein and downregulation of the *STAT3* gene at mRNA level suggest low transcriptional activity of this protein in the odontogenic cysts and tumors studied. In the cytoplasm, unphosphorylated STAT3 interacts with protein kinase R, blocking its enzymatic activity and inhibiting autophagy, and also regulates cell migration through microtubule polymerization [18, 19].

pSTAT3 was not included in the analysis because of technical difficulties with antibody staining. Despite this, the latent cytoplasmic STAT3 protein is activated by tyrosine phosphorylation mediated by Janus or Src kinases. Once phosphorylated (pSTAT3), the protein forms dimers and is translocated to the nucleus, where it binds to specific DNA promoter sequences for transcription of its target

genes [17]. Although the physiological performance of signaling is important for the standard cell response, disordered activation of the STAT3 pathway occurs in many human diseases, especially tumors. Thus, disruption of STAT3 signaling is related to the process of tumorigenesis, inducing the transcriptional activation of various genes that regulate inflammation, angiogenesis, apoptosis resistance, and metastasis [15, 20].

In contrast to the present study, in a series of only three cases analyzed by immunohistochemistry, absence of STAT3 in OKCs was revealed [4]. However, other authors found positive staining for this protein in follicular AM [5]. Given the higher immunostaining of STAT3 in AMs observed in the present study, even considering the lack of a significant difference in mRNA expression between OKC and AM, we suggest that STAT3 may participate at least in the tissue differentiation of these lesions. Regarding the type of keratocyst, we found significantly higher expression of the STAT3 gene at protein levels in sporadic OKCs than those associated with NBCCS. However, it is difficult to explain this difference because of the small number of syndromic cases. It is important to point out that, despite the lack of studies comparing STAT3 between different types of AM and OKC, there is no consensus in the literature regarding the distribution of tumor markers among different odontogenic cysts and tumors. However, this distribution is generally associated with proliferation, recurrence, and tumor aggressiveness, which cannot be inferred here [21, 22].

Immunostaining of STAT3 was observed in stellate reticulum-like cells, in areas of squamous metaplasia. It was also observed in peripheral cells of epithelial islands of the tumor parenchyma in AMs. In OKCs, STAT3 immunostaining was detected in superficial and intermediate layers. In addition, immunostaining was found in the suprabasal layer of the epithelium lining the fibrous cystic wall in unicystic AMs. These findings suggest the participation of STAT3 in the morphogenesis and differentiation of AM and OKC, indicating a role of this protein in physiological processes that are fundamental for tumor development [6, 7, 10, 11]. Activated STAT3 has been reported to participate in the differentiation of the stratified squamous epithelium by regulating the gene expression of cytokeratins [23]. These proteins are mainly found in stellate reticulum-like cells of the enamel organ and areas of squamous metaplasia in AMs, as well as in the intermediate and superficial layers of the cystic epithelial lining of OKCs, suggesting complete differentiation of the epithelial component of OKC [21, 24].

CONCLUSION

STAT3 expression at both mRNA and protein levels was detected in OKCs and AM regardless of their clinical presentation. This protein participates in the differentiation and maintenance of the cytoarchitectural pattern of these odontogenic lesions. This study provided insight into the role of STAT3 in OKC and AM. However, further studies investigating the Janus kinase / STAT3 signaling pathway, especially the phosphorylated form of the protein, would be useful to elucidate other aspects related to the pathogenesis of odontogenic tumors and cysts.

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Conflict of interest: None declared.

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Експресија гена *STAT3* код амелобластома и одонтогених кератоциста

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САЖЕТАК

Увод/Циљ *STAT3* (претварачи сигнала и активатори транскрипције) укључен је у различите физиолошке процесе, укључујући пролиферацију и преживљавање ћелија. Висока експресија овог протеина се примећује код различитих врста рака.

Ова студија је имала за циљ да истражи експресију гена и протеина *STAT3* у серији одонтогених циста и тумора како би пружила више информација о њиховом биолошком профилу.

Методе Експресија гена *STAT3* на мРНК је квантификована квантитативном ланчаном реакцијом полимеразе у реалном времену (*RT-kPCR*) у 23 одонтогене кератоцисте (ОКЦ) и седам амелобластома (АМ) и упоређена са ненеопластичном оралном слузницом. Такође смо проценили експресију гена *STAT3* на нивоима протеина, користећи имунохистохемију, у 43 ОКЦ и 47 АМ.

Резултати *STAT3* транскрипти су пронађени у 96,6% проучаваних тумора; међутим, ген је смањен у ОКЦ и АМ у поређењу са ненеопластичном оралном слузокожом. Експресија *STAT3* гена на нивоу мРНК била је већа код спорадичног ОКЦ него код синдромског *STAT3* ($p = 0,04$). Није било разлике у експресији *STAT3* гена на нивоу мРНА између ОКЦ и АМс ($p = 0,88$). Имунобојење *STAT3* није открило значајну разлику између спорадичног и синдрома ОКЦ ($p > 0,05$), нити између конвенционалних и уницистичних АМ ($p > 0,05$). Амелобластоми су показали значајно веће *STAT3* имунобојење од ОКЦ ($p = 0,03$). Код ОКЦ и АМ, *STAT3* имунобојење је било претежно цитоплазматско и није примећена разлика у ћелијској локализацији *STAT3* између ових лезија ($p = 0,58$).

Закључак Наши налази су показали ниску експресију гена *STAT3* у ОКЦ и АМс у односу на ненеопластичну оралну слузокожу. Међутим, примећено је веће *STAT3* имунобојење код АМ у поређењу са ОКЦ.

Кључне речи: одонтогене цисте; амелобластом; фактор транскрипције *STAT3*; експресија гена; имунохистохемија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Low-level laser efficiency in reparation of bone defects

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SUMMARY

Introduction/Objective Bone resorption is a common problem in dentistry, and bone reparation cannot be easily achieved. Several techniques of bone grafting and the use of low-level laser treatment (LLLT) as a new therapeutic optional recommended for improving bone repair were applied. The aim of the study was to investigate the influence of LLLT in bone repair of artificially made bone defects in the rat mandible using histomorphometry.

Methods The research was carried out on 60 female rats. Bone defects were made in the mandible, and animals were divided into two groups, each containing 30 animals. In the study group, the implantation site was submitted to GaAlAs laser irradiation 670 nm, 5 mW, 4 minutes per day for 5 days. The control group had no postoperative treatment. The animals were sacrificed after two, six, and eight weeks post LLLT, and preparations were analysed by histomorphometry, determining bone area fraction, bone area, integral density, mean density, and density variation.

Results Histomorphometric analysis revealed statistically higher values of area fraction, area, and integral density in the study group after two and six weeks. However, no beneficial laser effect was noticed after eight weeks.

Conclusion Low-level lasers have a stimulating effect on reparatory mechanisms in the early regeneration stage of artificially made bone defects in the rat mandible and can be used as a useful helping method in bone treatment.

Keywords: bone; low-level laser treatment; osteogenesis

INTRODUCTION

Alveolar bone resorption is a common problem in dentistry that occurs due to several pathologic and physiologic conditions. In such cases, reparative potentials of bone vary, and reparation cannot be easily achieved. Many techniques of bone grafting have been recommended for improving bone repair; the use of low-level laser treatment (LLLT) is a new therapeutic option [1]. The use of LLLT as a bio-modulation tool in dentistry has been continuously growing, and many studies have demonstrated its positive results on bone tissue healing after dental extraction, bone fractures, orthodontic treatments, and implant placement [2, 3]. When laser light enters the tissue and is absorbed, i.e. triggers biochemical processes that lead to activation of the mitochondrial chain and cell activities. LLLT induces proliferation of fibroblasts and production of collagen and increases enzyme activity and vascularisation of the treated area [3]. LLLT is painless and non-invasive, has no adverse effects, and there are almost no contraindications for its use [4].

Although LLLT is widely used, its effects on bone are still controversial. It is thought that LLLT stimulates bone through activation of osteoblasts, which induce faster formation and maturation of young bone. Yet, there is no universal opinion regarding the use of specific therapeutic dosage and time. Unlike pharmaceutical agents, LLLT involves a wide range of parameters in terms of laser properties and dosage, which has been shown to be important for the effects to occur. Under-dosage results in poor cellular response, but overdosage may paradoxically inhibit cell proliferation or induce apoptosis.

Different laser types with different wavelengths, including helium–neon (He-Ne), gallium aluminium arsenide (GaAlAs), and gallium arsenide (GaAs), have been used at different doses and different treatment schedules for the LLLT. In recent studies, the GaAlAs type of diode lasers has been shown to be more effective in bio-stimulation than He-Ne lasers due to the higher penetration ability into the deep tissues [5, 6]. The dose-dependent nature of LLLT results in stimulating effects at low doses

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(0.05–10 J/cm^2), while higher doses (over 10 J/cm^2) lead to bio-inhibition. It is difficult to compare studies about LLLT of bone because the dosage parameters, models, and duration of therapy are very distinct [2]. Thus, it is important that the cellular effects of LLLT are better understood and considered before formulation of clinical treatment protocols. Research is currently in progress and leads to finding common ground with universal recommendations for LLLT in everyday dental practice.

The aim of this study was to histomorphometrically investigate the influence of the LLLT on bone repair of artificial bone defects made in the rat mandible.

METHODS

This prospective randomized trial was carried out at the Faculty of Medicine, University of Niš, for nine weeks. The study was conducted according to the ethical principles in animal experimentation of the International Council for Laboratory Animal Science and the Committee for the Purpose of Control And Supervision of Experiments on Animals [7]. The study protocol was approved by the Faculty of Medicine Institutional Ethics Committee (No. 01-2800-7).

Sixty healthy white female rats of Wistar type, age 10 weeks, participated in the study. All of the animals were prepared for intervention by applying diazepam (Bensedin, ICN Galenika, Belgrade, Serbia) at a dose of 1.5 ml per animal and anesthetized with ketamine hydrochloride USP (Ketalar, Rotexmedica Gmbh, Trittau, Germany) at a dose of 0.5 ml per animal. Later on, defects 1.2 mm in diameter and 1.5 mm deep were made in the region between the medial line and mental foramen (region of maximum load in the mandible of rats) on the right side of the mandible. The animals were then divided into two equal groups. In the study group, the implantation site was submitted to GaAlAs laser irradiation (model Mils 94, Optica Laser, Sofia, Bulgaria), using 670 nm, power 5 mW, 4 minutes per day, for five consecutive days. The control group of animals had no postoperative treatment. Defects in the mandible were not large and did not require postoperative analgesia, nor were a threat of infection due to good vascularization of the treated area. Periodontal flap operation was a model after which the bone preparations in rat mandible were made, and it did not require the use of analgesics or antibiotics. The animals were kept in cages under appropriate conditions of light and temperature, and had water and food *ad libidum* according to institutional guidelines relating to animal experiments. The postoperative period was with no adverse consequences.

Animals were sacrificed two, six, and eight weeks post LLLT, with profound sedation and overdose of ketamine and xilazine, 0.5 ml each. Bone samples of the mandible, from the medial line to the mental foramen, were cut in the vestibulo-oral direction, washed in physiological solution, and fixed in 10% formaldehyde. Chemical decalcification was performed in a 15% solution of nitric acid, and the decalcification time ranged 24–72 hours. Decalcification by electrolysis was performed in an electrophoresis power supply MA 8903 apparatus (Elektronska industrija, Niš, Serbia), in an aqueous solution of 8% concentrated hydrochloric acid and 10% formic acid. The decalcification process by electrolysis was carried out for two hours at a voltage of 100 V and a current of 50 mA.

Afterwards, the samples were dehydrated in alcohol, molded into paraplast, cut and dyed. Thus 2–4 μm thick histological sections were dyed by haematoxylin-eosin (HE) and PAS methods. Digital pictures (640 × 480 pixels) were taken with a 63 × magnification objective of a NU-2 microscope (Carl Zeiss, Jena, Germany) and analysed by a Lucia 3.2G system (Laboratory Imaging, Prague, Czech Republic).

For histomorphometric analysis, the test area was determined (one field of view of each preparation), which included both the newly created bone tissue and the bone tissue immediately adjacent to the prepared defect. The following were measured: area fraction (the percentage of bone tissue at each visual area); area (the bone area which could be seen at each visual area); integral density (integrally collected optical density of investigated bone part); mean density (mean value of optical density), and density variation (density variation of newly formed compact and spongy bone).

Statistical processing of the results was performed with the SPSS 15.0 program (SPSS Inc., Chicago, IL, USA). MANOVA and t-test have been used for the analysis of the obtained results, which were shown as a mean value and standard deviation. The level of significance was set at $p \leq 0.05$.

RESULTS

After two weeks, histomorphometric analysis of bone revealed statistically higher values of area fraction, bone area, and integral density in the study group, while density variation was statistically higher in the control group (Table 1). A histological analysis of the mandibular cortical alveolar bone after two weeks in the study group showed a noticeable increase in bone tissue, with numeruous cement lines and reduction of Haversian canals compared to the cortical bone of the control group of animals (Figure 1).

After six weeks in the study group, submitted to the LLLT, histomorphometric analysis of bone still showed statistically higher values of area fraction, bone area, and integral density compred to the control group – in the study group, an increase in compact and cancellous bone tissue with numerious cement lines was noteiceable, compared to the samples of the control group of animals (Figure 2; Table 2).

There was no difference in histomorphometric findings between experimental groups after eight weeks (Table 3).

DISCUSSION

Alveolar bone loss represents a problem in dental rehabilitation. Although bone has good regenerative properties,

Table 1. Mean histomorphometric values of investigated bone after two weeks

Variable	Study group	Control group	p
	$\bar{x} \pm SD$		
area fraction (%)	0.59 ± 0.32	0.39 ± 0.16	< 0.05
area (μm2)	16,213.41 ± 3,133.04	11,072.31 ± 3,071.47	< 0.05
integral density (a.u.)	1,031,998.54 ± 42,443.07	82,724.4 ± 10,222.92	< 0.05
mean density (g/cm^2)	0.29 ± 0.06	0.27 ± 0.03	n.s.
density variation	0.02 ± 0.02	0.05 ± 0.02	< 0.05

Table 2. Mean histomorphometric values of experimental bone after six weeks

Variable	Study group	Control group	p
	$\bar{x} \pm SD$		
area fraction (%)	0.78 ± 0.06	0.58 ± 0.16	< 0.05
area (μm^2)	133,810.9 ± 12,007.13	108,499.9 ± 26,455.46	< 0.05
integral density (a.u.)	143,883.6 ± 20,550.68	1,100,468 ± 28,079.01	< 0.05
mean density (g/cm^2)	0.28 ± 0.03	0.28 ± 0.02	n.s.
density variation	0.08 ± 0.01	0.08 ± 0.02	n.s.

Table 3. Mean histomorphometric values of experimental bone after eight weeks

Variable	Study group	Control group	p
	$\bar{x} \pm SD$		
area fraction (%)	0.78 ± 0.17	0.68 ± 0.22	n.s.
area (μm^2)	136,174.4 ± 32,014.07	127,461.6 ± 36,342.66	n.s.
integral density (a.u.)	155,337.1 ± 55,853.52	133,247.3 ± 40,935.74	n.s.
mean density (g/cm^2)	0.28 ± 0.11	0.28 ± 0.04	n.s.
density variation	0.08 ± 0.35	0.08 ± 0.03	n.s.

a 650-nm wavelength increases orthodontic tooth movement more than other wavelengths (405 nm, 532 nm, and 940 nm). A similar wavelength was used in our investigation (670 nm).

It is widely accepted today that inflammation and bone resorption are basic responses of periodontal tissue to damage. LLLT reduces gingival inflammation, and many studies indicate that LLLT has capacity to alter bone cellular behaviour [8, 11]. Faster callus formation, revascularization, promotion of bone formation and denser trabecular networks have also been reported [12]. Liu et al. [13] investigated the healing of rat tibiae fractures irradiated with a low-level laser (830 nm, CW, 40 J/cm^2 once daily for five weeks) and suggested that LLLT causes an increase in callus volume. Lirani-Galvão et al. [14] investigated the effects of LLLT (GaAlAs laser, 780 nm, 30 mW) on bone repair in rats and noticed a significant increase in osteoblast number. Nagasawa et al. [15] irradiated bone defects with GaAlAs laser and noticed the active formation of spongy bone. Diker et al. [9] showed, through histomorphometry, that applying 10 sessions of LLLT

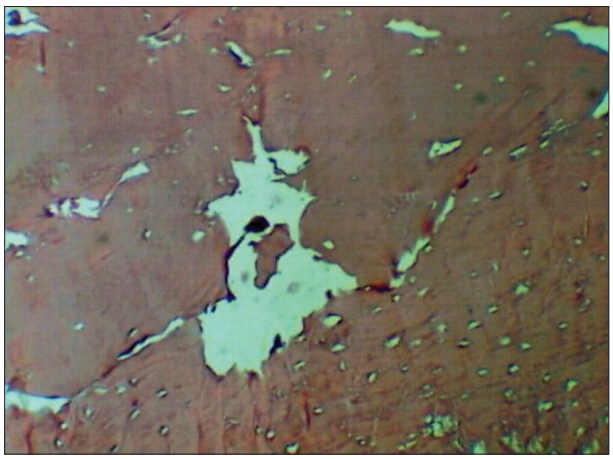

Figure 1. Intensive osteogenesis of compact bone two weeks after the completed low-level laser treatment; 25 ×

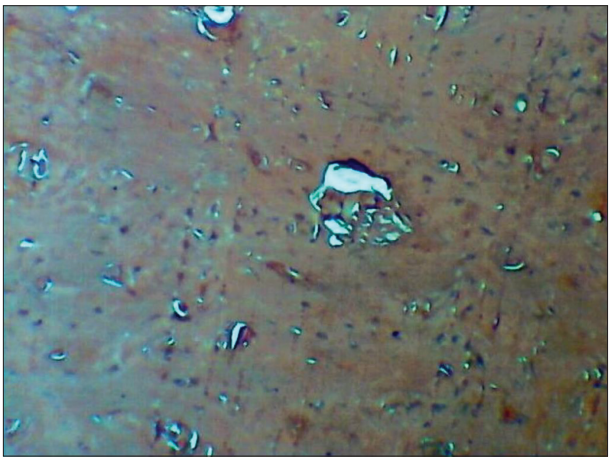

Figure 2. Osteogenesis of compact bone six weeks after the completed low-level laser treatment; 25 ×

its repair capacity may be impaired due to mechanical instability and the presence of other tissues with higher proliferative activity. The use of several techniques, including LLLT, has been studied in order to improve regeneration of alveolar bone and upgrading routine dental rehabilitation [8, 9]. In periodontal, oral, and maxillofacial rehabilitation, application of LLLT for assisting the treatment with bone grafts, distraction osteogenesis, peri-implant tissue healing, and wound healing, becomes an emerging trend, and has shown promising results [9]. It includes wavelengths 500–1100 nm and a dose of 1–4 J/cm^2, using lasers with output powers of 5–90 mW. The infrared portions of the spectrum have been shown to provide the best therapeutic results [2, 10]. Keklicki et al. [2] noticed that LLLT with stimulated osteoblastogenesis in bone defects of diabetic rats. Taha et al. [16] showed that LLLT (970 nm) could enhance the bone healing mechanisms and improve the outcome of the treatment in an animal study. These histological findings are similar to findings from our study, which emphasize the stimulating effect of LLLT on bone healing and reparation.

LLLT can stimulate bone cellular proliferation, which reflects osteoblastic activity. It is assumed that depending on the phase of bone repair, LLLT can accelerate bone resorption or formation [17, 18]. Prado et al. [1] evaluated *in vivo* osteogenesis on rough treated dental implants alone or in association with LLLT. LLLT was applied for seven days at the surgical site before and after placing the

implant. Bone-implant contact was measured after one, two, and six weeks using scanning electron microscopy and energy dispersion spectrophotometry. In short periods, significantly greater bone–implant contact was noticed. The investigators concluded that inducing cellular stimulation and improving bone–implant contact in short-term healing should be considered in clinical practice due to low cost and high effectiveness of the LLLT [1]. In our research, similar results have been shown through histomorphometric parameters during the investigated period of two and six weeks. In contrast, no differences between the groups were noticed during the later-investigated period of eight weeks. Pretel et al. [18] evaluated bone repair in artificially made rat mandible defects in three evaluation periods (15, 45, and 60 days) after stimulation with infrared LLLT. The histological results showed an advanced bone tissue response compared to the control group, abbreviating the initial inflammatory reaction and promoting rapid new bone matrix formation at 15 and 45 days. There were no significant differences between the groups after 60 days. The authors concluded that LLLT showed a stimulating effect on bone remodelling by stimulating modulation of the initial inflammatory response. In our study, histomorphometric analysis revealed statistically higher values of bone area fraction, bone area, and integral density in the group submitted to LLLT. Based on the obtained results, it has been noticed that LLLT's beneficial effect was more pronounced in a shorter investigated period [19]. However, it is still difficult to compare LLLT studies on bone due to different dosimetrical parameters, experimental models and duration of treatments [4, 20, 21].

The treatment protocol used in our study is in agreement with other authors, as no existing LLLT parameters are universally accepted [1]. It is possible that laser treatment's effect on bone regeneration depends not only on the total dose of irradiation but also on the duration and mode of irradiation. Further studies are needed to determine optimal parameters, particularly dosage and treatment period, to establish universal recommendations for the use of LLLT in everyday dental practice. The increase of knowledge about the low-level laser influence on regeneration and reparation of bone defects creates a sound basis for a broader application of this therapeutic procedure that involves the implantation of artificial bone in regeneration. In such a way, impaired regeneration present in patients with poor general health, like in certain systemic diseases, could be successfully overcome and regeneration of alveolar bone achieved.

CONCLUSION

Histomorphometric analysis of artificially made defects in rat mandible revealed that low-level lasers have a stimulating effect on reparatory mechanisms in the early bone regeneration stage, after two and six weeks of the applied treatment. No beneficial laser effect was noticed after an investigated period of eight weeks. These findings suggest that LLLT can be useful as a helping method in the early stages of alveolar bone regeneration.

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Ефикасност ласера мале снаге у репарацији коштаних дефеката

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САЖЕТАК

Увод/Циљ Ресорпција кости представља чест проблем у стоматологији, а тешко се постиже надокнада коштаног ткива. Као нови терапијски приступ у побољшању регенерације кости сада се препоручују различите технике коштане трансплантације и третман ласером мале снаге (*low-level laser treatment – LLLT*).

Циљ овог истраживања је био да се хистоморфометријски испита утицај *LLLT* на регенерацију кости вештачки направљених коштаних дефеката мандибуле пацова.

Методе У истраживању је учествовало 60 пацова женског пола. После препарације коштаних дефеката доње вилице, животиње су подељене у две групе од по 30 животиња. У студијској групи, место имплантације је подвргнуто зрачењу ласером *GaAlAs* 670 *nm*, 5 *mW*, четири минута дневно, пет дана, док у контролној групи није било постоперативног третмана. Животиње су жртвоване после две, шест и осам недеља од завршене *LLLT*. Исечци су хистоморфометријски анализирани мерењем ареалне фракције кости, ареје кости, интегрисане густине, средње густине и варијације густине.

Резултати Хистоморфометријска анализа је показала статистички значајно веће вредности ареалне фракције, ареје и интегрисане оптичке густине у групи подвргнутој ласеротерапији после две и шест недеља. Није уочено посебно дејство ласера у периоду после осам недеља.

Закључак Ласери мале снаге имају биостимулативни ефекат на процесе репарације у раној фази регенерације вештачки направљених дефеката кости мандибуле пацова и могу се користити као помоћно средство у третману кости.

Кључне речи: кост; терапија ласерима мале снаге; остеогенеза

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Real-world data of cardiotoxicity during long-term therapy with trastuzumab in human epidermal growth factor receptor-2-positive metastatic breast cancer

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SUMMARY
Introduction/Objective This study aims to investigate the cardiotoxicity of long-term therapy with trastuzumab in patients with HER-2-positive metastatic breast cancer.
Methods A total of 48 patients with metastatic HER-2-positive breast cancer were analyzed. The patients received long-term trastuzumab (time of application was longer than 20 months). The analyzed characteristics of the patients were the following: age, initial stage of the disease, application of anti-HER-2 therapy and anthracyclines in the adjuvant setting, the number and type of applied systemic therapies concomitant with trastuzumab in the metastatic setting. Cardiac toxicity was assessed using left ventricular ejection fraction (LVEF) values at three time-points: at the beginning, in the middle, and at the end of treatment period for each patient separately.
Results In 17 (35.4%) patients the trastuzumab treatment was temporary discontinued. The average time of trastuzumab therapy interval was 52.2 ± 23.5 months. The mean LVEF values were 66.73 ± 7.02%, 64.62 ± 5.7%, and 63.44 ± 6.1%, respectively. The mean values of LVEF differed significantly in the three observed time-points (F = 4.9 p = 0.009). *Post hoc* pairwise comparison, using Bonferroni correction, confirmed significantly lower mean LVEF values at the end point (at the end of treatment) compared with the mean LVEF values at the beginning of anti-HER-2 treatment (p = 0.019), but within the reference range of LVEF ≥ 50%.
Conclusion The data confirm good safety profile of long-term trastuzumab therapy in HER-2 positive metastatic breast cancer patients considering cardiotoxicity.
Keywords: breast cancer; cardiotoxicity; ejection fraction; safety; trastuzumab

INTRODUCTION

Cancer and cardiovascular diseases represent the leading cause of death and an important contributor to mortality rates worldwide [1]. Female breast cancer (BC) has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases [2]. The human epidermal growth factor receptor 2 (HER-2) status in BC has a prognostic and predictive value and provides information about the prognosis of the disease and the type of appropriate specific treatment, and thus helps in selecting the optimal therapy for patient treatment. HER-2 testing is recommended for all newly diagnosed invasive BCs. The most commonly used methods for testing are immunohistochemical staining and *in situ* hybridization. Approximately 15% of BCs have excessive expression or amplification of HER-2 and have aggressive clinical behavior [3]. There has been a general consensus that the HER-2 oncogene, when overexpressed, is the dominant driver of BC biology, regardless of hormone receptor status [4]. The use of trastuzumab in combination with chemotherapy dramatically improves the prognosis in all stages of HER-2-positive BC [5]. Trastuzumab is a humanized monoclonal antibody directed against the extracellular domain of HER-2. It represents the standard of care in BC patients with HER-2 amplification and/or overexpression, both in the advanced and (neo) adjuvant setting. Adding trastuzumab to standard chemotherapy has led to a significant improvement in survival outcomes [6].

Trastuzumab-mediated cardiotoxicity

Cardiotoxicity is an important segment in HER-2-targeted therapy. Unlike anthracycline-induced cardiotoxicity, trastuzumab-mediated

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cardiotoxicity is not dose-dependent and is reversible. Trastuzumab may lead to an exacerbation and augmentation of cardiotoxicity induced by prior anthracycline treatment by interfering in the mechanism of homeostasis and cell survival and repair pathways [7]. In a leading phase 3 study for trastuzumab [8], as well as in subsequent studies, a significant number of patients with cardiac dysfunction were observed. Those patients received trastuzumab therapy, with special consideration for those patients in which anthracyclines and trastuzumab were administered concomitantly. Additional studies have shown that, in general, cardiotoxicity is reversible and that trastuzumab can be reintroduced in the treatment after establishing regular cardiac function. The U.S. Food and Drug Administration (FDA) has evaluated trastuzumab-mediated cardiotoxicity in four adjuvant trials (NCCTG N9831, NSABP B-31, HERA, BCIRG 006) and found a four- to six-fold increased symptomatic heart dysfunction in patients that received trastuzumab [9]. Myocardial dysfunction and heart failure, mostly described as cardiotoxicity, are the most concerning cardiovascular complications of cancer therapies. Most trials use the definition of cardiotoxicity related to cancer therapeutics defined by the European Society of Cardiology (ESC) as a decrease in left ventricular ejection fraction (LVEF) > 10% points to a value below 50% [10]. According to ESC Guidelines, LVEF should be determined before and periodically during treatment for early detection of cardiac dysfunction in patients receiving potentially cardiotoxic chemotherapy [10]. This group considers the lower limit of normal (LLN) of LVEF in echocardiography as 50%. If LVEF decreases more than 10% to a value below the LLN (LVEF < 50%), angiotensin-converting enzyme inhibitors in combination with beta-blockers are recommended to prevent further LV dysfunction or the development of symptomatic heart failure.

A cardio-oncology expert panel from the French Working Group of Cardio-Oncology has tried to harmonize the most recent American and European guidelines to propose decision algorithms that would be easy for clinicians in their daily practice [11]. The French Working Group proposes complete cardio-oncological evaluation every three months during HER-2 treatment in all patients. Original trastuzumab-related FDA prescription instructions recommend cardiology consultation and withholding trastuzumab for four weeks if the LVEF falls by ≥ 16% from the baseline, or if LVEF falls ≥ 10% below the baseline and below the LLN. According to the prescribing information, trastuzumab can be safely restarted if the LVEF returns to normal and within 15% of the baseline [12]. According to the European Society for Medical Oncology consensus recommendations for cardiac disease management in cancer patients, asymptomatic patients undergoing trastuzumab treatment who have LVEF decrease of ≥ 10% from the baseline or a drop in LVEF to ≥ 40%; a value < 50% indicates a need for referral for cardiology consultation, preferably with a cardio-oncology specialist and consider initiation of cardioprotective treatments [13]. If trastuzumab is stopped, LVEF within three to six weeks should be repeated and it is recommended to resume trastuzumab therapy if LVEF has normalized to a value > 50%. Screening with an LVEF assessment should be considered at 6–12 months and possibly two years post-treatment, and consideration for reassessment periodically thereafter [14]. Recommendations are that patients undergoing trastuzumab therapy should have a baseline cardiovascular assessment of cardiac function including history, physical examination, EKG with QTc interval and determination of LVEF by quantitative 3D transthoracic echography, cardiac magnetic resonance or multigated acquisition scan. Routine use of cardiac biomarkers (cardiac troponins) in patients receiving or potentially receiving cardiotoxic therapy is insufficiently established. There are different views on the pre-treatment use of angiotensin-converting enzyme inhibitors or beta-blockers in patients at high cardiac risk [11].

Objective

The objective of this longitudinal observational analysis is to examine the safety profile and tolerability of long-term anti-HER-2 therapy with trastuzumab and its real efficacy in the treatment of metastatic HER-2-positive BC (HER-2+ MBC) patients in everyday clinical practice.

METHODS

A total of 48 HER-2+ MBC patients were analyzed retrospectively in this study. All the patients received long-term anti-HER-2 therapy with trastuzumab (period of application was longer than 20 months) simultaneously with other systemic treatment modalities (chemotherapy, hormonal therapy) regarding to characteristics of the disease since July 2004 at University Clinic for Radiotherapy and Oncology in Skopje. In 10 patients, the disease was initially diagnosed in stage IV, while in 38 patients, disease relapse was registered after initial treatment for early-stage BC. The study also included patients who had previously received trastuzumab as part of adjuvant treatment. HER-2 status was determined locally by immunohistochemical analysis or with *in situ* hybridization in accordance with the recommendations of the American Society of Clinical Oncology / College of American Pathologists, initially at primary diagnosis or with analysis of tumor tissue obtained by biopsy of the metastatic (secondary) lesion [15].

In 38 patients diagnosed initially with early breast cancer (eBC) in whom metastatic disease occurred later after disease-free interval, parameters related to the clinical and pathological features of the primary tumor and the adjuvant oncological treatment were analyzed: primary stage of the disease, hormone receptor status (estrogen receptor and progesterone receptor), adjuvant treatment with anthracyclines, and trastuzumab. The characteristics of metastatic disease and the type of treatment were analyzed in all 48 patients with HER-2+ MBC. Analyzed parameters included: presence of visceral metastases (lung, liver, pleura, peritoneum, pleural effusion, ascites), presence of non-visceral metastases (bones, skin, lymph nodes, contralateral breast),

presence of brain metastases, number and type of systemic therapies for metastatic disease treatment applied concurrently during trastuzumab therapy. The median time to the first progression of the disease (invasive disease-free survival) in 38 patients with initially diagnosed eBC was obtained by this analysis. In all 48 patients, the duration of trastuzumab therapy and trastuzumab toxicity were analyzed by obtaining LVEF values by echocardiography. The initial time-points for the above-mentioned statistical analyses were as follows: the date of initial diagnosis, the date of first relapse of the disease for patients with initially diagnosed eBC, and the date of initiation of trastuzumab therapy for metastatic disease in all 48 patients. The patients who were alive were censored with the date of the final observation point (data cut-off at April 2021).

Cardiac toxicity was assessed by obtaining of LVEF values according to standard clinical practice every three months during anti-HER-2 therapy application period with regard to the protocol (or more frequently as it was indicated). LVEF values were collected and evaluated at three time-points for each individual patient: at the beginning (LVEF before initiating trastuzumab treatment as the first-line treatment for MBC), in the middle (LVEF in mid-treatment period for each individual patient), and at the end of treatment period (LVEF final measurement) for each patient separately. Informed consent from the patients or family members of deceased patients who were included in the analysis was obtained to use their data for scientific purposes. The database with clinical and demographic characteristics of the patients was formed using the medical records and the electronic database of the Clinic.

This study was done in accordance with the institutional standards on Ethics.

Statistical analysis

Statistical analysis of the data was performed in the statistical program IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). The obtained data are presented in tabular and graphical form. Categorical variables are represented by absolute and relative numbers. Quantitative variables are presented with descriptive statistics (mean ± SDi, minimum and maximum values, median value, and interquartile range). The Kolmogorov–Smirnov test was used to test the normality of data distribution. The repeated-measures ANOVA analysis was used to compare the value of EF% in the three time-points. Kaplan–Meier survival analysis was used for invasive disease-free survival (iDFS). Statistical significance was defined at the level of $p < 0.05$.

RESULTS

The study included 48 patients with pathohistologically verified HER-2+ MBC. At the time of the study closure, 24 patients were alive and 24 were deceased. Analyzed patients were 27–69 years old, with a mean age of 47.2 ± 9.9 years. In patients with eBC as initial diagnosis (38 in total), the most common stage of disease was II – 19 (39.6%) patients. The distribution of patients by stage of disease is shown in Table 1.

Table 1. Distribution of patients by disease stage

Stage of disease	n (%)
I	3 (6.25)
IC	1 (2.08)
IIA	6 (12.5)
IIB	13 (27.08)
IIIA	5 (10.42)
IIIB	2 (4.17)
IIIC	8 (16.67)
IV	10 (20.83)
Total	48
live	24 (50)
deceased	24 (50)

Analysis of clinical parameters of patients with early breast cancer until metastatic disease onset

Analysis of data for applied adjuvant therapy in patients with initially diagnosed eBC showed that 28 (73.7%) patients received anthracyclines in the adjuvant setting, 18 (47.4%) patients were treated with adjuvant trastuzumab. Treatment with adjuvant trastuzumab in all included patients was conducted after treatment with anthracyclines (sequentially), concomitant with taxane therapy until the completion of one year adjuvant treatment. Locoregional relapse was initially reported in 10 (26.32%) patients and distant metastases in 28 (73.68%) patients. Time to onset of the first relapse of the disease (iDFS) ranged 13–216 months, with an average time of 79.2 ± 52 months. In half of the patients, the time to the first relapse occurrence was less than 55.5 months.

Analysis of clinical parameters of patients with metastatic disease

This group includes 48 patients (10 patients who were initially diagnosed with stage IV disease and 38 patients with eBC beginning from the moment of metastatic disease diagnosis). Table 2 shows the most common sites of distant metastases, the data for applied systemic therapies concomitant with trastuzumab, types of systemic therapy (lines of chemotherapy and endocrine therapy applied sequentially), treatment discontinuation, and causes for treatment discontinuation. The average duration of trastuzumab treatment was 52.2 ± 23.5 months. The shortest time of receiving trastuzumab was 20 months (in one patient), while the longest was 113 months (also in one patient). In half of the patients, the duration of trastuzumab treatment was longer than 48 months. Data on duration of trastuzumab treatment are shown in Table 3.

In 17 (35.4%) patients the treatment was discontinued; in 11 (22.92%) patients the interruption lasted longer than four months, while in six (12.5%) the interruption was shorter than four months. The reason for discontinuation of trastuzumab treatment longer than four months was initiation of a

Table 2. Sites of relapses, types and lines of applied concomitant systemic therapies, treatment discontinuation and causes for treatment discontinuation in patients with metastatic breast cancer

DM type	n (%)
visceral	15 (31.25)
non-visceral	17 (35.4)
brain	2 (4.17)
mixed (visceral + non-visceral)	11 (23.4)
mixed (visceral + brain)	3 (6.25)
Applied lines of chemo +/- hormone therapy	
1	18 (37.5)
2	15 (31.25)
3	6 (12.5)
4	6 (12.5)
5	2 (4.17)
6	1 (2.08)
Type of therapy	
chemotherapy	17 (35.42)
chemo+ hormone therapy	26 (54.17)
hormone therapy	5 (10.42)
Discontinuation of treatment	
yes	17 (35.42)
no	31 (64.58)
Cause of treatment discontinuation	
Decline of LVEF	3
Anthracyclines toxicity	8
other	6
Discontinuation of treatment (months)	
< 4 months	6 (12.5)
> 4 months	11 (22.92)

DM – distant metastases; LVEF – left ventricular ejection fraction

Table 3. Duration of anti HER-2 treatment with trastuzumab (months)

Descriptive statistics		
Duration of trastuzumab treatment (months)		
Mean ± SD	Median (IQR)	Min–max
52.2 ± 23.5	48 (35–66)	20–113

IQR – interquartile range

new line of treatment with chemotherapy regimen containing anthracyclines or therapy with T-DM1 (ado-trastuzumab emtansine). There was no discontinuation in trastuzumab treatment in 31 (64.58%) patients. Three (6.25%) patients had discontinuation of trastuzumab because of a decline of LVEF below 50%. In two of these three patients, discontinuation of treatment was longer than four months and anti-HER-2 treatment was resumed after normalization of LVEF. In one patient, anti-HER-2 treatment was not continued due to low LVEF and disease progression. Table 2 presents the causes for treatment discontinuation.

The mean LVEF values were 66.73 ± 7.02%, 64.62 ± 5.7% and 63.44 ± 6.1%, at the beginning, median, and end of treatment, respectively (Table 4). According to the results in Table 5, the mean values of LVEF differed significantly in the observed three time-points (F = 4.9 p = 0.009). *Post hoc* pairwise comparison, using Bonferroni correction, confirmed significantly lower mean LVEF values at the endpoint (at the end of treatment) compared with the mean LVEF values at the beginning of anti-HER-2 treatment (p = 0.019) but within the reference range of LVEF ≥ 50%.

DISCUSSION

This longitudinal observational analysis included 48 patients with metastatic BC treated with trastuzumab for more than 20 months. The mean duration of trastuzumab treatment was 52.2 ± 23.5 months. In 17 (35.4%) patients the treatment with trastuzumab was temporarily discontinued, in 11 (22.92%) of them the discontinuation was longer than four months, while in six (12.5%) the discontinuation was shorter than four months. Discontinuation of trastuzumab therapy was mostly due to initiation of anthracycline-containing regimen because of disease progression. Only in three (6.25%) patients, the reason for discontinuation of treatment was decline in LVEF below 50%. Of these three patients, in two patients the discontinuation of treatment was longer than four months and anti-HER-2 treatment was resumed after normalization of LVEF, while in one patient anti-HER-2 treatment was not continued due to low LVEF and disease progression. The mean LVEF was 66.73 ± 7.02%, 64.62 ± 5.7%, and 63.44 ± 6.1%, at the beginning, middle, and the end of treatment, respectively. *Post hoc* pairwise comparison, according to Bonferroni correction, showed significantly lower mean value of LVEF in the third analyzed point (last evaluation of LVEF at the end of treatment) compared to the starting point (p = 0.019), but this difference did not exceed the referent values. These results support favorable safety profile of long-term therapy with trastuzumab, which was 52 months in this study. Trastuzumab is a milestone in the treatment of HER-2-positive BC. However, the data on the safety of long-term use of trastuzumab in a metastatic setting are scarce. One review study detected four trials for long-term safety of trastuzumab in metastatic BC [16]. The LHORA study reported 2.2% of trastuzumab-related

Table 4. Mean values of left ventricular ejection fraction (beginning, median, and end of treatment)

Variable	Descriptive statistics		
	Mean ± SD	median (IQR)	Min–max
1. LVEF% (start of treatment)	66.73 ± 7.02	66 (61.5–69)	58–101
2. LVEF% (median of treatment)	64.62 ± 5.7	64 (60–68)	55–83
3. LVEF% (end of treatment)	63.44 ± 6.1	64 (60–68)	46–75

IQR – interquartile range; LVEF – left ventricular ejection fraction

Table 5. *Post hoc* pairwise comparison (Bonferroni correction)

Repeated measures ANOVA F = 4.9; p = 0.009 sig		
	2	**3**
1	0.20ns	**0.019 sig**
2		0.459 ns

ns – non-significant; sig – significant; ANOVA – analysis of variance; adjustment for multiple comparisons: Bonferroni

cardiotoxicity in patients with progression-free survival > 3 years receiving first-line treatment with trastuzumab without discontinuation of the treatment [17]. This may be particularly important in the context of the new clinical reality which is the use of novel anti-HER-2 targeted therapies that enable significant increase in disease-free survival and overall survival in patients MBC (pertuzumab, trastuzumab emtansine, lapatinib, neratinib, trastuzumab deruxtecan, margetuximab, tucatinib).

Trastuzumab deruxtecan (T-DXd) is a HER-2-targeting antibody-drug conjugate approved for patients with advanced HER-2+ MBC based on the results from the DESTINY-Breast01 study [18, 19]. According to the DESTINY-Breast03 study, the most common treatment emergent adverse event associated with treatment discontinuation for T-DXd was interstitial lung disease / pneumonitis, while LVEF decline was seen in 2.7% [20]. Many ongoing trials evaluate toxicity profile and safety of combined anti-HER-2 agents. Ongoing DESTINY-Breast09 trial will evaluate the efficacy and safety of trastuzumab deruxtecan, either alone or in combination with pertuzumab, in treating patients with HER-2-positive BC as a first line of treatment (San Antonio Breast Cancer Symposium 2021 Abstract OT1-14-02).

The data obtained in our study related to cardiac toxicity, which was registered in 6.25% of the patients, are comparable to those obtained in the CLEOPATRA study [21]. In this study, the median follow-up time was longer than 50 months, with reported left ventricular dysfunction lower in the pertuzumab group than in the control group (6.6% *vs*. 8.6%). The data on the toxicity of long-term use of trastuzumab in daily clinical practice are of great importance in decision making for patients' treatment with poorer performance status, comorbidities, older age, symptomatic disease, or the combination of the aforementioned characteristics. The patient population in daily clinical practice is generally less selected or unselected compared to randomized clinical trials, while data from these studies may be somehow limited in terms of data generalization.

The presented data are obtained mostly from observational studies prior to the introduction of dual anti-HER-2 therapy with pertuzumab/trastuzumab and taxanes as standard of care in a first-line treatment setting for MBC HER-2-positive patients. A particularly interesting research area is a possible increase in cardiotoxicity due to dual anti-HER-2 blockade. In addition, future trials for new agents targeting HER-2 should focus on cardiotoxicity, as they represent a new standard in subsequent treatment settings. Women's Heart Centers are a globally adopted follow-up solution and can offer comprehensive care for women cancer survivors [22].

CONCLUSION

The presented data are in correlation with the favorable safety profile and tolerability of trastuzumab in patients with MBC treated with prolonged trastuzumab therapy. The low incidence of registered cardiac events confirms the favorable safety profile of long-term therapy. However, cardiac monitoring on regular intervals tailored to each patient during and after treatment is necessary, especially for high-risk patient subgroups. In patients with stable heart function and low cardiac risk, it is possible to adjust the period of cardiac monitoring to longer time intervals. Data from daily clinical practice confirm the efficacy of trastuzumab in an unselected patient population.

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Кардиотоксичност код дуготрајне анти-HER2 терапије трастузумабом у случају болесника са метастатским карциномом дојке позитивним на HER2 – подаци из свакодневне клиничке праксе

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САЖЕТАК

Увод/Циљ Ова анализа има за циљ да истражи кардиотоксичност дуготрајне терапије трастузумабом код болесника са метастатским карциномом дојке позитивним на HER2.

Методе Студија је ретроспективно анализирала 48 болесница са метастатским карциномом дојке позитивним на HER2. Болесници су примали дуготрајну анти-HER2 терапију трастузумабом (време примене дуже од 20 месеци). Анализиране су следеће карактеристике болесника: старост, почетни стадијум болести, примена анти-HER2 терапије трастузумабом и антрациклинима у адјувантном контексту, број и врста аплицираних системских терапија конкурентно са трастузумабом у лечењу метастатске болести. Кардијална токсичност је процењена коришћењем вредности левовентрикуларне ејекционе фракције (ЛВЕФ) у три временске тачке: на почетку, на средини и на крају периода лечења сваког болесника.

Резултати Код 17 (35,4%) болесника лечење трастузумабом је привремено прекинуто. Просечна вредност ЛВЕФ-а била је 66,73 ± 7,02%, 64,62 ± 5,7% и 63,44 ± 6,1%, појединачно. Просечне вредности ЛВЕФ-а су се значајно разликовале у евалуиране три временске тачке ($F = 4,9$; $p = 0,009$). Пост-хок анализа парова, коришћењем Бонферонијеве корекције, показала је значајно ниже средње вредности ЛВЕФ-а на крају третмана (на крајњој тачки), у поређењу са средњим вредностима ЛВЕФ-а на почетку анти-HER2 третмана трастузумабом ($p = 0,019$), али унутар референтне вредности ЛВЕФ-а ≥ 50%.

Закључак Резултати потврђују добар безбедносни профил дуготрајне анти-HER2 терапије трастузумабом код болесника са метастатским карциномом дојке позитивним на HER-2 у погледу кардиотоксичности.

Кључне речи: рак дојке; кардиотоксичност; ејекциона фракција; безбедност; трастузумаб

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Neuropsychological manifestations in rheumatic patients with chronic pain

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SUMMARY

Introduction/Objective Patients with rheumatoid arthritis (RA), osteoarthritis and fibromyalgia, alongside chronic pain, often suffer from functional disabilities, as well as cognitive dysfunction.
The objective of this study is to compare the intensity of pain, symptoms of depression, anxiety, and memory ability among rheumatic patients with chronic pain and to compare rheumatic patients to a control group of healthy participants.

Methods The cross-sectional study, which included 110 (82 female; 28 male) patients with chronic pain, was done at the Special Hospital for Rheumatic Diseases, Novi Sad. Depression was determined by Beck's Depression Inventory, anxiety was diagnosed by Spielberger's anxiety test, and memory was assessed by the Wechsler Memory Scale.

Results Mean pain intensity in patients with fibromyalgia were statistically significantly higher compared to patients with osteoarthritis and RA ($p < 0.05$). A statistically significant difference in the psychological status of patients ($p < 0.001$) and patient memory ($p < 0.05$) with chronic pain was established, compared to patients in the control group. There was no statistically significant difference in the psychological status of patients, patient memory level, and pain intensity in patients with positive fibromyalgia test results in comparison to rheumatic patients not meeting the criteria for fibromyalgia. Patients with osteoarthritis had a statistically significantly lower memory coefficient in comparison to patients with RA and fibromyalgia.

Conclusion In RA, osteoarthritis, and fibromyalgia patients, clinical factors such as pain, depression, and anxiety play an active role in cognitive impairment and should be considered when planning treatment.

Keywords: chronic pain; rheumatic diseases; emotions; memory

INTRODUCTION

Pain is an unpleasant and complex sensory and emotional experience [1]. A mutual interaction between pain and cognitive processing was determined [1]. Pain impairs cognitive functioning, while cognitive functioning may reduce the level of pain perception [1].

Chronic pain and depressive symptoms are often associated clinically, and together they make treating patients difficult. Depressive symptoms could prolong duration of pain as well as increase its intensity [2].

Depression and anxiety are psychiatric disorders which could be associated with rheumatoid arthritis (RA), osteoarthritis (OA), and fibromyalgia (FM). The explanation for this association could be biological, possibly cytokine-related, or it can be explained by prolonged negative impact of medical condition on mental health of patient [3].

Cognitive impairment is defined by memory loss, difficulties in learning new things, solving problems, decision making, or problems with concentration. There is evidence that RA influences cognitive processing [4].

Patients with chronic pain and FM have deficits in working memory [5].

Several studies showed a high level of affective disorders in patients with chronic pain [6]. Severe depression is prevalent among patients with chronic pain. With the development of pain into a chronic condition, disorders such as anxiety, anhedonia, sleep disorders, cognitive impairments, and suicide may occur along with negative emotional states [6].

Pain, anxiety, and depression may impair work ability in different chronic rheumatic diseases [7]. There is association of chronic pain and neurocognitive impairment [7]. The pain may cause an emotional distress, which also contribute to reduce the patient ability to cope with chronic pain self-management as well as with the overall functioning [7]. However, it is not easy to define the correlation between pain and neurocognitive impairment. There is a possibility that pain can cause cognitive impairment or vice versa, that cognitive impairment is associated with higher risk of chronic pain. [7]

The challenges of chronic disease could potentially be decreased, and patient-oriented care could be improved by interventions

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Table 1. Demographic characteristics and pain among groups

Characteristics		CO	RA Medium DA	RA High DA	OA	FM
N		30	26	14	40	30
Sex (n, %)	M	3 (10)	7 (26.92)	4 (28.57)	14 (35)	3 (10)
	F	27 (90)	19 (73.08)	10 (71.42)	26 (65)	27 (90)
Years (X ± SD)		48.3 ± 6.42	47.81 ± 7.53	48.47 ± 5.9	59 ± 10.31	55.1 ± 12.71
Pain (X ± SD)		Without pain	53.19 ± 22.01	58.54 ± 19.25	52.10 ± 18.79	*66.07 ±22.05

X – mean value; SD – standard deviation; CO – control group; RA – rheumatoid arthritis; DA – disease activity; OA – osteoarthritis; FM – fibromyalgia; *statistically significant ($p < 0.05$)

Table 2. Results of psychological tests in patients with chronic pain compared to the control group

Parameter	CO	RA-MDA	RA-HDA	OA	FM
BDI (X ± SD)	6.93 ± 4.49	16.39 ± 12.65*	19.13 ± 10.25*	12.72 ± 8.4	18.18 ±12.61*
STAI-S (X ± SD)	41.63 ± 7.83	52.25 ± 5.66	55.17 ± 5.01*	53.12 ± 6.94	54.68 ± 6.34
STAI-T (X ± SD)	40.73 ± 8.01	49.06 ± 6.86	50.04 ± 6.84	49.15 ± 6.24	49.32 ± 6.57
WMS (X ± SD)	136.37 ± 6.07	125.11 ± 14.18*	124.08 ± 13.03*	108.95 ± 19.05**	123.75 ± 15.39*

X – mean value; SD – standard deviation; CO – control group; DA – disease activity; RA – rheumatoid arthritis; MDA – medium disease activity; HDA – high disease activity; OA – osteoarthritis; FM – fibromyalgia; BDI – Beck's Depression Inventory; STAI-S – Spielberger Trait Anxiety Inventory State; STAI-T – Spielberger Trait Anxiety Inventory Traité; WMS – Wechsler Memory Scale;
*statistically significant ($p < 0.05$);
**highly statistically significant ($p < 0.001$)

focused on the symptoms. To adequately inform clinical practice, additional studies of treatment interventions are necessary. Continued workplace engagement in patients with chronic health conditions could be supported by further improvement of workplace accommodation and other such interventions [8].

METHODS

The study included 110 (40 with RA, 40 with OA, 30 with secondary FM) (82 female, 28 male) patients with chronic pain. The control group consisted of 30 healthy subjects (CO group). The research was started in April of 2021 and finished in November of 2021, in the Special Hospital for Rheumatic Diseases, Novi Sad. The RA group satisfied the criteria for the diagnosis of RA of American College of Rheumatology Association (2010 ACR/EULAR criteria) [9], the FM group met the modified ACR criteria [10], and the OA group met ACR criteria [11, 12, 13]. The exclusion criteria were as follows: neurological and psychiatric diseases, hearing impairment, dementia, head trauma, any use of psychoactive substances and antidepressants at least a month before the study. The data from history, clinical examination, and questionnaires were used in the study. The following parameters were collected in the RA group: joint pain defined by a visual analogue scale (VAS) of 0–100 mm, painful and swollen joint count, erythrocyte sedimentation rate, and Disease Activity Score (DAS 28), which divided patients into two groups (RA-MDA – medium disease activity – DAS 28 > 3.2 < 5.1; and RA-HDA (high disease activity – DAS 28 > 5.1). The test for evaluation of emotional manifestations, Spielberger's anxiety test (Spielberger Trait Anxiety Inventory State and Trait), was applied for determination of anxiety, which includes STAI-S and STAI-T questionnaires for standardized measurement of current and general anxiety. A score below or equal to 30 points defines a low level of anxiety, while a score of 31–44 indicates a moderate level, and a score above 45 represents a high level of anxiety. Beck's depression scale (Beck's Depression Inventory – BDI) was used for the evaluation of depression. BDI total score defines the severity of depression. The scores above 30 imply severe clinical depression. Wechsler Memory Scale (WBsp Form 1) was used to detect verbal and non-verbal memory functioning with correction for the age group.

The study has been approved by the Ethics Committee of the Special Hospital for Rheumatic diseases, Novi Sad, Serbia (ethical approval number 14/28-5/1-21).

Statistical methods

The descriptive statistics measures were applied as follows: mean, frequency, measures of variation. Parametric (Student's t-test) and nonparametric (Mann–Whitney test, χ^2 test) were used to compare two groups. The analysis of variance ANOVA and Kruskal–Wallis test were used to compare three or more groups. Pearson correlation coefficient was used for correlation of continuous variables.

RESULTS

There were 40 patients with RA (26 RA-MDA; 14 RA-HAD), 40 patients in the OA group, and 30 patients in the FM group, as well as the control group consisting of 30 subjects of good health (CO group) who had no significant age difference in comparison to the chronic pain groups (CV < 30) (Table 1). The average pain intensity values determined using VAS were statistically significantly higher in patients with FM than in patients with RA and patients with degenerative disorders (Table 1).

Average depression score assessed by BDI was 7.41 ± 0.84 in the CO group, 16.39 ± 12.65 in the RA-MDA group, 19.13 ± 10.25 in the RA-HDA group, 12.72 ± 8.4 in the OA group, 18.18 ± 12.61 in the FM group (Table 2). There was statistically significant difference of depression scores between all three groups and the control group ($p < 0.001$) (Table 2), and a statistically significant difference of the depression scores between the FM, RA-HDA, RA-MDA, and OA group ($p < 0.05$) (Table 2).

Average state anxiety score assessed by STAI-S test was 41.63 ± 7.83 in the CO group, 52.25 ± 5.66 in the RA-MDA group, 55.17 ± 5.01 in the RA-HDA group, 53.12 ± 6.94 in the OA group, and 54.68 ± 6.34 in the FM group. Statistically significant difference in state anxiety scores were found between all three groups and the control group ($p < 0.001$) (Table 2). However, no statistically significant difference in state anxiety scores were detected between the groups of patients with chronic pain (Table 2).

Average general anxiety score by STAI-T test was 40.73 ± 8.01 in the CO group, 49.06 ± 6.86 in the RA-MDA group, 50.04 ± 6.84 in the RA-HDA group, 49.15 ± 6.24 in the OA group, 49.32 ± 6.57 in the FM group. Statistically significant difference of general anxiety scores was found between the control group and all three groups of patients with chronic pain ($p \leq 0.001$) (Table 2).

The results of the STAI-S test differ in patients with RA grouped against DAS 28 disease activity (RA-MDA, DAS 28 ≥ 3.2 < 5.1; RA-HAD, DAS 28 > 5.1); no differences in BDI, STAI-T, WMS in relation to activity of the disease in patients with RA were found (Table 2).

There was a statistically significant difference in memory coefficients using the Wechsler Memory Scale between RA patients (RA-MDA 125.11 ± 14.18; RA-HDA 124.08 ± 13.03; OA group 108.95 ± 19.05; FM 123.75 ± 15.39) and healthy controls (136.37 ± 6.06) (RA *vs.* CO $p < 0.05$; FM *vs.* CO $p < 0.05$), (OA *vs.* CO $p < 0.001$). The statistically significant lower memory values were present in the OA group compared with the RA and the FM group ($p < 0.001$).

DISCUSSION

Our results showed the highest depression score (BDI) and current and general anxiety score (STAI -S / STAI-T) in patients with RA-HDA, and the lowest BDI score in patients with OA.

Patients with RA have a high prevalence of psychiatric comorbidities, which aligns with our findings. The lifetime prevalence of depression is estimated to be 41–66%, and for anxiety disorders it amounts to 70% [14, 15, 16].

By examining the differences in the degree of anxiety and depression of patients with RA and the control group, a statistically significant difference was established in the coefficients of depression among people with RA (BDI, RA-MDA 16.39 ± 12.65; RA-HAD 19.13 ± 10.25), and CO (6.93 ± 4.49) ($p < 0.001$), as well as in instantaneous scores (STAI-S) and general anxiety (STAI-T) ($p < 0.001$), which is in accordance with the literature data [17, 18].

Ozcetin et al. [19] compared patients with RA, FM, and knee OA in regards to anxiety and depression, and found the highest average BDI score in FM patients and the lowest in patients with OA knee, the lowest values of anxiety in FM, and the highest in RA.

FM often coexists with RA, which we can see in our results. Of the 30 patients with a positive test for FM, 25 (83.33%) already had a diagnosis of RA. These results are confirmed by the research of Wolfe et al. [20], which indicate a significant prevalence of FM and FM-associated findings in patients with RA.

We noted statistically significantly higher scores for anxiety and depression as well as the lower values of memory coefficients in people with FM compared to the CO group, which was found in previous studies [21, 22]. Cognitive impairment was found in patients with FM, which is contrast to the healthy controls, and the level of emotional disorders (depression and anxiety) may explain the heterogeneity of studies [23].

Additionally, data from the literature indicate that most patients with FM who have anxiety and depression also have poor quality of life and do not have adequate social support [24].

Our study did not find a statistically significant difference in the psychological status of patients (depression, current and general anxiety of patients), patient memory level, and intensity of pain in patients with positive FM test results in comparison to patients with rheumatic diseases who do not meet the criteria for FM. In contrast, the findings of Katz et al. [24] found that in patients with FM there are more incidences of memory impairment (70.2–24.6%), mental confusion (56.1–12.3%), and speech difficulty (40.4–3.5%), compared to patients with rheumatic diseases who do not meet the criteria for FM.

Our results also showed statistically significantly lower values of memory coefficients in patients with chronic pain (RA *vs.* CO $p < 0.05$), (OA *vs.* CO $p < 0.001$), (FM *vs.* CO $p < 0.05$) compared to the CO group. The previous study on cognitive impairment in patients with RA compared to healthy subjects indicates a higher incidence of the presence of memory problems in patients with RA [25]. Furthermore, Roldán-Tapia et al. [26] have shown that patients with RA and FM developed substantial cognitive impairments, which could not be easily explained by the pathology of these chronic diseases.

Our results have shown that patients with OA had statistically significantly lower memory level than patients with RA and FM. The association between memory loss and OA is not fully explored. OA and joint pain are clearly linked with memory loss. The sleep and mood disorders could be associated with memory loss [27].

Pain has an immense impact on functional capacity and quality of life in patients with knee OA. The focus of the assessment should not be solely on the afflicted organ, but rather the importance should be given to functional disorders (physical, emotional, social), which patients may experience because of their illness [28].

The link between FM and depressive disorders could be the similarity of symptoms, such as sleep problems and

fatigue and shared biological and psychological mechanisms [29].

It is likely that depression in patients with RA is not only a consequence of the distress and disability caused by RA, but also contributed to by immunological changes. Targeting immunological pathways can, therefore, be used to lessen the mental burden in addition to treating RA. Regardless of immunological intervention, multidisciplinary approach including the psychiatrist and psychologist support is important in successful disease management [30]. Chronic pain is responsible for making patients more susceptible to mood disorders that can further deteriorate the perception, intensity, and duration of nociception, thereby making treatment and diagnosis more challenging [31]. Studies have shown that a genetic predisposition to chronic pain is linked to an increased risk of depression [32].

CONCLUSION

Our study established a significant difference in the psychological status of patients (depression, anxiety) and the level of memory in patients with chronic pain (RA, OA, FM) compared to the CO group. Patients with FM had more intense pain compared to patients with OA and RA. Patients with OA had significantly lower memory coefficient values, as well as significantly lower average BDI values compared to patients with RA and FM. These results should be incorporated into the treatment approaches of such chronic and debilitating conditions. Longitudinal studies are required to confirm the cross-sectional findings.

Conflict of interest: None declared.

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Неуропсихолошке манифестације код реуматолошких болесника са хроничним болом

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САЖЕТАК

Увод/Циљ Болесници са реуматоидним артритисом, остеоартритисом и фибромијалгијом често поред хроничног бола имају функционалну онеспособљеност, као и когнитивне дисфункције.

Циљ рада је био поређење интензитета бола, симптома депресије, анксиозности и способности памћења између болесника са реуматским болестима и хроничним болом, као и поређење болесника са реуматским болестима са контролном групом здравих испитаника.

Методе Студија пресека у коју је укључено 110 (82 жене, 28 мушкараца) болесника са хроничним болом спроведена је у Специјалној болници за реуматске болести у Новом Саду. Депресија је процењивана применом Бекове скале, анксиозност коришћењем Спилбергеровог упитника, меморија применом Векслерове скале меморије.

Резултати Средње вредности интензитета бола код болесника са фибромијалгијом биле су статистички значајно више у односу на болеснике са остеоартритисом и реуматоидним артритисом (РА) ($p < 0,05$). Утврђена је статистички значајна разлика у психолошком статусу ($p < 0,001$) и памћењу болесника ($p < 0,05$) са хроничним болом у односу на болеснике у контролној групи. Није било статистички значајне разлике у психолошком статусу, нивоу памћења и интензитету бола код болесника са секундарном фибромијалгијом у односу на болеснике са РА који не испуњавају критеријуме за фибромијалгију. Болесници са остеоартритисом имали су статистички значајно нижи коефицијент меморије у односу на болеснике са РА и фибромијалгијом.

Закључак Код болесника са РА, остеоартритисом, и фибромијалгијом, клинички фактори као што су бол, депресија и анксиозност играју значајну улогу у когнитивним поремећајима, те би на то требало обратити пажњу при планирању лечења.

Кључне речи: хронични бол; реуматске болести; емоције; меморија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Diagnostic significance of immunophenotyping of peripheral blood lymphocytes in pediatric patients from the Autonomous Province of Vojvodina, Republic of Serbia

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SUMMARY

Introduction/Objective Although lymphocyte immunophenotyping based on flow cytometry is a powerful tool in the diagnosis of many primary immunodeficiences (PID), there has been an increasing awareness of associated costs and the need for its reassessment as a screening tool.
We present the results and diagnostic impact of immunophenotyping performed by flow cytometry in the University Children's Hospital, Belgrade, in a series of patients referred from the Institute for Child and Youth Health Care of Vojvodina from July 2008 to July 2018.

Methods We reviewed the laboratory reports on numbers of B lymphocytes (CD19+), T lymphocytes (CD3+), natural killer cells (CD3–CD16/CD56+) and activated T cells (CD3+HLA-DR+), as well as CD4+ and CD8+ T cells in 198 children.

Results Patients were grouped by stated indication into the following eight categories: hypogammaglobulinemia (34), selective IgA deficiency and/or IgG subclass deficiency (43), various infections with no immunoglobulin deficiencies (67), asthma and/or allergies with no immunoglobulin deficiencies or infections (23), known or suspected autoimmune disorders (24), and miscellaneous diagnoses not accompanied by infections (7). In total, 159 (80.3%) findings were either completely within the respective reference range or exhibited only minimal aberrations. Four patients were diagnosed with Bruton's disease and one with Artemis immunodeficiency. Nineteen patients were given immunoglobulin substitution to control infections and/or maintain immunoglobulin G levels.

Conclusion Lymphocyte immunophenotyping aids the diagnosis of PID in selected patients. We venture some thoughts on how the usefulness of this laboratory method could be improved in real-life tertiary care pediatric hospital settings.

Keywords: immunophenotyping; flow cytometry; lymphocytes; immunodeficiency; children

INTRODUCTION

Immunophenotyping of blood lymphocytes by flow cytometry is a valuable diagnostic tool in many primary and some secondary immunodeficiencies. In its basic capacity, this method usually yields information about absolute and relative abundances of main lymphocyte populations: B lymphocytes ($CD19^+$), T lymphocytes ($CD3^+$) and natural killer (NK) cells ($CD3^-CD16/CD56^+$), as well as main T-cell subpopulations ($CD4^+$ helper T cells and $CD8^+$ cytotoxic/suppressor T cells, with calculation of the CD4:CD8 ratio), and the percentage and absolute number of activated T cells ($CD3^+HLA\text{-}DR^+$). A rare, but diagnostically important subpopulation of "double negative" T cells ($CD4^-CD8^-$) can also be detected [1].

Primary immunodeficiency disorders (PID) that can be diagnosed and evaluated in this way include severe combined immunodeficiency, Omenn syndrome, Wiskott–Aldrich syndrome, agammaglobulinemia (X-linked or autosomal recessive), and primary NK cell deficiencies, while in many other PID immunophenotyping can significantly contribute to the establishment of diagnosis or the assessment of disease severity [2]. This is particularly true for 22q11.2 deletion syndrome (Di George syndrome), where enumeration of immune cells is an obligatory part of patient workup [3, 4], and autoimmune lymphoproliferative syndrome (ALPS; [5, 6]. Likewise, in children (as in adults) with hypogammaglobulinemia, selective IgA deficiency or IgG subclass deficiency, most diagnostic protocols require a checkup of absolute number of B cells in order to uncover potential B-cell immunodeficiencies [7, 8]. In addition, many other PID, such as hyper-IgM syndrome, are amenable to diagnosis by flow cytometry using antibodies specific to the molecule with impaired expression [9].

Among secondary immunodeficiencies, by far the most important indication for

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immunophenotyping is the need for enumeration of $CD4^+$ T lymphocytes in human immunodeficiency virus-infected persons [10]. The method is also used in monitoring the effects of immunosuppressive treatment, particularly when rituximab is administered, or planned to be administered [11]. In other secondary immunodeficiency settings, immunophenotyping usually plays only a limited diagnostic role; it is, however, often necessary to perform it in order to rule out (some) PID in cases when the etiology is less than clear [12]. It should be emphasized that, according to most diagnostic protocols and recommendations, immunophenotyping does not constitute a first- (nor even a second-) level laboratory test [13, 14, 15]. Following the appropriate ordering of tests is crucial for both diagnostic efficiency and cost-effectiveness. On the other hand, early diagnosis of PID is imperative, since it is a major determinant of long-term prognosis.

In this paper, we present the general results and diagnostic impact of immunophenotyping performed by flow cytometry in the Laboratory for Immunology, University Children's Hospital, Belgrade, in a series of patients referred by immunologists from the Child and Youth Health Care of Vojvodina.

METHODS

We reviewed the laboratory reports of 198 children aged from two months to 16 years (76 girls and 122 boys) who were referred by a pediatric immunologist from the Institute for Child and Youth Health Care of Vojvodina for immunophenotyping of peripheral blood lymphocytes to the University Children's Hospital in the period from July 2008 to July 2018.

Briefly, immunophenotyping was performed on Beckman Coulter FC500 Flow Cytometer (Beckman Coulter Inc., Brea, CA, USA) using commercial fluorochrome-conjugated monoclonal antibodies (Miltenyi Biotec, Bergisch Gladbach, North Rhine-Westphalia, Germany) with specificity to human molecules CD3, CD4, CD8, CD16, CD19, CD45, CD56 and HLA-DR. Lymphocyte population was gated on the diagram representing the intensity of CD45 expression and side scatter. In this population, percentages of B lymphocytes ($CD19^+$), T lymphocytes ($CD3^+$), NK cells ($CD3^-CD16/CD56^+$) and activated T cells ($CD3^+HLA\text{-}DR^+$) were determined. Absolute numbers of these subpopulations were calculated based on total lymphocyte number determined using an automated cell counter. The CD4/CD8 ratio was also calculated. Measured abundances of all subpopulations were compared to age-specific reference ranges [16]. For the purpose of this study, we separately evaluated deviations from reference values in the following categories: increase in absolute number of T, B, or NK cells, and alterations of the CD4/CD8 ratio not accompanied by abnormal absolute number of $CD4^+$ or $CD8^+$ cells. These were designated as minimal aberrations (MA).

This paper has been approved by the Ethical Committee of the University Children's Hospital in Belgrade.

RESULTS

According to stated indications for analysis, all patients can be grouped into following eight categories: hypogammaglobulinemia (34), selective IgA deficiency and/or IgG subclass deficiency (43), various infections with no immunoglobulin deficiencies (67) asthma and/or allergies with no immunoglobulin deficiencies or infections (23), known or suspected autoimmune disorders (24), and miscellaneous diagnoses not accompanied by infections (7). The miscellaneous category was comprised of one child with short bowel syndrome after ileostomy performed after repeated episodes of gastroenterocolitis of unknown etiology in infancy, one with ill-defined neutrophil defects and a developmental disorder, one with ataxia, one with unexplained lymphocytosis, one with fever of unknown origin and one whose records, including reasons for referral, have been lost.

All investigated populations were found to be in their reference ranges in 11 (32.4%) of patients with hypogammaglobulinemia; 23 (53.5%) of patients with selective IgA deficiency and/or IgG subclass deficiency; 32 (47.8%) of patients with infection without immunoglobulin deficiencies; 14 (60.9%) of patients with asthma and/or allergies with no immunoglobulin deficiencies or infections, 11 (45.8%) of patients with autoimmune disorders and four (57.1%) patients in the miscellaneous group, for a total of 95 (48.0%) completely normal findings overall.

However, when we add to normal findings those in the MA category, as defined above, the numbers of patients with unremarkable immunophenotype were as follows: 26 (76.5%) of patients with hypogammaglobulinemia; 40 (93.0%) of patients with selective IgA deficiency and/or IgG subclass deficiency; 51 (76.1%) of patients with infection without immunoglobulin deficiencies; 17 (73.9%) of patients with asthma and/or allergies with no immunoglobulin deficiencies or infections, 19 (79.2%) of patients with autoimmune disorders and six (85.8%) patients in the miscellaneous group, for a total of 159 (80.3%) findings that are either completely within the respective reference range or exhibit only MA.

By year of analysis, the proportion of normal findings was 2/5 (40%) in 2008, 3/5 (60%) in 2009, 2/5 (40%) in 2010, 2/12 (16.7%) in 2011, 2/8 (25%) in 2012, 1/9 (11.1%) in 2013, 6/11 (54.5%) in 2014, 16/28 (57.1%) in 2015, 23/46 (50%) in 2016, 35/43 (81.4%) in 2017, and 10/26 (38.4%) in 2018.

B-cell defects

A reduced absolute number of B cells ($CD19^+$) was found in eight patients (23.5%) with hypogammaglobulinemia (five of whom, or 14.7% of all hypogammaglobulinemia patients, had severely decreased number of B cells, defined as < 2% of total lymphocytes); two (4.6%) patients with selective IgA deficiency and/or IgG subclass deficiency; eight (11.9%) patients with infections, two of whom (3%) had a severe decrease (with one of those two showing prompt recovery of B-cell number, returning to normal

range on follow-up examination two months later); three (13%) patients with asthma/allergies; six (25%) patients with autoimmune disorders, one of whom with pancytopenia (4.1%) had a severe decrease exhibiting a reduction of all lymphocyte subpopulations, with recovery on subsequent investigations (although the absolute B-cell number was rather slow to normalize, remaining somewhat below the reference range after two months); and none of the patients in the miscellaneous group. An increase in the absolute number of B lymphocytes, regarded as MA in this study, was noted in two patients (5.9%) with hypogammaglobulinemia, one patient (2.3%) from the selective IgA deficiency/IgG subclass deficiency group, two patients (3%) with infections, and one patient (4.2%) in the autoimmunity group.

Among the patients immunophenotyped for hypogammaglobulinemia, four were genetically diagnosed with a hereditary B-cell defect (Bruton's disease), while one turned out to have a combined (Artemis) deficiency. All of those five patients had relative B cell numbers below 2%.

T-cell defects

The absolute number of T cells ($CD3^+$) was found to be below the lower boundary of the age-appropriate reference range in one patient with hypogammaglobulinemia (2.9%), one patient in the infections group (1.5%), two patients with autoimmunity (4.2%) and one patient in the miscellaneous group (14.3%). On the other hand, an increase of the absolute number of T cells above the reference range (MA finding) was noted in two patients with hypogammaglobulinemia (5.6%), three patients with selective IgA deficiency and/or IgG subclass deficiency (7%), one patient with allergies/asthma (4.3%), 10 patients with infections (14.9%), one patient with autoimmunity (4.2%), and one patient in the miscellaneous group (14.3%).

The patient with reduction in both T and B lymphocytes was diagnosed with Artemis deficiency, as noted above.

The CD4/CD8 ratio was reduced in four patients with hypogammaglobulinemia (11.8%), two patients with selective IgA deficiency and/or IgG subclass deficiency (4.7%), two patients with allergies/asthma (8.7%), 12 patients with infections (17.9%), and four patients with autoimmunity (16.7%). Of these, the absolute number of $CD4^+$ T cells was reduced in just one patient from the infections' group and one from the autoimmunity group. Conversely, the CD4/CD8 ratio was found to be increased in two patients with hypogammaglobulinemia (5.6%), three (6.8%) patients with selective IgA deficiency and/or IgG subclass deficiency (7%), three (13%) patients with allergies/asthma, two patients with infection, two patients with autoimmune phenomena (8.3%), and one patient classified as miscellaneous (14.3%). In all of the above instances except one patient with hypogammaglobulinemia, increased CD4/CD8 ratio was not accompanied with a reduction of the absolute number of $CD8^+$ T cells below the reference range, and were therefore regarded as MA.

An increased number of activated T lymphocytes ($CD3^+HLA\text{-}DR^+$) was noted in one patient with hypogammaglobulinemia (2.9%), one patient with selective IgA deficiency and/or IgG subclass deficiency (2.3%), six patients with infections (9.0%), and two patients with autoimmune disorders (8.3%).

Natural killer cell deficiency

A reduction in the absolute number of NK cells was found in one patient (2.9%) in the hypogammaglobulinemia group, two (8.7%) of patients in the asthma/allergy group, five (7.5%) in the infections group, and three (12.5%) in the autoimmunity group.

Hypogammaglobulinemia

As noted above, out of 34 patients in this category, eight (23.5%) had a reduced number of B cells, including five (14.7%) with severe reduction. The remaining 26 (76.5%) patients had normal or MA findings. Apart from five patients with genetically confirmed primary immunodeficiencies, who were all in this group, five more patients received immunoglobulin substitution, one of whom had a moderately decreased B-cell count. Thus, immunoglobulins were received by 10 (29.4%) children with hypogammaglobulinemia overall.

Selective IgA/IgG subclass deficiency

In this group, as noted above, two (4.6%) patients had a reduction of absolute number of B cells, while one (2.3%) had an increased number of activated T lymphocytes. The remaining 40 (93%) patients had either normal or MA immunophenotype. Substitution therapy was introduced by attending immunologist in 13 patients in this group: one patient with selective IgA deficiency solely and a B-cell count decrease, three patients with both IgA and IgG subclass deficiency and nine with IgG subclass deficiency alone, all of whom had normal B-cell counts.

Asthma/allergy with no immunoglobulin deficiencies or infections

Three (13%) patients in this group demonstrated reduced B-cell numbers, one of whom also had a reduction of NK cells. Another patient (4.3%) had an isolated reduction of NK cells. In addition, two (8.7%) patients had an increased number of activated T cells. The remaining 17 (73.9%) patients had normal or MA findings. Out of 23 patients with isolated asthma/allergy, none received immunoglobulin substitution.

Asthma/allergy combined with immunoglobulin deficiencies, infection or autoimmunity

In addition to the 23 patients with isolated asthma/allergy, 15 patients had asthma or allergy combined with hypogammaglobulinemia. Of these, 13 (86.7%) had normal findings or MA, one had a significant reduction of B-cell number (diagnosed as Bruton's disease), and one

had a combined reduction of B and T-cell numbers with subsequent recovery. Another 15 patients had a combination of asthma/allergy and selective IgA deficiency, with 14 (93.3%) exhibiting normal/MA immunophenotype and the remaining one a modest reduction of B cells. Thirteen children had a combination of asthma/allergy with IgG subclass deficiency. In this group, all findings were either normal or MA. Eighteen patients had a combination of asthma/allergy and infection without immunoglobulin abnormalities, twelve of whom had normal and three MA findings (83.3%). Two patients had increased number of activated T lymphocytes, one had moderate reduction in both B and NK cells. All six patients whose asthma/allergy was combined with autoimmunity had findings within the reference range.

Infections with no immunoglobulin deficiencies

In this group, eight (11.9%) patients had a decreased number of B cells, (with one of those two showing recovery of B-cell number, returning to normal range on follow-up examination two months later) and another patient is lost for follow-up. Three of eight patients with low number of B cells also had a low number of NK cells. Low number of NK cells was found in two additional patients (one who also had a reduction of $CD4^+$ T cells and the other with an isolated reduction of NK cells). Thus, in total, 5 (7.5%) patients had a reduced number of NK cells. Six patients in this group displayed an increased number of activated T cells. The total number of patients in this group who had normal or MA findings was 51 (76.1%). One patient with asthma and bronchiectasis in this category received immunoglobulin substitution, with all investigated lymphocyte populations within reference values.

Autoimmune diseases

Of the 24 patients with known or suspected autoimmune diseases, 12 (50%) had connective tissue disorders. Eleven of these (91.7%) had normal findings or MA, while one showed an increased number of activated T cells. Five patients (20.8%) had inflammatory bowel disease, all with normal/MA findings. Three (1.2%) were evaluated because of cytopenias; two of them, with thrombocytopenia, had a normal immunophenotype, while the third child, who had pancytopenia, exhibited a reduction of all lymphocyte subpopulations attributable to the pancytopenia itself, with recovery on subsequent investigations (although the absolute B-cell number was rather slow to normalize, remaining somewhat below the reference range after two months). The remaining four children were subjected to immunological examination for various reasons: two for lymphadenitis, one accompanied by *erythema multiforme*, a combination of glomerulonephritis and Hashimoto thyroiditis, and isolated splenomegaly, respectively. They all had normal findings or MA. None of these patients received immunoglobulin substitution.

DISCUSSION

Although lymphocyte immunophenotyping based on flow cytometry is a powerful tool in the diagnosis of many primary immunodeficiencies, there has been an increasing awareness of associated costs and the need for its reassessment as a screening tool in the diagnosis of PID [17].

The results we present here constitute referrals by the only tertiary center for immunodeficiencies for peripheral blood lymphocyte immunophenotyping to the University Children's Hospital in Belgrade. Pediatric population of the Autonomous Province of Vojvodina counts around 300,000 children, but we cannot exclude that patients from Vojvodina were refereed to another tertiary care pediatric institution in Belgrade directly, without being seen by immunologists in Novi Sad. The exact prevalence of primary immunodeficiencies in Serbia (or Vojvodina) is unknown at this time. Using the data from the German National Registry of Primary Immunodeficiencies (PID-NET), where the minimal prevalence of PID is stated to be 2.72 per 100,000 inhabitants [18] we should expect 45–50 people with PID living in Vojvodina (approximately 1.7 million inhabitants). We actually found five patients with genetically confirmed immunodeficiency and additional 19 patients requiring therapy for immunodeficiencies during a ten-year period. In our patients, lymphocyte subpopulation analysis was practically performed as an initial test, together with immunoglobulin levels. As shown by our results, this approach yielded a relatively low proportion of findings of diagnostic importance. All patients except one that were diagnosed with PID or received therapy suffered from either hypogammaglobulinemia or IgG subclass deficiency. Nineteen patients were given immunoglobulin substitution in order to control infections or/and to maintain immunoglobulin G levels without confirmed immunodeficiencies, an approach used by other authors as well [19]. A moderately decreased B cell count was found in only one patient in this group.

The rationale for our decision to subsume non-specific findings under the category of MA was that these particular lymphocyte abnormalities are not, by itself, diagnostic or strongly indicative of any particular PID according to the guidelines of the European Society for Immunodeficiencies [20]. In our experience, such findings appear to be of quite limited clinical value, and thus we considered the sum of patients with this type of findings and those with all findings within the normal range informative regarding the diagnostic value of the analysis. Thus only ~20% of analyses in this series resulted in "positive" findings. Even among the latter, we noted that isolated aberrations of NK cells also appear to be of limited value. We have to emphasize that we did not have patients with severe combined or other complex immunodeficiencies where enumeration of NK cells might be of diagnostic significance. NK cell deficiency-associated PID are quite rare, and none of our patients with a low absolute number of NK cells exhibited the clinical signs of respective disorders (as stated in the European Society for Immunodeficiencies guidelines), such as *GATA2* deficiency, accompanied by

susceptibility to mycobacteria, papillomaviruses, histoplasmosis and lymphedema [20]. Furthermore, the number of activated T cells ($CD3^+HLA\text{-}DR^+$) usually reflects ephemeral changes related to some current infection or other factors. This might justify their inclusion in the MA category, although T-cell activation status could be useful as part of an extended immunophenotype, particularly in patients with autoimmune disorders. Finally, reduction in absolute numbers of $CD4^+$ or $CD8^+$ T cells was also rare in our patients, and not particularly informative. We are therefore inclined to agree with Dias et al. [17] that the enumeration of the above subpopulations can, as a screening test, hardly be cost-effective on a large scale.

Furthermore, if we analyze the proportion of normal findings by year of analysis, we did not see an increase in "positive" findings, indicating that there was no change in referral policy. However, here we must add a *caveat* that in some cases (e.g., lymphopenia in the first months of life, early-onset inflammatory bowel disease, or clinical suspicion of a severe combined immunodeficiency) a normal immunophenotype can be diagnostically important. We would like to highlight the need for more precise guidelines and standardized indications for testing, as well as for improving the communication between clinicians who order tests and specialists who perform and evaluate them. On the other hand, for the diagnosis of specific PID, a more detailed immunophenotypic analysis is necessary: one that would include further B- and T-cell subpopulations (such as naïve and memory cells) or subtypes (e.g., Th1, Th2, Th17, Treg). Flow cytometry could also be helpful in the investigation of immune cell function (oxidative burst, cytotoxicity), cytokine production, mitogen- or antigen-induced cell proliferation, signaling pathways, or specific protein expression pertinent to the diagnosis of PID. Such tests are planned to be introduced in our center in the near future, highlighting the need to ensure that they will be used in accordance with proper indications, supported by relevant European Society for Immunodeficiencies or other guidelines.

CONCLUSION

Lymphocyte immunophenotyping can contribute to the diagnosis of PID in selected patients. However, the usefulness of this laboratory method in real-life tertiary care pediatric hospital settings could be significantly improved by strict adherence to indications and further integration towards a comprehensive diagnostic approach.

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Conflict of interest: None declared.

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Дијагностички значај имунофенотипизације лимфоцита периферне крви код педијатријских болесника из Аутономне Покрајине Војводине, Република Србија

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САЖЕТАК

Увод/Циљ Мада је имунофенотипизација лимфоцита заснована на проточној цитометрији моћно оруђе у дијагностици многих примарних имунодефицијенција, постоји нарастајућа свест о трошковима које ова метода повлачи и потреба за преиспитивањем њене улоге у скринингу на поменута обољења. Приказујемо резултате и дијагностички значај имунофенотипизације помоћу проточне цитометрије изведене у Имунолошкој лабораторији Универзитетске дечје клинике у Београду код серије болесника упућених из Института за здравствену заштиту деце и омладине Војводине у периоду од јула 2008. до јула 2018.

Методе Анализирали смо лабораторијске налазе броја Б-лимфоцита (*CD*19+), Т-лимфоцита (*CD*3+), урођено убилачких ћелија (*CD*3–*CD*16/*CD*56+) и активираних Т-ћелија (*CD*3+*HLA-DR*+), као и *CD*4+ и *CD*8+ Т-ћелија код 198 деце.

Резултати Болесници су груписани према назначеним индикацијама у следећих осам категорија: хипогамаглобулинемија (34), селективна *IgA* дефицијенција и/или дефицит поткласе *IgG* (43), разне инфекције без имуноглобулинских дефицијенција (67), астма и/или алергије без имуноглобулинских дефицијенција или инфекција (23), потврђене или суспектне аутоимунске болести (24) и разне дијагнозе које нису биле праћене инфекцијом (7). Укупно 159 (80,3%) налаза је у целини било у одговарајућем референтном опсегу или је показивало тек минимална одступања. Код четири болесника је постављена дијагноза Брутонове болести, а код једног је откривена имунодефицијенција Артемис. Деветнаест болесника је примало имуноглобулинску супституцију ради сузбијања инфекција и/или одржавања нивоа *IgG*.

Закључак Имунофенотипизација лимфоцита доприноси дијагностици примарних имунодефицијенција код одабраних болесника. Прилажемо одређена размишљања о томе како би се учинак ове лабораторијске методе у реалним условима терцијарних педијатријских установа могао побољшати.

Кључне речи: имунофенотипизација; проточна цитометрија; лимфоцити; имунодефицијенција; деца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Soluble interleukin-2 receptor in pediatric patients investigated for hemophagocytic lymphohistiocytosis – a single-center, 10-year-long experience

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SUMMARY
Introduction/Objective Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory condition characterized by fever, splenomegaly, and cytopenias. Diagnosis of HLH requires at least five of the eight criteria set by the Histiocyte Society and poses a significant challenge to physicians. HLH-2004 criteria include measurement of plasma levels of soluble receptor for interleukin-2 (sIL-2R), an invaluable tool in the diagnosis of HLH, particularly because it can be measured swiftly and inexpensively.
Methods We retrospectively analyzed medical records of 45 pediatric patients (28 boys and 17 girls, median age 8.1 years) who were investigated for suspected HLH in University Children's Hospital in Belgrade, during the period from 2012 to 2022.
Results Ten children were diagnosed with HLH, while 35 did not have HLH. All 10 HLH patients had secondary HLH: eight suffered from infection or inflammatory condition, one from an autoimmune disease, and one from malignancy. Level of sIL-2R was above the HLH-2004 cutoff value of 2400 IU/ml in 9/10 patients with HLH (sensitivity 90%) and 9/35 of patients who did not have HLH (specificity 74.2%).
Conclusion Soluble IL-2 receptor measurement is valuable in children suspected to have HLH. Sensitivity and specificity of this analysis can be further improved by strict patient selection and a comprehensive diagnostic approach.
Keywords: hemophagocytic lymphohistiocytosis; soluble IL-2 receptor; children

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a severe and life-threatening hyperinflammatory condition characterized by fever, splenomegaly and cytopenias, as well as elevated serum ferritin levels, hypofibrinogenemia and hypertriglyceridemia [1]. The cornerstone of HLH pathogenesis is abnormal macrophage activity causing overabundance of proinflammatory cytokines, thought to be a consequence of insufficient function of cytotoxic lymphocytes ($CD8^+$ T cells and natural killer [NK] cells), hampering the physiological termination of immune response and initiating a vicious circle of immune activation. Hemophagocytic lymphohistiocytosis can be primary or secondary. The former may occur as familial HLH or as part of certain primary immune deficiencies, while the latter can be triggered by a wide variety of infectious, autoimmune, malignant, and other conditions [2].

Diagnosis of HLH requires at least five of the eight criteria set by the Histiocyte Society (HLH-2004) and poses a significant challenge to physicians, since one or more criteria may be absent in some patients, particularly early in the course of the disease, and timely treatment is life-saving [3]. HLH-2004 criteria include measurement of plasma levels of soluble receptor for interleukin-2 (sIL-2R), a molecule shed in great quantities by activated T cells, and thus a marker of T-cell activation [4]. With a cut-off value of 2400 IU/ml, sIL-2R measurement is an invaluable tool in the diagnostic workup of patients suspected to have HLH, particularly because it can be measured swiftly and inexpensively. However, the real-life impact of sIL-2R on the diagnosis of HLH may vary. We will review plasma sIL-2R levels found in pediatric patients investigated for HLH in the past 10 years and assess their diagnostic significance.

METHODS

We retrospectively analyzed medical records of 45 pediatric patients (28 boys and 17 girls, median age 8.1 years) who were investigated for suspected HLH in University Children's Hospital, Belgrade, during the period 2012–2022. Data on disease course and outcome, initial and final diagnosis, treatment modalities, status of HLH-2004 criteria (fever, splenomegaly, bi/pancytopenia, hemophagocytosis in bone marrow, serum levels of fibrinogen, ferritin and triglycerides), other relevant laboratory parameters (cerebrospinal fluid findings [CSF],

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C-reactive protein [CRP], procalcitonin, transaminases and activated partial thromboplastin time [APTT]) were retrieved from the patients' histories, as were the results of immunological tests (cytotoxic lymphocyte function) and genetic analyses, if performed.

Serum levels of sIL-2R were measured as part of the diagnostic workup. For this purpose, 2 ml of peripheral blood was drawn in a tube containing 0.38% Na-citrate as anticoagulant. Upon separating the serum by centrifugating the samples at 1600 × g, the analysis was either performed on the same day or the serum was kept frozen at -20°C for a maximum of two months until the time of analysis. The level of sIL-2R was measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The results were expressed in standardized international units (IU/ml).

The values of appropriate parameters were expressed as median, range and standard deviation. Statistical significance of differences between patient groups was determined by two-tailed Mann–Whitney's U test.

All procedures on human subjects were performed in accordance with the Helsinki Declaration and were approved by the Ethical Committee of University Children's Hospital, Belgrade.

RESULTS

We classified all patients (n = 45) in four groups according to the type of underlying condition:

1) detected or suspected infection or an inflammatory state of unclear origin (n = 18);
2) known or suspected autoimmune disease (n = 10);
3) pre-existing malignant disease (n = 11);
4) a transplanted organ (n = 6).

Patients diagnosed with hemophagocytic lymphohistiocytosis

The diagnosis of HLH was established in 10 patients (six boys and four girls, median age 4.5 years). Of these, eight were in the infection/inflammation group and one in the autoimmune and malignant groups, respectively. All HLH patients except one (9/10, 90%) had sIL-2R levels above 2400 IU/ml. All patients (10/10) were febrile, six (60%) had an enlarged spleen, while seven (70%) displayed bi- or pancytopenia. Hemophagocytosis was found in bone marrow aspirate in four patients (40%). Hypofibrinogenemia was present in six (60%), hypertriglyceridemia in five (50%), and hyperferritinemia in eight patients (80%). Functional capacity of cytotoxic T lymphocytes and NK cells was investigated in three patients, with results in the physiological range. Genetic investigations for HLH (clinical exome sequencing) were performed in one patient, who exhibited two heterozygous variants of undetermined significance in the UNC13D gene. Of the 10 HLH patients, one satisfied six HLH-2004 criteria, while the remaining nine satisfied five criteria. Median time from the onset of HLH to diagnosis was 2½ weeks (range 1–8 weeks).

In all 10 children, HLH was classified as secondary. In three children, the underlying disorder was a clinically diagnosed pneumonia (without identification of the causative agent, although one child had a positive serologic test for West Nile virus). In two children, clinical and laboratory findings indicated a viral upper respiratory infection (with negative results of specific virological tests). One child had septicemia/systemic inflammatory syndrome (with sterile blood culture). This child also had a positive serological test for *Leishmania*, but without the finding of this organism on bone marrow examination. One child had an antineutrophilic cytoplasmic antibody-positive systemic vasculitis accompanied by respiratory problems, with a positive serological test for *Chlamydia pneumoniae*. One child had a positive serological test for Epstein–Barr virus and clinical signs of Kawasaki disease. One child developed secondary HLH during maintenance therapy for acute lymphoblastic leukemia, while another was eventually diagnosed with lymphoma.

Five children were treated according to HLH-2004 protocol for secondary HLH (dexamethasone and cyclosporine without etoposide), two of whom also received intravenous immunoglobulins enriched for IgM fraction. The remaining four children were treated by glucocorticoids with or without intravenous immunoglobulins. In total, disease had a fatal outcome in four children (40%), while six (60%) recovered. Among the fatalities, one child (aged 2 years 11 months) received glucocorticoids and died of systemic vasculitis complicated by pneumonia five days after the diagnosis of HLH. One child (aged 15 years one month) suffered from acute lymphoblastic leukemia, also received glucocorticoids, and died two months after the onset of secondary HLH. One child had unexplained septicemia/systemic inflammatory response syndrome (age 12 years 11 months), was treated by dexamethasone, cyclosporine and immunoglobulins, and died two weeks after the diagnosis of HLH. One child (age 13 years six months) was treated with dexamethasone and cyclosporine and died after being transferred to another center; the underlying disorder was later determined to be lymphoma.

Patients who were not diagnosed with hemophagocytic lymphohistiocytosis

Among the 35 children who did not have HLH, 6 (17.1%) fulfilled only one HLH-2004 criterion, 11 (31.4%) fulfilled two criteria, 12 (34.3%) three, three (8.6%) four, two (5.7%) five, and one (2.9%) six criteria. The three children with 5–6 criteria were not deemed by their physician to suffer from HLH, and were given other diagnoses (febrile neutropenia complicated by sepsis in two children with leukemia, and a poorly defined viral infection in the remaining child).

In this group, six children died: four with malignant disease (three with acute leukemia and one with Langerhans cell histiocytosis), one as a consequence of systemic cytomegalovirus infection complicated by acute respiratory distress, and one child with transplanted kidney who was

killed in a traffic accident unrelated to medical issues. Only two of these six patients (both with leukemia) had an sIL-2R level above 2400 IU/ml. One of these two did fulfill five HLH-2004 criteria, although his condition was attributed to disease progression and not HLH. The other child had three criteria.

Levels of soluble IL-2 receptor

Median level of sIL-2R in patients with HLH was 3489.3 (range 2101.0–5536.0 IU/ml, SD 1664.9 IU/ml). This was significantly higher ($p < 0.01$) compared to patients without HLH (1145.0; range 0.0–8955.0 IU/ml, SD 1663.1 IU/ml). Highest levels of sIL-2R were found in the infection/inflammation group (2921.5; range 328.0–8955.0 IU/ml, SD 2044.2 IU/ml), followed by malignancy (1425.0; range 322.0–4110.0, SD 1366.3 IU/ml), autoimmunity (1103.5; range 0.0–2872.0 IU/ml, SD 807.0 IU/ml), and transplantation (787.0; range 142.0–3070.0 IU/ml, SD 1065.3 IU/ml). The difference was statistically significant between the infection/inflammation and autoimmunity groups ($p < 0.01$) and between infection and transplantation groups ($p < 0.05$).

Level of sIL-2R above 2400 IU/ml was found in 9/10 patients with HLH (sensitivity of analysis 90%) and 9/35 (25.8%) of patients who did not have HLH, yielding a specificity of 74.2%. Of the nine patients with high sIL-2R and no HLH, five (55.6%) were in the infection/inflammation group, three (33.3%) in the malignancy group, and one (11.1%) in the transplantation group. Of the five children in the first group, one had culture-confirmed staphylococcal septicemia, while four had a febrile condition of unknown origin. Of these, one was eventually diagnosed with multisystemic inflammatory syndrome (MIS-C) as a consequence of COVID-19. All five of the aforementioned children fully recovered – three with glucocorticoids with intravenous immunoglobulin (with or without IgM enrichment), and one without any anti-inflammatory treatment. Two of the three children with malignant disease (acute lymphoblastic leukemia) died – one of febrile neutropenia and consequent septicemia, the other due to disease progression. The third child recovered with glucocorticoids alone. The only patient with high sIL-2R in the transplantation group had positive serological and virological findings for Epstein–Barr virus and was successfully treated.

Fever, splenomegaly and cytopenias

Fever was present in all 10 patients with HLH and 20/35 (57.1%) patients without HLH. It was found in 16/18 (88.9%) children with infection/inflammation, 5/10 (50%) of children with autoimmunity, 7/11 (63.6%) of those with malignancy, and 2/6 (33.3%) of those with transplanted kidney. Among the patients with high sIL-2R and no HLH, 7/9 (77.8%) were febrile.

An enlarged spleen was found in 6/10 (60%) of children with HLH and 12/35 (34.3%) without HLH. In the infection/inflammation group, splenomegaly was present in 10/18 (55.6%); in the autoimmunity group, in 3/10 (30%); in the malignancy group, in 3/11 (27.3%); and in the transplantation group, in 1/6 (16.7%). The frequency of splenomegaly among children with high sIL-2R and no HLH was 6/9 (66.7%).

Bi- or pancytopenia existed in 7/10 (70%) patients with HLH and 16/35 (45.7%) patients without HLH. In the subgroup of the latter with high sIL-2R levels, bi- or pancytopenia was found in 4/9 (44.4%) children. Among patient groups, those with malignancy had the highest frequency of bi/pancytopenia (8/11, 72.7%), followed by transplantation (4/6, 66.7%), autoimmunity (4/10, 40%), and infection/inflammation (7/18, 38.9%).

Hemophagocytosis

Hemophagocytosis was observed in the bone marrow in 4/10 (40%) of children with HLH and 1/35 (2.9%) without HLH. The child with hemophagocytosis did not have sIL-2R level above the cutoff. All four patients with hemophagocytosis and HLH were in the infection/inflammation group, while the patient with hemophagocytosis without HLH belonged to the transplantation group.

Fibrinogen and triglycerides

Median fibrinogen level of children with HLH was 1.44 g/l (range 0.80–4.90 g/l, SD 1.41 g/l) and of those without HLH 3.60 (range 1.13–14.30 g/l, SD 3.08 g/l; $p < 0.01$). Fibrinogen was lowest in the infection/inflammation group (median 2.05; range 0.80–8.00 g/l, SD 1.97 g/l), followed by the autoimmunity (median 3.05 g/l; range 1.16–14.30 g/l, SD 4.49 g/l), malignancy (median 3.96 g/l; range 1.13–14.00 g/l, SD 3.49 g/l) and transplantation (median 4.27 g/l; range 3.40–5.11 g/l, SD 0.67 g/l) groups. None of these differences were statistically significant.

Median level of triglycerides was 3.07 mmol/l (range 1.60–9.14 mmol/l, SD 2.53 mmol/l in children with HLH and 2.29 mmol/l (range 0.45–10.78 mmol/l, SD 2.45 mmol/l) in children without HLH. This difference was not statistically significant. By group, the highest triglyceride level was in the infection/inflammation group (median 3.15 mmol/l; range 0.86–10.06 mmol/l, SD 2.74 mmol/l), followed by the autoimmunity (median 2.63 mmol/l; range 0.45–10.78 mmol/l, SD 3.17 mmol/l), transplantation (median 2.54 mmol/l; range 1.17–6.48 mmol/l, SD 1.92 mmol/l) and malignancy groups (median 1.99 mmol/l; range 0.63–3.20 mmol/l, SD 0.74 mmol/l). The only significant difference was between infection/inflammation and malignancy groups ($p < 0.05$).

In total, 8/10 (80%) children with HLH had hypofibrinogenemia and/or hypertriglyceridemia, as did 28/35 (80%) children without HLH and 8/9 (88.9%) children without HLH who had sIL-2R above 2400 IU/ml.

Ferritin

Serum ferritin level was above 500 ng/ml in 8/10 (80%) children with HLH and 26/35 (74.2%) children without HLH. In the subgroup of the latter with high sIL-2R,

hyperferritinemia was present in 7/9 (77.8%). Median level of ferritin in children with HLH was 1734.0 ng/ml (range 155.6–5001.0 ng/ml; SD 1825.2 ng/ml), compared to 1490.0 (range 34.2–7985.2 ng/ml; SD 2069.8 ng/ml) in children without HLH. This difference was not statistically significant. The highest ferritin level was found in patients with malignancy (median 2699.7 ng/ml; range 1351.9–5401.7 ng/ml, SD 1660.9 ng/ml), followed by autoimmunity (median 1495.4 ng/ml; range 34.2–7378.7 ng/ml, SD 2311.8 ng/ml), transplantation (median 1296.8 ng/ml; range 295.8–3507.8 ng/ml, SD 1203.1 ng/ml), and infection/inflammation (median 762.0 ng/ml; range 50.1–7985.2 ng/ml, SD 2094.0 ng/ml). Statistically significant were the differences between infection/inflammation and malignancy ($p < 0.01$) and between malignancy and transplantation ($p < 0.05$).

C-reactive protein

In patients with HLH, CRP levels were somewhat higher (median 87.2 mg/l; range 7.3–196.9 mg/l, SD 74.5 mg/l) compared to patients without HLH (median 33.5; range 0.6–349.8 mg/l, SD 99.2 mg/l). However, this difference was not statistically significant. Highest CRP levels were found in malignancy (median 150.6 mg/l; range 1.2–323.8 mg/l, SD 100.3 mg/l), followed by infection/inflammation (median 53.5 mg/l; range 1.4–349.8 mg/l, SD 101.9 mg/l), transplantation (median 14.2 mg/l; range 1.2–45.3 mg/l, SD 16.3 mg/l), and autoimmunity (median 9.7 mg/l; range 0.6–171.0 mg/l, SD 60.7 mg/l). Statistical significance is reached between autoimmunity and malignancy ($p < 0.05$) and transplantation and malignancy ($p < 0.01$). Nine patients with high sIL-2R and no HLH had median CRP level of 58.7 mg/l (range 4.5–349.8 mg/l, SD 109.7 mg/l). This was not significantly different from the HLH group.

Procalcitonin

Procalcitonin levels were available for only three of the 10 HLH patients and were 0.47, 0.48 and 0.51 ng/ml, respectively (median 0.48 ng/ml, SD 0.02 ng/ml). Procalcitonin levels were also available for 19 patients without HLH (median 0.32 ng/ml; range 0.06–4.99 ng/ml, SD 1.43 ng/ml). The highest median procalcitonin level was in children with malignancy (0.59 ng/ml; range 0.25–2.06 ng/ml, SD 0.75 ng/ml; n = 8), followed by infection/inflammation (0.49 ng/ml; range 0.19–4.99 ng/ml, SD 2.13 ng/ml; n = 7), transplantation (0.28 ng/ml; range 0.25–0.32 ng/ml, SD 0.04 ng/ml; n = 3) and autoimmunity (0.24 ng/ml; range 0.06–0.54 ng/ml, SD 0.20 ng/ml; n = 4). None of these differences were statistically significant; however, this mainly reflects small sample sizes.

Cerebrospinal fluid findings

Of children with HLH, 8/10 (80%) had normal cytological and biochemical CSF findings. Two children (20%) had a pathologic finding: one had leukocytosis with predominance of lymphocytes (83%) and marked proteinorachia (1159.0 g/l), while the other had moderate amounts of protein (0.4 g/l) with no cellular elements. Moderate leukocytosis and proteinorachia were also found in 2/35 (5.7%) children without HLH, none of whom had sIL-2R above 2400 IU/ml.

Transaminases

In total, 9/10 (90%) children with HLH and 17/35 (46.8%) children without HLH had elevated serum levels of aspartate-aminotransferase (AST, above 36 U/l) and/or alanine-aminotransferase (ALT, above 68 U/l). In children without HLH and sIL-2R > 2400 IU/ml, 7/9 (77.8%) had elevated AST and/or ALT. This finding was present in 14/18 (77.8%) children in the infection/inflammation group, 5/10 (50%) in the autoimmunity group, 7/11 (63.6%) in the malignancy group, and 1/6 (16.7%) in the transplantation group.

Blood coagulation defect

A prolonged APTT (> 35 seconds) was found in 4/10 (40%) children with HLH and 5/30 (16.7%) children without HLH for whom this analysis was performed. In patients without HLH who had sIL-2R above 2400 IU/ml, APTT was measured in seven children, and was prolonged in three (42.8%). This abnormality was noted in 6/15 (40%) patients with infection/inflammation, 1/8 (12.5%) patients with autoimmunity, 2/11 (18.2%) patients with malignancy and none of the six patients with a transplanted kidney.

DISCUSSION

Primary HLH was not diagnosed in our patient series. This may partly be due to the unavailability of genetic testing in all patients but one. Notably, no sharp demarcation exists between primary and secondary HLH, since primary HLH is often initiated by an infection or other trigger and secondary HLH is often associated with a genetic predisposition [5, 6]. Primary and secondary HLH are, however, quite distinct in their response to treatment and prognosis: in the absence of timely diagnosis and hematopoietic stem cell transplantation, patients with primary HLH may experience only a brief remission and their long-term prognosis is dismal, while secondary HLH can be cured by immunosuppressive agents [7]. For the purpose of this analysis, we decided to group patients according to the type of underlying condition or trigger (infection/hyperinflammatory state, autoimmune disease, pre-existing malignancy, organ transplantation). Most of our HLH patients belonged to the infection/inflammation group. We chose to form such a heterogenous group because causative agent or trigger is rarely identified. Of the 18 children in this group, only five had a defined or probable infection. An infectious trigger was also present in three of the 10 patients with autoimmune disease, one of the 11 children with malignancies, and two of the six children with transplanted kidney. Since all children suspected of HLH underwent extensive bacteriological, virological, and (if necessary) parasitological and mycological investigations, a small percentage of patients with an identified microbiological agent in our series may be an additional sign of the

magnitude of challenge faced by clinicians in the attempt to uncover the trigger of secondary HLH.

Macrophage activation syndrome, a variant of HLH, most often accompanies systemic juvenile idiopathic arthritis [8] and systemic lupus erythematosus [9], but can be encountered in a wide range of autoimmune disorders [10]. The only child in our series with autoimmune disease who was diagnosed with HLH suffered from antineutrophilic cytoplasmic antibody-positive systemic vasculitis. The remaining patients (not diagnosed with HLH) in this group had autoimmune hemopathies (5), systemic juvenile idiopathic arthritis (2), systemic lupus erythematosus (1) and polymyositis/dermatomyositis (1). Since all received immunosuppressive treatment, we cannot exclude the possibility that some of them – less than five HLH-2004 criteria notwithstanding – really exhibited a sort of incomplete, decapitated, or abortive form of macrophage activation syndrome.

In children, as in adults, HLH can arise in various hematological malignancies [11, 12]. Although only one of eleven children with malignancies in this series was diagnosed with HLH, additional three had sIL-2R level above 2400 IU/ml, two of whom even formally satisfied HLH-2004 criteria. In this group, the main differential diagnostic problem is febrile neutropenia due to malignancy itself and its treatment. Some children with malignancy display a sort of inflammatory syndrome that may overlap with HLH, but responds promptly to glucocorticoid treatment. In such children, measurement of plasma sIL-2R levels may aid differential diagnosis. Diagnosis of HLH in pediatric oncology is in many ways a peculiar diagnostic problem, necessitating a specific approach [13]. Finally, although none of our patients with a transplanted organ had HLH, the investigation of sIL-2R in this group may be additionally justified by the fact that rising sIL-2R levels could be a harbinger of transplant rejection [14].

In our patient series, measurement of sIL-2R alone (with the HLH-2004-prescribed cutoff of 2400 IU/ml) displayed a sensitivity of 90% and specificity of 74.3% in the diagnosis of HLH. True sensitivity could, however, be even higher, given that the only patient with HLH and sIL-2R below the cutoff value was investigated early in the course of the disease. The specificity observed in our series was also in broad agreement with published data [15]. From the differential diagnostic perspective, the group of nine patients who had sIL-2R above 2400 IU/ml and no HLH comes to attention. One child in this group had Kawasaki disease and another had MIS-C, while four had an acute inflammatory condition of unknown cause. This resonates well with literature data indicating that the specificity of sIL-2R measurement in the diagnosis of HLH could be significantly improved by the exclusion of patients with inflammatory conditions known to be accompanied by high sIL-2R levels (such as Kawasaki disease, systemic inflammatory response syndrome, MIS-C) [16, 17, 18]. MIS-C is documented to be associated with extremely high plasma levels of proinflammatory cytokines, and consequently very high levels of sIL-2R [19, 20]. Similar appears to be true of systemic inflammatory response syndrome of any etiology [21]. This is important because treatment of choice in these conditions significantly differs from that of HLH: the above hyperinflammatory states respond well to intravenous administration of immunoglobulins, with or without IgM enrichment [22].

Most often satisfied HLH-2004 criteria in our patients were febrility, cytopenias and hyperferritinemia, and this confirms their importance in the triage of patients suspected to have HLH. Hemophagocytosis was noted in the bone marrow in only four patients (40%), which is by no means unusual [23], and in just one child without HLH, even though this phenomenon may be encountered in a wide range of other conditions [24]. Notably, CRP level does not appear to be of great assistance in the diagnosis of HLH. The same applies to procalcitonin levels, APTT and CSF findings. However, these tests are indispensable in the vigilance for potential complications.

Although the function of cytotoxic lymphocytes is intimately connected to the pathogenesis of HLH, appropriate laboratory tests are not routinely performed in our institution (or indeed available) at this moment. Thus, we were able to obtain data on cytotoxic lymphocyte function in just three patients, all with normal findings. The inability to perform this analysis places the treating physician into a difficult situation to diagnose HLH based on five of seven, rather than five of eight criteria, potentially reducing the sensitivity, and to a lesser degree also the specificity of analysis. Similarly, genetic analysis was performed in just one child with HLH, uncovering two variants of undetermined significance in UNC13D gene. This corresponds to secondary HLH arising upon variable genetic predisposition [25]. Although costly, extensive genetic testing of HLH patients could be expected to uncover such predisposition in many more instances.

The cure rate of children with HLH in our series (60%) is in broad agreement with literature data [26]. Time elapsed before the diagnosis of HLH (median 2½ weeks) may also be considered acceptable, but it can – and should – be improved by timely and judiciously performing appropriate laboratory tests, including plasma sIL-2R measurement and cytotoxic lymphocyte functional analyses.

CONCLUSION

Soluble IL-2 receptor measurement is valuable in children suspected to have HLH. Sensitivity and specificity of this analysis can be further improved by strict patient selection and a comprehensive diagnostic approach.

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Солубилни рецептор за интерлеукин-2 код педијатријских болесника испитиваних на хемофагоцитну лимфохистиоцитозу – десетогодишње искуство једног центра

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САЖЕТАК

Увод Хемофагоцитна лимфохистиоцитоза (ХЛХ) тешко је хиперинфламаторно стање које се одликује грозницом, спленомегалијом и цитопенијама. Дијагноза ХЛХ изискује најмање пет од осам критеријума које је поставило Хистиоцитно друштво и значајан је изазов лекарима. Критеријуми ХЛХ-2004 обухватају мерење нивоа солубилног рецептора за интерлеукин-2 у плазми (сИЛ-2Р), драгоцено оруђе у дијагностици ХЛХ, посебно јер га је могуће брзо и економично мерити.

Методе Ретроспективно смо анализирали медицинску документацију 45 педијатријских болесника (28 дечака и 17 девојчица, медијана узраста 8,1 година) који су испитивани због сумње на ХЛХ у Универзитетској дечјој клиници у Београду у периоду 2012–2022.

Резултати Код десеторо деце је постављена дијагноза ХЛХ, док 35 није имало ХЛХ. Код свих десет болесника ХЛХ је била секундарна: осам је патило од инфекције или запаљенског стања и по један од аутоимунске и малигне болести. Ниво сИЛ-2Р је био изнад граничне вредности од 2400 *IU/ml* прописане критеријумима ХЛХ-2004 код деветоро од десеторо деце са ХЛХ (сензитивност 90%) и деветоро од 35 деце без ХЛХ (специфичност 74,2%).

Закључак Мерење сИЛ-2Р је драгоцено код деце са сумњом на ХЛХ. Сензитивност и специфичност ове анализе могуће је додатно побољшати строгом селекцијом болесника и свеобухватним дијагностичким приступом.

Кључне речи: хемофагоцитна лимфохистиоцитоза; солубилни рецептор за ИЛ-2; деца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Cardiac tumors in the pediatric population – surgical experience of four decades

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SUMMARY
Introduction/Objective Although cardiac tumors in the pediatric population are found infrequently, their occurrence is constant and with occasional variations.
The purpose of this study is to show our experience with surgical options of these masses in two leading national university-level pediatric centers over four decades.
Methods This retrospective study is presenting a total number of 24 surgically treated pediatric patients who have been operated on 1998–2020. There were 16 children with primary masses and eight children with metastatic cardiac tumors. Two patients with tumor-like intracardiac masses were not included in the series but had been mentioned as diagnostic challenges. Our patients did not have cardiac transplantation options.
Results The average age of our patients was five and a half years, and the most frequent operated tumor was the cardiac myxoma. Four children had neurological symptoms. There were two deaths, one in the primary tumor group and one metastatic patient misdiagnosed as a primary tumor in the early ages of our department. We had two recurrent cases, a girl with Carney complex, and an infant with an extremely rare form of cardiac malignancy after a myxoma extraction. One child required a permanent pacemaker insertion.
Conclusion Although rare, the pediatric cardiac tumors can be a source of different life-threatening conditions and lifelong sequelae. Therefore, special considerations should be paid to the diagnostic and surgical modalities of their treatment.
Keywords: cardiac tumors; pediatrics; surgical approach; follow up

INTRODUCTION

Primary cardiac tumors in the pediatric population are rare. They present with a variety of clinical presentations and different histological findings. Surgical strategies should be oriented towards the embologenic potential of the mass but also to the compression and obstruction symptoms. Benign tumors are dominant over malignant masses. Secondary cardiac tumors are less seen. The secondary (metastatic) tumors are more frequent in adults than in children [1, 2, 3], whilst the last national 30-years' series was published nearly a decade ago [4].

We present our series with modalities of surgical treatment and available follow up. Our results differ somewhat from the reported series so far.

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METHODS

Data collection / patients

The patient data was obtained by using institutional protocols (two tertiary pediatric cardiac surgery centers), operative notes, discharge summaries, histopathological reports and follow up reports. The Committee of Ethics of the Dr Vukan Čupić Institute for Mother and Child Health Care of Serbia has approved this investigation on March 24, 2021 (No 2263/1). Pre- and postoperative events (cardiac and non-cardiac), the operation itself and early and late complications had been analyzed. The starting date was September 1988 and the end date April 2020. The postoperative cardiac follow-up examinations (echocardiography [ECHO], and/or computed tomography [CT], nuclear magnetic resonance [NMR]) were performed at 15 days, three, six, 12, and 24 months respectively. One of the patients had a surgical reintervention abroad. The patients were transferred to adult medical care at the age of 18.

Background

Our paper encompasses a period of 32 years in which the population of Yugoslavia / Serbia decreased from 22 million to 7 million inhabitants in the current era. The majority of cases (20 out of 24) were operated after 1992. Individual referrals were accepted from surrounding

countries. Thus, the real incidence of the pediatric cardiac tumors cannot be adequately calculated and is limited to Caucasian population.

Sixteen cases of primary cardiac tumors were operated on a total of 11,840 cases in 38 years (0.135% of all operated cardiac cases).

One patient, initially presenting as a primary tumor in 1989, was excluded from the group of primary cardiac tumors and transferred into the secondary group. Intraoperative findings had shown a metastatic tumor with a complete occlusion of the inferior vena cava (IVC). This patient was the one of the two deaths in our series where the attempt of cannulating the IVC resulted in a fatal bleeding.

Metastatic cardiac tumors which were referred to our centers were eight children who were sent to our centers as metastatic Wilms' tumors and one child with non-Hodgkin lymphoma (NHL). Four children with Wilms' were inoperable with disseminated metastases and three were older adolescents who had minimal cardiac involvement but dominant infracaval expansion of the tumor. These children were referred to the vascular surgeons. The NHL patient was treated at the oncological department and was lost for follow up when he transferred to adult care.

Two secondary tumors were excluded from our study as the mass being non-tumorous. They shall be mentioned as masses which were initially thought to be metastases. The number of incidental findings at autopsies was one infant with a right ventricular rhabdomyoma who died of other causes. Four fetal discoveries of rhabdomyomas had been made in our series, one had been operated.

Our country has no pediatric cardiac transplant service and this option is not a feasible as a treatment modality.

RESULTS

The mean age of our patients with primary cardiac tumors was 64.26 months (5.35 years), with female predomination (12:4). The dominant tumors in our series of operated children were myxomas (six children) with rhabdomyomas in the second place (four children). There were two patients with fibromas, three with intrapericardial teratomas and one with a hemangioma.

All the primary tumors were classified as benign. Because of the extension of myocardial involvement, one child with a fibroma was only biopsied and estimated as a patient with an unacceptable high surgical risk. There were two deaths, a neonate with a cardiac fibroma after a biopsy, and the first child in our series who was mistaken for a primary tumor in 1988.

Clinical presentations were varying. All of our patients have been diagnosed by clinical examinations, electrocardiogram, Chest X ray and transthoracic ECHO. Cardiac catheterization/angiography had not been utilized. CT and NMR imaging were readily available to all patients since 2006 [3, 5].

Table 1. Cardiac tumor demographics

Total number of patients	24		
Age (month, mean age)	64.3 (1 day – 220 months)		
Co-existing lesions	5		
Atrial septal defect Bicuspid aortic valve Patent ductus arteriosus	3 1 1		
Clinical signs			
Aortic regurgitation	1	Chest pain	2
Mitral regurgitation	2	Arrhythmias	5
Tricuspid regurgitation	1	LVOTO/RVOTO (> 30 mmHg)	2
Congestive heart failure	4	Pericardial effusion	3
Heart murmur	5	Tamponade	1
Dyspnea	4	Neurological symptoms	4
Cyanosis	1	Cerebrovascular Insults	3
Mode of diagnosis (heart and central nervous system)			
ECHO Cardiac catheterization CT NMR	16 (one prenatal) 0 7 6		
Type of tumor	Benign (16)	Malignant (8)	
	Myxoma 6 (37.5%) Rhabdomyoma 4 (25%) Fibroma 2 (12.5%) Teratoma 3(18.7%) Hemangioma 1 (6.25%)	Primary 0 Secondary 8 (100%) NH 1(12.5%) Wilms tumor 7 87.5%)	
Sex (male:female)	4:12	4:4	
Recurrent tumors	Myxoma – Carney complex (after six months) Myxoma – Myoepithelial carcinoma of the LV (after three months)		

CT – computerized tomography; ECHO – echocardiography; LV – left ventricle; LVOTO – left ventricular outflow tract obstruction; NMR – nuclear magnetic resonance; RVOTO – right ventricular outflow tract obstruction; NHL – non-Hodgkin lymphoma

All our patient cases had been presented at regular institutional heart team meetings. The timing and scheduling of the operation was in function of severity of symptoms and localization of the masses.

Concomitant cardiac lesions occurred as follows: five congenital heart lesions, a patent ductus arteriosus (one child), a secundum atrial septal defect (three children) and one competent bicuspid aortic valve. Atrial septal defects were not diagnosed in the patients who suffered stroke. All lesions had been corrected intraoperatively (ligation and closure) (Table 1).

There were four patients in our series with neurological symptoms. Three presented with stroke. The child most severely damaged by the brain tumor embolization was a teenager with a clinical history of migraines and participation in competitive sports. Postoperatively, she was diagnosed as Carney complex and had remained with devastating sequelae. The other two patients with myxomas had cerebral infarctions with concomitant hemiparesis. The older patient recovered completely, the infant had a recurrent malignant tumor and remaining sequelae. One patient with rhabdomyoma had convulsions which responded well to conventional therapy.

Initial treatment during the investigation of stroke etiology in our hospital is low

Table 2. Localization of the tumors

Type of tumor	Localization								Total
	RA	RA/LA	RV/RVOT	LA	LA/LV	LV/LVOT	RV/LV	OTHER	16
Myxoma	1			3	1	1			6
Rhabdomyoma			1			1	2		4
Fibroma							2		2
Teratoma								3	3
Hemangioma						1			1

RA – right atrium; RV – right ventricle; RVOT – right ventricular outflow tract; LA – left atrium; LV – left ventricle; LVOT – left ventricular outflow tract

molecular weight heparin. Since cardiac disease is complicated by stroke in a number of pediatric patients, but the cardiac tumors are extremely rare, the number of patients in whom the first clue to cardiac tumor diagnosis was the acute neurological deterioration is also small. This is the reason why the type and duration of anticoagulation treatment in this subgroup of cardioembolic patients could not be evaluated in a randomized prospective way. After the removal of cardiac tumor, in our opinion, the rationale is to continue either low molecular weight heparin or aspirin for at least three months. This approach could be modified according to the coexistence of underlying disease, as well as personal and family history for thrombophilia [6].

The timing of the surgical procedure after stroke should be individualized, the embologenic potential of the remaining mass being the dominant factor for the urgent operation [5, 7].

Surgical approach

All of our patients have been operated via median sternotomy on cardiopulmonary bypass (CPB), except for the two children with intrapericardial teratomas where extracorporeal circulation was not initially utilized. One child had an intraoperative iatrogenic aortic tear which required the repair on CPB. Ascending aorta and bicaval cannulation, systemic hypothermic to normothermic temperatures (28–36°C), aortic cross clamp, crystalloid/blood cardioplegia were used, the technique being the surgeon's preference. No deep hypothermic circulatory arrest techniques were used [1].

Once on CPB, all of the tumors were approached initially through the right atriotomy. The left sided masses were approached via the atrial septum and in one case through aortotomy. The huge right ventricle (RV) fibroma required detachment and re-attachment of the septal tricuspid leaflet. The iatrogenic injury to the ventricular free wall had been managed by individual pledgetted sutures. In all patients with myxomas, additional circles of endocardial tissue were excised around the tumor (Table 2). Since 2003, transesophageal and/or epicardial echocardiograms were performed on all patients, after coming of CPB.

Myxomas

Myxomas were the most frequent tumors. Four patients (66.7%) were adolescents (12–16 years old). Two patients (33.3%) were younger than three years (three months, 18 months). They presented as pedunculated masses of different textures: two patients with myxomas had recurrence of the disease. A 13-year-old girl was operated after a stroke. A large myxoma was extracted from the left atrial septum, following all postulates of myxoma removal. A new mass was detected five months after the initial operation originating from the left atrial appendage. Genetic testing confirmed a *de novo* (nonfamilial) Carney complex [7, 8]. The patient had further multiple endocrine surgical procedures operations on her endocrine glands (thyroid, breast), polycystic ovaries and concomitant clinical depression.

One child had a right atrial myxoma with propagation into the IVC. Two patients had solitary peduncular myxomas in the left atrium attached to the superior wall of the chamber.

An unusual case was seen in a previously healthy infant with a sudden onset of afebrile convulsions and cerebral infarction. A large mass was found in the left ventricle protruding through the left ventricular outflow tract (LVOT) into the aorta. The mass originating from the posteromedial papillary muscle was completely surgically removed. The histological diagnosis of a myxoma was confirmed at our institution. Four months later, the child was reoperated for a rapid-growing recurrent mass abroad. A very rare malignant myoepithelial carcinoma was diagnosed and the child had been started on chemotherapy. The histological findings from the first operation had no presence of malignant cells [9] (Figure 1).

Rhabdomyomas

Although rhabdomyomas are the most frequent pediatric cardiac tumors, not all of them require surgical treatment [10]. Out of four operated children in our series one had positive family history. Female patients dominated (3:1). Tuberous sclerosis was confirmed in three cases (75%). One baby had prominences on the lateral ventricles of the brain without neurological symptoms. One infant had a solitary but obstructive tumor in the right ventricular outflow tract (RVOT), the other three were neonates with multiple masses in both ventricles and right atrium. The only child that developed postoperative moderate residual mitral regurgitation (detachment from the posteromedial papillary muscle) and was reoperated later in life (mitral valve reconstruction). Reduction of the masses was noted in two patients, six- and nine-months post-surgery.

An incidental finding of a RV rhabdomyoma was found on an autopsy of a child who died of other causes (Figure 2).

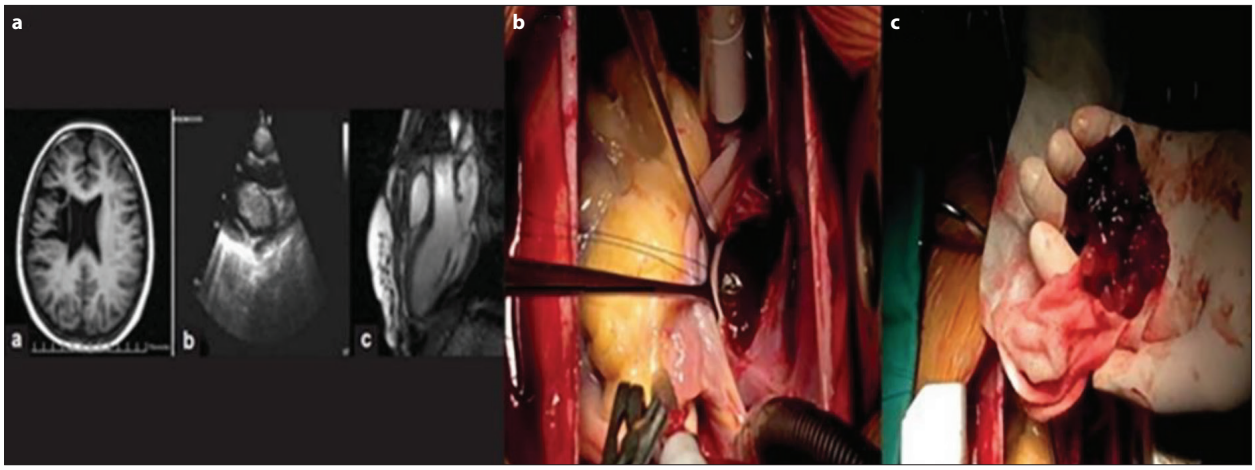

Figure 1. Preoperative and intraoperative myxoma findings; a – preoperative brain and heart computed tomography scans of a huge myxoma in a patient with Carney complex; b – intraoperative finding of a myxoma; c – the tumorous mass after enucleation

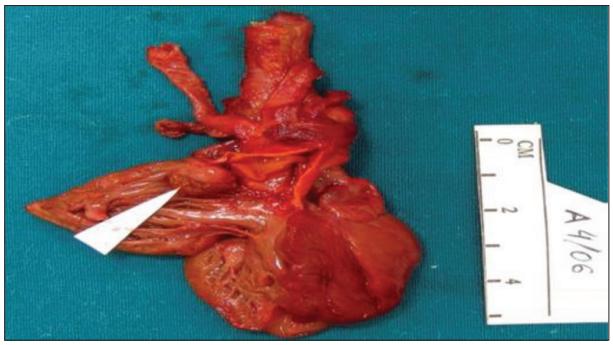

Figure 2. Rhabdomyoma in the right ventricle – incidental finding at an autopsy

Fibromas

The two patients with fibromas were approached by biopsy and relief of RVOT obstruction (RVOTO). The hemodynamically compromised premature neonate weighing 1400 grams, was biopsied with a fatal postoperative outcome. The myocardium was completely invaded by fibrous tissue. The second child had a significant right ventricular involvement with RVOT protrusion. The flow through the pulmonary artery was laminar and sufficient to maintain adequate oxygenation of the child but the patient developed arrhythmias and dyspnea. The clinical symptoms and distant place of residence pushed us towards the attempt of extracting the tumor. Intraoperatively, the septal tricuspid valve leaflet was found to be incorporated and adherent over the fibroma. The leaflet was mobilized, detached, repaired with autologous pericardium, and subsequently reattached during surgery. The fibroma was originating from the RV free wall which was damaged during the enucleation, and had to be sutured with interrupted pledgetted sutures. The child required an insertion of a permanent pace maker ten days after the surgery for complete atrioventricular block, in spite of sinus rhythm in the immediate postoperative period [11, 12] (Figure 3).

Teratomas

Two intrapericardial teratomas in our series were diagnosed in the neonatal age and one in infancy. Both neonates presented with signs of severe respiratory distress after birth [13, 14]. The intrapericardial teratomas were adherent to the right atrium and the adventitia of the ascending aorta, and to the aorta in the second case. The third case with the aortic attachment was an infant who was operated by a general pediatric surgeon. During the operation, the aortic wall was damaged with profuse bleeding. The aorta had been repaired on CPB with a good outcome (Figure 4).

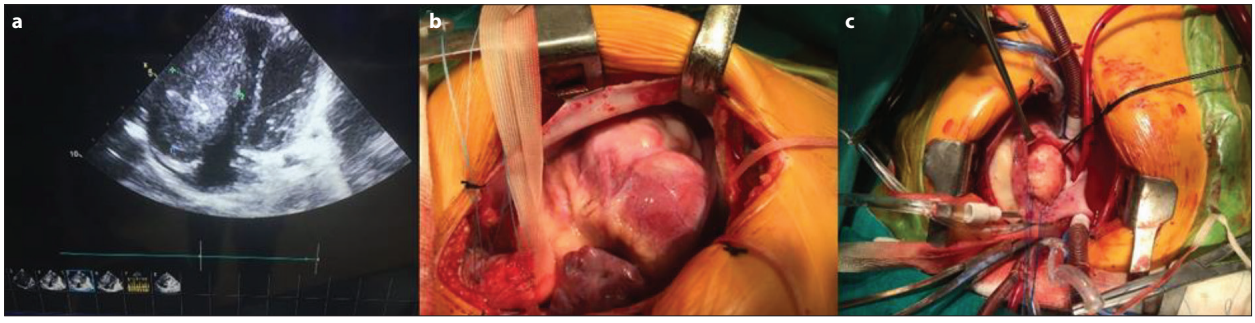

Figure 3. Fibroma; a – echocardiographic view of the tumor; b – macroscopic view of the heart with a huge fibroma; c – "delivery" of the egg-shaped fibroma through the tricuspid valve

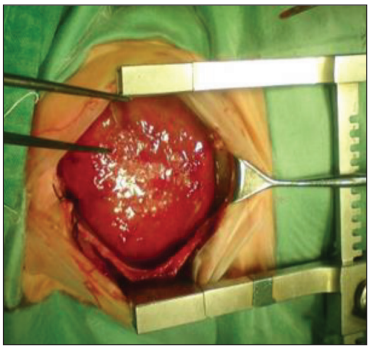

Figure 4. Intrapericardial teratoma adherent to the right atrium and ascending aorta

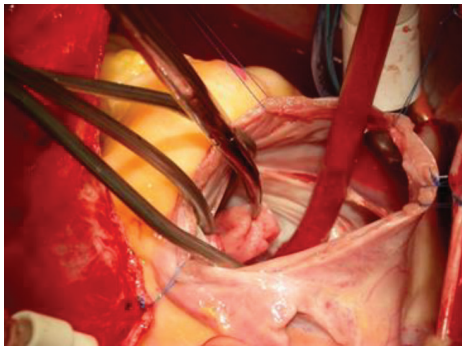

Figure 5. Transseptal and transmittal removal of a hemangioma

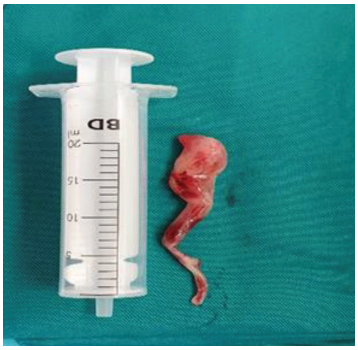

Figure 6. White thrombus originating from the superior vena cava in a patient operated from epipharyngeal rhabdomyosarcoma

Hemangiomas

Cardiac hemangiomas are very rare [15, 16]. Solitary case of a left ventricular hemangioma was diagnosed and surgically removed from an 18-year-old girl who complained of migraines six months prior to the diagnosis. Intraoperatively, the pedunculated multicystic formation was partially attached to the posteromedial papillary muscle and the ventricular free wall. No residual clinical or echocardiographic sequelae were seen (Figure 5).

Secondary cardiac tumors

Seven patients with secondary cardiac tumors were metastatic Wilms' tumors and one had been an adolescent with a NHL [17, 18].

Wilms' tumors

All Wilms' tumor patients with intracardiac involvement were referred from other centers after unsuccessful treatment with chemotherapy and radiation [18]. The first patient in our Wilms' series was mistaken for a primary right atrial tumor. This operation was performed in the early 1980s as our first cardiac tumor operation. The problems with cannulating the IVC resulted with the vein tear and fatal abdominal bleeding. Three patients in critical conditions (Wilms' tumor grade III/IV) were denied surgery. The remaining three patients were referred to the adult cardiovascular centers. One child was operated and died on the second postoperative day due to hepatorenal failure.

Non-Hodgkin lymphoma

The NHL patient had an incidental finding of his condition after a car accident and diagnosis of a pericardial effusion with a threatening tamponade. After draining more than 1 liter of serosanguinous fluid from the pericardium, layers of white fragile tissue were found to be incorporated in the epicardium. Biopsies of both pericardium, pericardial effusion and the epicardial layers of the heart confirmed the diagnosis of NHL. The echocardiographic examination showed intracardiac opacity and thickening of the right ventricular cavity but with no hemodynamic compromise. He was transferred and treated by hematology oncologists. The regression of both extracardiac and intracardiac components after chemotherapy was decisive to abandon the intracardial biopsy attempt. He went into remission and was transferred into adult care.

False alarms

Two patients were referred to the cardiac team as children with masses in the right atrium after previous treatments for malignant diseases: NHL and oropharyngeal rhabdomyosarcoma. The boy who was treated for NHL developed a mass around the long-standing Hickman catheter. Although we were confident that the mass was not of tumorous origin, macroscopically the mass did not resemble a thrombus. The histopathology confirmed a thrombus.

The second patient had an epipharyngeal rhabdomyosarcoma surgically removed three months before a mass appeared in the right atrium. The cardiac examination followed after a systolic murmur was heard during a routine auscultation. The child did not have previously cannulated neck vessels, yet intraoperatively, we found a mass originating in the superior vena cava on a thin peduncle, extending and free-floating into the right atrium. Histopathology confirmed to be a white thrombus (Figure 6).

Postoperative course, outcomes, and follow-up

All operated patients were easily weaned off CPB. The inotrope protocols changed and decreased over time. Nowadays, inotropes are used only if needed in younger patients. The extubation protocol is individualized and dependent on the status of the patient. Three patients were extubated on the operating table, 12 were transported to the Intensive Care Unit intubated. The mean intubation time was 15.7 hours, SD 19.5 (3–48 hours). No patient needed reintubation.

Postoperative bleeding / thoracic drainage was not significant. All thoracic drains were removed within 72 hours.

The patient with a right ventricular fibroma developed a complete atrioventricular block after initial postoperative

period of sinus rhythm. A permanent transvenous pacemaker was implanted on the 10th postoperative day.

The girl with a huge atrial myxoma and stroke had an early recurrence of the myxoma. The rapidly growing mass was noted five months after the initial operation. The patient reported generalized pruritus as main complaint and the characteristic "cafe au lait" spots became visible only at the time of the tumor recurrence. She was consequently operated from a thyroid adenoma, breast adenoma, and a ovarian torsion. She was under psychiatric care for depression.

We had only one case of early mortality; neonate who was biopsied for a cardiac fibroma. Except from the myxoma recurrence that was treated surgically again abroad, all other patients were free of reintervention.

The patients who are still in our care receive no medical treatment.

We had seen no late mortality. All the patients exceeding the pediatric age group, were not under our care and we have not included the latter findings.

The mean follow up was 43.13 months (SD 34.001 months), while one patient moved abroad two months after the operation.

Limitations of the study

This is a retrospective study of a 32-year-long period hallmarked by demographic changes of our country and heavy regional displacement, different availability of diagnostics and approaches to data collection. Until 1998, no uniform system was present nationally, yet data were collected per surgeons' training. Regrettably, we have limited knowledge of the patient follow-up in the adult care. The absence of a national data base and limited possibilities of fetal screening in our country decreases the prenatal detection of intracardial masses.

DISCUSSION

Cardiac tumors in the pediatric age group are a rare entity [19]. The first patient who survived a cardiac mass operation was a child in whom Crafoord removed a myxoma on CPB in 1954.

The true incidence of cardiac tumors is children is not known, but it can be quoted that is between 0.0017 and 0.28%. The majority of cardiac tumors in the pediatric population are primary masses.

In our series, the exact tumor incidence could not be calculated due to geopolitical changes in our country. We can state that cardiac tumor operations make 0.13% of all operated cases. The female to male ratio is inverse in our series compared to the data reported in other series. Female patients were dominantly more affected, 12 compared to four males.

We had one accidental finding of a rhabdomyoma on an autopsy of an infant who had died from non-cardiac causes. Fetal examinations have also discovered four hearts with rhabdomyomas, but as the adequate fetal scans are limited only to high volume centers this number is probably larger. There are currently 12 patients with rhabdomyomas under our care, who do not need surgical treatment. Central nervous involvement is seen in more than 50% of the cases.

All the primary masses in our series were benign except for one recurrent tumor. Our series differs from the majority of papers by the dominance of operated myxomas. Myxomas have been seen in children as young as three and 18 months. The child who was initially operated at the age of three months as a left ventricular myxoma had an early recurrence of a rare malignant form of myoepithelial carcinoma. It is difficult to postulate that this child had two different tumors in such a short period of time, but the pathohistological specimens of the first removed had no malignant cells and were typical of a benign myxoma. The early recurrent mass was malignant. This type of recurrence has not been described so far.

The myxomas were, in spite of their benign nature, tumors with the worst general outcomes: only one patient did not have a prior stroke. Unfortunately, the symptoms usually have a sudden onset in children who are healthy or have no other comorbidities. The Carney complex, hereditary or *de novo*, is an absolute indication for additional and more detailed and frequent postoperative examinations as they are inevitably linked to other medical conditions.

Rhabdomyomas were the second most frequent tumors in our series. The dominant clinical symptoms had been of obstruction and compression. The majority have confirmed tuberous sclerosis. The greatest challenge is the extensiveness of the surgical resection. Our approach is to remove only the hemodynamically compromising mass. These patients are younger than the others and have a potential for tumor regression. Over aggressive surgery has the potential of myocardial injury and damage of vital structures.

Fibromas are rare but from the surgical point of view the most demanding cases and tumors where cardiac transplantation is the most feasible option. Therefore, in one of our two cases, extensive resection was recognized as an option of treatment because of threatening obstruction and the dysrhythmias. These tumors do not regress and are incorporated in the myocardium itself beside the intracavitary formations. Risks of valvular or myocardial injuries are recognized risks.

The secondary tumors in the pediatric population are less frequent than in adults. Our series comprised of seven patients with Wilms' tumor and one with an accidentally discovered NHL. The patients with Wilms' tumor who have an intracardiac (right atrial) propagation through the IVC are often in the end-stage disease with unfavorable outcome. Although authors report successful extraction of tumor masses and/or thrombus, our experience with this type is nonexistent except for one case in the early stages of our department who had a fatal outcome after mistaking the metastatic tumor for a primary mass. The solitary case of NHL cardiac involvement in our series had been an incidental finding. We had operated on two children (treated for other malignancies) with right atrial masses, but both tumors proved to be organized intracardiac thrombi.

CONCLUSION

Our study illustrates that despite the benign histology of most primary pediatric cardiac tumors there is a significant associated comorbidity and occasional mortality. Clinicians should keep in mind that although these tumors are rare, they have a wide and unusual spectrum of presentations. Total resection is not always the therapeutic aim; an important factor is the restoration of the best possible hemodynamic cardiac function without additional cerebrovascular or pulmonary embologenic accidents. Stroke should be treated medically like any other etiology. Although intrapericardial teratomas might attract general surgeons, our hospital policy is that all thoracic tumors should be operated by cardiac surgeons. The absence of a pediatric cardiac transplant program can push the surgeon towards palliative interventions (partial obstruction relief).

Conflict of interest: None declared.

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Тумори срца у педијатријској популацији – хируршко искуство од четири деценије

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САЖЕТАК

Увод/Циљ Мада су тумори срца у педијатријској добној групи ретки, њихова инциденција је константна са повременим варијацијама.

Намера ове студије је да покаже искуства и хируршка решења лечења тумора срца у два водећа национална универзитетска педијатријска центра у распону од четири деценије.

Методе Ова ретроспективна студија укључује 24 хируршки лечена болесника који су оперисани у периоду између 1988. и 2020. године. Било је 16 деце са примарним масама и осморо са метастатским туморима у срцу. Два болесника са масама у срцу које нису биле тумори нису укључени у серију, али су представљени као дијагностички изазови. Болесници нису имали могућности за трансплантацију срца.

Резултати Просечан узраст наших болесника је био пет и по година, а најчешће оперисан тумор је био миксом срца. Четворо деце је имало неуролошке симптоме. Била су два смртна исхода – један у групи примарних тумора и један случај где је код болесника метастаза погрешно дијагностикована као примарни тумор. Пријављујемо и два рекурентна случаја – девојчицу са Карнијевим комплексом и одојче са екстремно ретком формом малигнитета срца после примарне екстракције миксома. Један болесник је захтевао уградњу трајног пејсмејкера.

Закључак Иако ретки, тумори срца у дечјој популацији могу бити узрок различитих животно угрожавајућих стања, као и доживотних последица. Стога, посебну пажњу треба обратити како на дијагностичке тако и на хируршке врсте лечења.

Кључне речи: тумори срца; педијатрија; хируршко лечење; период праћења

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Recognition and treatment of mild cognitive impairment in Serbian general practice

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SUMMARY

Introduction/Objective Mild cognitive impairment (MCI) is a state of progressive cognitive decline, rarely recognized by general practitioners (GPs), which is a reason of late treatment and fast progression towards more serious conditions. The main obstacles for the timely treatment of MCI are lack of diagnostic protocols and clinical guidelines as well as lack of knowledge and disbelief in the pharmacological therapeutic possibilities.

The aim of this investigation was to assess level of recognition of MCI symptoms by GPs, and to estimate their perception of distinct risk factors significance for MCI development.

Methods Participants of the "Days of General Medicine" Conference (Serbia, March 2018), n = 340, completed 12 items questionnaire about recognition and treatment of the MCI patients. We have used descriptive statistics, χ^2, Mann–Whitney U tests, binary logistic regression analysis for results presentation, sub-groups comparison, to assess predictors of drug therapy selection, respectively.

Results Study showed GPs recognize diabetes as most important factor for MCI, then hypercholesterolemia, smoking and sedentary behavior, while hypertension and obesity are perceived as less important. Those GPs who estimated diabetes and hypercholesterolemia as more important for all patients are significantly more prone to prescribe symptomatic therapy (pentoxifylline and vinpocetine), $p < 0.05$ according to χ^2 test. Logistic regression analysis regarding therapy predictions showed that years of GP experience is the most important predictor of drug therapy selection ($p < 0.01$).

Conclusion Results of this investigation pointed a need for MCI education for young physicians, in order to improve diagnosis and treatment of these patients.

Keywords: mild cognitive impairment; diagnosis; pharmacological therapy; general practice physicians

INTRODUCTION

Mild cognitive impairment (MCI) is defined as mental decline which is not severe enough to cause dependence in daily functioning [1]. The main obstacle in MCI defining and, thus, diagnosis is its similarity with dementia and also lack of appropriate and sufficiently sensitive diagnostic (psychometric) tests. MCI is frequently evolved as secondary consequence of several diseases such as neurologic, neurodegenerative, psychiatric or vascular, but also could be a manifestation of Alzheimer disease [2]. Regarding subtypization of this disease there are two main types: amnestic (the basic problem is related to memory loss) and non-amnestic where the focus is on other cognitive problems such as visuo-spatial skills, language and/or executive functions [3]. Of particular interests are reversible MCIs caused by metabolic, vascular, systemic or psychiatric conditions which could be controlled and cured; along with that, the MCI also has a chance to be alleviated [4]. According to some definitions, MCI is patients' mental status placed between normal aging and dementia [5]. Because of that, it is of crucial importance to enable primary care physicians more information about signs, symptoms and clinical tools for its recognition and diagnostics. Moreover, if we are aware that Alzheimer's or non-Alzheimer's dementia will develop in 10–15% of patients 12 months after the MCI diagnosis [6], we should make every effort to broaden the window of possible disease postponement or even eradication. Neurobehavioral MCI estimation relies on several tests: mini mental state examination, clock-drawing test and frontal function tests [7]. Modern clinical practice recommended several biomarkers as a part of regular diagnostic procedure, but also imaging techniques [4]. Selection of neuropsychological tests for MCI diagnosis is challenging, because simple tests are lacking sensitivity and overly complicated tests could not resolve disease development. MCI diagnosis obviously depends upon primary healthcare practitioners' judgment; therefore, it is important to prepare powerful tools and assure MCI awareness for those physicians dealing with susceptible population in everyday practice.

The aim of this investigation was to assess the regular practice of Serbian physicians, and increase their awareness about the risk factors (RF) and signs and symptoms of cognitive impairment (CI).

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METHODS

This survey is conceptualized as cross-sectional and performed by using specifically constructed questionnaire for the "General Medicine Days" conference (Belgrade, Serbia March 2018; http://www.opstamedicina.org/default.asp?ID=1368), by several members of the Serbian Medical Society, Section of General Practice. The Scientific Committee of the Section of General Practice has approved the questionaries' structure, content and purpose of the investigation. The subjects of the survey were medical doctors, general practitioners (GPs) and general medicine specialists (n = 340) taking a part in the Conference. All participants voluntarily filled the questionnaire and thus took part in the investigation. The study was conducted in accordance with the principles of the Helsinki Declaration, regarding participants' name and identity protection. The questionnaire consisted of 12 questions. This questionnaire analysis gives us an opportunity to compare opinions, attitudes and common practice of GPs involved in medical treatment of MCI patients, as a first line "warriors" of any healthcare system. Questionnaires' analysis leads to several conclusions, which could serve for real life practice overview and improvement. The integral version of the questionnaire in English and Serbian is uploaded as a supplementary file that can be found here: http://srpskiarhiv.rs/global/pdf/SupplementaryQuestuonnaireEngSer173-213272B3_1.pdf

Questionnaire structure description

In total, 30 medical doctors selected by study organizers (random sample selection method), filled the questionnaire to test its comprehension, readability and questions formatting. Questionnaire equivalence reliability is estimated by using Cronbach's α analysis. After this preliminary analysis, slight modifications were performed to make the questions more understandable and better formatted. This questionnaire included 12 items classified into four key domains. The first domain consisted of three study subjects (physicians) related items: number of years of service, data about specialization, and total number of patients per physician.

The first three questions were formatted as gaped sentences formats (questions 1 and 3) and question 2 was of multiple-choice type. Second domain (questions 4 and 5) addressed items regarding patients' age and percentage distribution of number of RF related to cardiovascular disease (CVD) (both formatted as gaped sentences). Third domain was a core section comprising questions about cognitive decline related issues (questions 6–11 queried whether physicians notice cognitive symptoms in their patients, whether patients are aware of the existence of cognitive decline symptoms, at which frequency do physicians perceive cognitive symptoms as normal aging, at which frequency do physicians use cognitive decline tests, physicians' estimation of cardiovascular RF importance in cognitive decline development and percent of cognitive declined patients who were directed to neurologist, respectively). Questions 6, 7, and 10 were multiple-choice and questions 8, 9, and 11 gaped sentences type. The fourth part of the questionnaire was devoted to the choice of cognitive decline therapy (question 12, multiple choice type). Data from all completed questionnaires were entered in primary excel database, coded and statistically analyzed using the descriptive and inferential methods and IBM SPSS Statistics for Windows software Version 21.0. (IBM Corp., Armonk, NY, USA).

Questionnaire's validation analysis

Cronbach's analysis showed acceptable reliability of the questionnaire (Cronbach's $\alpha = 0.726$). No deleted items produced significantly higher reliability index, which means that all items are consistent with the main topic. The intraclass correlation coefficient (single measures) was 0.525 (95% confidence interval 0.438–0.642; $F = 12.5$, $df1 = 28$, $df2 = 243$, $p < 0.01$). Average inter-item correlation coefficient was 0.311 ± 0.040 which suggested relatively strong correlation between items (the average correlation coefficient is acceptable if it is larger than 0.300). Test-retest analysis showed good correlation between different items in two time points (average Spearman's $\rho = 0.875$ (0.700–0.983; $p < 0.01$).

Statistical analysis

Data are presented as frequencies (%) for categorical variables and median values (interquartile range) in the case of continuous variables and graphically with pie and bar charts. For the relation between variables estimation, the χ^2 test for categorical variables was used because the data are presented as the Likert scale. Mann–Whitney U test was used for continuous variables comparison. Binary logistic regression analysis was used in order to test predictive capability of different factors for a. physicians' CVD RF importance for MCI awareness and b. drug prescription *vs.* supplements and/or without therapy attitude. Criterion for low and high CVD RF awareness was determined from sum of all six RF marks given by physicians, where tertile values (first tertile = 22, and second tertile = 27 points) were cut-offs for low, medium and high awareness. For binary logistic regression analysis, we used only low and high awareness subjects. For all tests p value < 0.05 was considered as statistically significant.

RESULTS

Physicians' data

Physicians, participants in this current study have broad level of experience (1–41 years) with majority of them having 21–30 years in medical service. Average number of patients per doctor according to practice protocol was about 1800 patients (range: 5–5000). Usual number of medical check-ups per physician per day is about 40. Almost half of participants were without medical specializations (44.7%) and other half had specialization in general medicine (Table 1).

Table 1. Physicians' related data: working experience, number of patients per physician and proportion of general medicine specialist *vs.* general practitioner

Variables	Minimum–Maximum	Median (25–75%)
Physicians' years of service (n = 340)	1–41	24 (13–30)
Frequencies, number (%) < 10 y. 11–20 y. 21–30 y. 31–40 y.	72 (21.2) 69 (20.3) 128 (37.6) 60 (17.6)	
Number of patients in physician's protocols	5–5000	1800 (1400–2000)
Distribution of frequencies, number (%) < 1000 1001–1500 1501–2000 2001–2500 > 2500	28 (8.2) 74 (21.8) 138 (40.6) 65 (19.1) 18 (5.3)	
Physicians' specialization *vs.* general practitioner number (%)	186 (54.7) *vs.* 152 (44.7)	

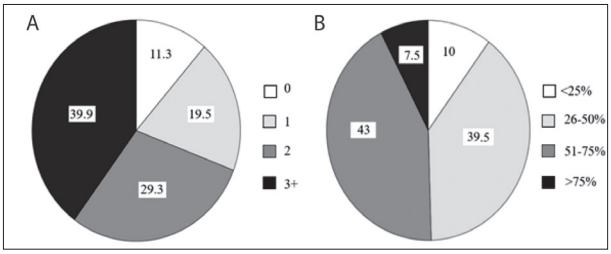

Figure 1. Percentage of physicians' estimation of: A – proportion of patients with different risk factors (arterial hypertension, diabetes mellitus, hypercholesterolemia, obesity, smoking, physical inactivity) in their practice, (%); B – proportion of patients older than 65 (%)

Physicians' estimation of patient population characteristics regarding age and the number of risk factors

Higher number of different comorbidities (arterial hypertension, diabetes mellitus (DM), hyperlipidemia, obesity) and risk social habits (smoking and physical inactivity) assumes higher probability of cognitive declines being observed in patients, by either themselves or by their physicians. Results indicate that according to physicians' estimation high percent of patients (about 40%) had three and more conditions connected with changes in cognition, which is expected because about 50% of physicians reported that they have more than 50% of patients older than 65 years (Figure 1).

The majority of physicians (80%) noticed MCI symptoms in less than 30% of their patients. Physicians reported that less than 30% of patients averagely have complained about MCI symptoms (Figure 2A and 2B).

The highest proportion of physicians (73.2%) estimated MCI symptoms as normal aging in low percent of their patients (below 30%). Almost 28% of physicians referred more than 50% of their patients to neurologist for more specialized diagnostics and therapy, while 54% of physicians send 30–50% of patients to the neurologist (Figure 2C).

The most important MCI risk factor assumed by almost 85% of physicians is DM (sum of opinions "important for older" and "for all" patients), in smaller percent as important RF are estimated hypercholesterolemia (76%), smoking (76%) and physical inactivity (76%), and arterial hypertension (73%). Obesity was obviously underscored

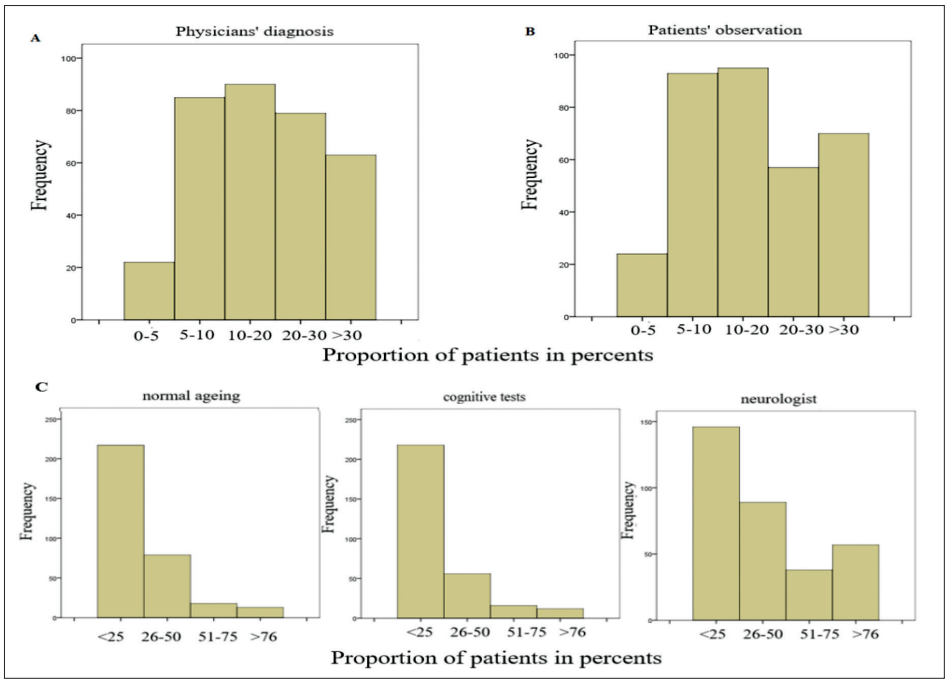

Figure 2. Distribution of physicians' estimation of patients proportion whose cognitive deterioration was noticed by physicians (A) or patients themselves (B); proportion of three characteristics of physicians' attitude towards patients' cognitive decline signs and symptoms: perceiving as normal aging, cognitive tests performance and referring to neurologist (C); A – distribution of frequency of physicians noticed patients' forgetfulness, decreased concentration, walking instability, thought slowness (symptoms of brain's small blood vessels damage); B – distribution of frequency of patients complains about forgetfulness, mood swings, walking instability, or brain fog; A and B data expressed in % (0–5, 5–10, 10–20, 20–30, > 30); C – distribution of frequency of physicians opinion about mild cognitive impairment as normal aging, cognitive tests implementation and neurologist inclusion (results are presented in %: < 25, 26–50, 51–75, > 76)

Table 2. Average physicians' categorization (in %) of six traditional cardiovascular disease risk factors importance for cognitive impairment development

Disease/life style- importance	Unimportant	Low importance	Undefined / unspecified attitude	Important for older patients	Important for all patients
Arterial hypertension	3.9	7	16.6	30.8	41.7
Diabetes mellitus	0.9	5.1	9.6	28.8	55.6
Hypercholesterolemia	1.8	6	15.9	37.4	38.9
Obesity	1.8	17.6	25.1	22.7	32.7
Smoking	1.8	8.4	14.1	21.9	53.8
Physical inactivity	2.4	6.4	15.2	25.9	50

Table 3. General practitioners' and general medicine specialists' comparison regarding cardiovascular diseases risk factors perception and mild cognitive impairment therapy practice

Variable	General practitioner	General medicine specialist	p
Risk factors for cognitive impairment – physicians' perception[#]			
Arterial hypertension 0/1/2	47/53/49 (31.5/35.6/32.9%)	44/48/88 (24.4/26.7/48.9%)	0.014
Hypercholesterolemia 0/1/2	47/60/44 (31.1/39.7/45.5%)	32/64/85 (17.7/35.4/54.5%)	0.001
Smoking 0/1/2	45/37/68 (30/24.7/45.3%)	36/35/110 (19.9/19.3/60.8%)	0.017
Physical inactivity 0/1/2	41/45/62 (27.7/30.4/41.9%)	38/39/101 (21.3/21.9/56.7%)	0.028
Patients without risk factors *	10 (5–20%)	10 (5–15%)	0.048
Cognitive symptoms assumed as normal aging *	20 (10–30%)	10 (6–30%)	< 0.01
Therapy			
Prescription drug (pentoxifylline or vinpocetine alone or in any combination with different supplements) no/yes	112/40 (74%/26%)	120/66 (64%/36%)	0.045

*Mann–Whitney U test; otherwise χ^2 test for categorical variables;
[#]level of risk factor estimation: 0 – unimportant; 1 – important only for old patients; 2 – important for all patients

and considered as important CI factor for 56% responders (Table 2).

Comparison between general practitioners' and general medicine specialists' perception of mild cognitive impairment and related risk factors

CVD related RF were estimated as more important by specialists compared to GPs ($p < 0.05$). Accordingly, specialists noticed significantly lower percent of patients without CI RF, and also assumed less frequently cognitive decline signs and symptoms as "normal aging" consequence ($p < 0.05$, 0.01, respectively). Subsequent analysis of possible factors determining patients' referral to neurologist revealed physicians who estimated DM as important factor for all patients are more prone to cooperate with neurologist to provide better diagnostics and treatment for potentially CI jeopardized patients (Table 3). Table 3 also presents data about therapy practice of GPs/general medicine specialists regarding MCI patients, revealing more specialist are likely to prescribe the symptomatic therapy ($p < 0.05$).

Comparing the physicians who dealt with less than 35% of patients older than 65 years and those with more than 70% of older ones, we found that those who dealt with younger patient population, in significantly lower percent referred patients to neurologist to diagnose their cognitive decline (data not shown here). As expected, more experienced physicians (more than 30 years of medical service) considered hypercholesterolemia, obesity, and physical inactivity as more important inductors of cognitive disturbances, than less experienced participants (supplementary Table S1). In contrast to expectations, results of the questionnaire showed that physicians with larger number of patients (> 2000 according to medical protocol registration) performed some of the functional tests (one or more) on a larger number of patients, compared to colleagues with less than 2000 patients [15% (10–30) *vs.* 10% (5–28), $p = 0.049$ by Mann–Whitney U test].

Physicians' mild cognitive impairment treatment attitude and practice

Only 5% of physicians did not advice any medication to patients with CI symptoms. For this indication physicians usually use two kinds of drugs (pentoxifylline, vinpocetine), many different supplements or different combinations which are commonly used in clinical practice in Serbia. The most popular supplement for this treatment area is *Ginkgo biloba* preparation, recommended from 20.6% of physicians, and the most frequently in combination with antioxidants (33.2%). Prescription drugs indicated for circulatory disorders (pentoxifylline and vinpocetine) were given from 104 (30.6%) physicians, while the use of dietary supplements was advised from 64.4% physicians. Pentoxifylline was prescribed from 62 (18.2%) physicians, alone or in combination with other preparations; seven physicians (2%) prescribed it as the only therapy. Vinpocetine, alone or in combination with other preparations was prescribed from 56 (16.4%) physicians,

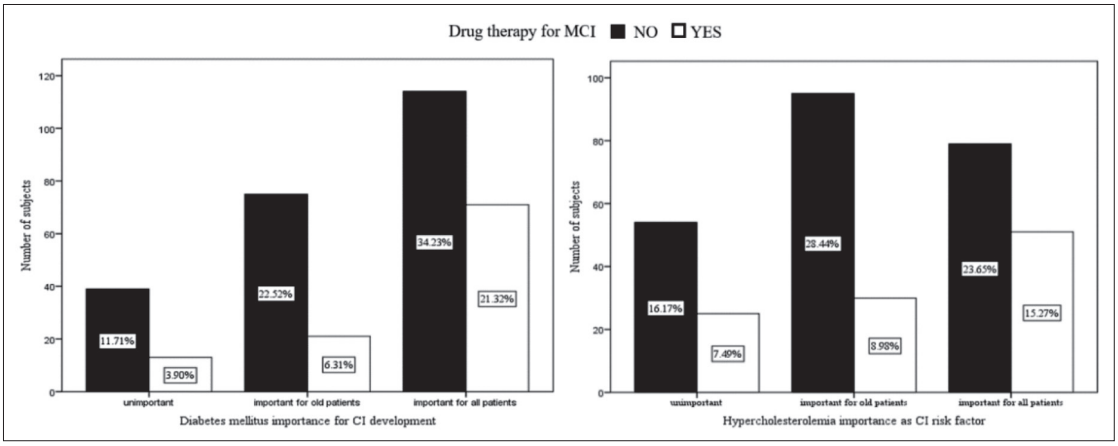

Figure 3. Distribution of physicians with different attitude towards hypercholesterolemia and diabetes mellitus importance as cognitive impairment risk factor who prescribed drug therapy *vs*. supplements for cognitive impairment; χ^2 test for proportion comparison is used; $p < 0.05$ *vs*. physicians' stance estimated distinct risk factor as less important for both risk factors, respectively

while only in 4.7% of cases as the only drug, according to data gathered in this current study. Circulatory system disorders related drugs was prescribed significantly more by specialists than by GPs (21% *vs.* 9%, $p < 0.01$). Detailed data regarding MCI therapy practice are presented in supplementary Table S2.

Results of this investigation enabled therapy modality choice analysis and its dependence from physicians' attitude or judgment of distinct CVD RF as triggers of patients' CI (Figure 3). DM and hypercholesterolemia rating as significant factors for CI occurrence, regardless of patients age, leads to significantly higher proportion of any of the two prescription drugs (pentoxifylline and/or vinpocetine); $p < 0.05$ for both RF according to χ^2 test.

Logistic regression analysis was performed to assess possible factors (predictors) for physicians' awareness of CV RF importance and also for providing prescription drugs (pentoxifylline and/or vinpocetine) among this study group (Tables 4 and 5). This analysis was a surrogate quantitative measure of physicians' MCI recognition. After the primary univariate analysis of all possible factors from the questionnaire, we have selected all predictors with $p \leq 0.100$ and included it in multivariate logistic regression analysis with backward selection to get the best Models of physicians' awareness of CV RF importance and therapy prescription predictors, respectively (Table 4 and 5).

The most significant predictors of RF recognition were relate to physicians' experience, number of patients per protocol, number of their CVD-CI RF and percent of physicians who assumed MCI as normal aging, while predictors of drugs' prescription were years of experience, number of their CVD-CI RF and number of physicians who performed cognitive tests.

DISCUSSION

Worldwide absolute number of people with dementia was estimated to 35.6 million in 2010, and it is predicted to 115.4 million people by 2050 [8].

Epidemiological studies revealed MCI prevalence in people over 65 about 5–10% [9, 10]. MCI is difficult to predict because diagnosis depends on the precise definitions/subtypization [11]. One half of the physicians taking a part in this study were general practitioner with specialization in general medicine and other half was without specialization (basic data about study participants are presented in Table 1). General insight in regular medical practice showed a large work load of the Serbian physicians with about 40 patients per day. Majority of the participants were experienced physicians with more than 20 years of service. They reported about 70% of patients had two or more CVD RF which could be predisposing indicators of MCI (Figure 1). Detailed physicians' categorization of six traditional CVD RF' importance for CI progression is presented in Table 2. In the last decade is recognized that CVD RF like arterial hypertension, DM, dyslipidemia and obesity could cause brain alterations, hence problems in cognition, especially in older patients became evident. RF are clustered with aging, so intellectual problems in elderly are not incidental. This relation between number of RF and cognition impairment are also a part of normal aging and this is a main obstacle for appropriate MCI diagnosis [12]. Even subclinical cognitive changes could seriously disturbed patients' daily activities and many important aspects of their general well-being like medication adherence, comorbidity recognition and overall safety [13]. Majority of GPs noticed forgetfulness, decreased concentration, walking instability, thought slowness (symptoms of damage to small blood vessels in the brain) in 5% to 30% of their patients, while 5–20% of their patients complained of forgetfulness, mood swings, walking instability, or a feeling of brain fog (Figure 2). These data are in accordance with other studies which reported MCI in about 16–20% of patients over 65 [6]. Number of patients with MCI is certainly in positive correlation with proportion of older patients (older population is defined as subjects over the age of 65, which is in agreement with period of retirement for working population).

Recommendations from the American Academy of Neurology [4] are decisive regarding assuming cognitive

Table 4. Univariate and multivariate logistic regression analysis of predictors for physicians' awareness of cardiovascular risk factors importance for cognitive impairment development

Predictor	B (SE)	Wald coefficient	OR (95th CI)	p
Years of physicians' experience	-0.043 (0.013)	10.30	0.958 (0.934–0.983)	**0.001**
Physicians' specialization *vs.* general practitioner	-0.763 (0.265)	8.30	0.466 (0.277–0.784)	**0.004**
Number of patients in physician's protocols	0.000 (0.000)	3.40	1.000 (0.999–1.00)	**0.066**
Percent of patients older than 65 years	0.004 (0.007)	0.30	1.004 (0.990–1.018)	0.573
Percent of patients without CVD risk factors	0.003 (0.014)	0.03	1.003 (0.976–1.030)	0.854
Percent of patients with 1 CVD risk factor	0.023 (0.013)	3.20	1.023 (0.998–1.048)	**0.072**
Percent of patients with 2 or more CVD risk factors	-0.015 (0.007)	4.00	0.985 (0.971–1.000)	**0.046**
Percent of physicians perceiving MCI symptoms as normal aging	0.011 (0.006)	3.40	1.011 (0.999–1.023)	**0.065**
Percent of physicians who perform MCI tests	-0.006 (0.006)	0.90	0.994 (0.983–1.006)	0.341
Percent of physicians who referred MCI suspected patients to neurologist	-0.006 (0.004)	2.00	0.994 (0.986–1.002)	0.153
Multivariant analysis – the best model*				
Years of physicians' experience	**-0.051 (0.015)**	**11.9**	**0.950 (0.923–0.978)**	**0.001**
Number of patients in physician's protocols	-0.001 (0.000)	2.8	0.999 (0.998–1.000)	0.094
Percent of patients with 2 or more CVD risk factors	-0.020 (0.008)	6.2	0.980 (0.964–0.996)	**0.013**
Percent of physicians perceiving MCI symptoms as normal aging	0.013 (0.007)	4.0	1.013 (1.000–1.027)	**0.047**

CVD – cardiovascular disease; MCI – mild cognitive impairment; B (SE) – beta coefficient (standard error), OR – odds ratio with 95th confidence interval;
*Variables selected for multivariant analysis according to p from the univariant analysis ≤ 0.100

Table 5. Univariate and multivariate logistic regression analysis of predictors for providing prescription drugs (pentoxifylline and/or vinpocetine) by physicians

Predictor	B (SE)	Wald coefficient	OR (95th CI)	p
Years of physicians' experience	0.037 (0.012)	9.2	1.038 (1.013–1.064)	**0.002**
Physicians' specialization *vs.* general practitioner	0.432 (0.240)	3.247	1.54 (0.96–2.46)	**0.072**
Number of patients in physician's protocols	0.000 (0.000)	0.885	1.00 (0.99–1.00)	0.347
Percent of patients older than 65 years	-0.001 (-0.006)	0.014	0.99 (0.99–1.01)	0.906
Percent of patients without CVD risk factors	0.004 (-0.012)	0.136	1.00 (0.98–1.03)	0.712
Percent of patients with one CVD risk factor	0.008 (-0.010)	0.652	1.01 (0.99–1.03)	0.419
Percent of patients with two or more CVD risk factors	-0.024 (0.011)	4.607	0.98 (0.96–0.99)	**0.032**
Percent of physicians perceiving MCI symptoms as normal aging	-0.002 (0.006)	0.137	0.99 (0.98–1.01)	0.711
Percent of physicians who perform MCI tests	0.012 (0.005)	5.686	1.01 (1.00–1.02)	**0.017**
Percent of physicians who referred MCI suspected patients to neurologist	0.006 (0.001)	2.640	1.01(0.99–1.02)	**0.100**
Physicians' estimation of arterial hypertension importance	0.113 (0.110)	1.058	1.12 (0.90–1.39)	0.304
Physicians' estimation of diabetes mellitus importance	0.300 (0.143)	4.373	1.35 (1.02–1.79)	**0.037**
Physicians' estimation of hypercholesterolemia importance	0.182 (0.126)	2.077	1.20 (0.94–1.54)	0.150
Physicians' estimation of obesity importance	0.011 (0.102)	0.011	1.01 (0.83–1.23)	0.918
Physicians' estimation of smoking importance	0.121 (0.114)	1.139	1.13 (0.90–1.41)	0.286
Physicians' estimation of physical inactivity importance	0.184 (0.119)	2.392	1.20 (0.95–1.52)	0.122
Multivariant analysis – the best model*				
Years of physicians' experience	0.039 (0.014)	7.8	1.04 (1.01–1.07)	**0.005**
Percent of patients with two or more CVD risk factors	-0.029 (0.013)	5.1	0.97 (0.95–0.99)	**0.024**
Percent of physicians who perform MCI tests	0.013 (0.005)	5.8	1.03 (1.01–1.05)	**0.016**

CVD – cardiovascular disease; MCI – mild cognitive impairment; B (SE) – beta coefficient (standard error), OR – odds ratio with 95th confidence interval;
*Variables selected for multivariant analysis according to p from the univariant analysis ≤ 0.100

symptoms as normal aging, late diagnosis will lead to failed recognition of reversible CI symptoms. This in turn may affect patients' life quality, course of the disease, progression to dementia, as well as the cost increase for the healthcare system. Serbian physicians generally reported CI symptoms as "normal aging" in less than 25% of their patients (Figure 2).

Undiagnosed MCI in older population ranged from 50–75% according to results of different studies and is a consequence of various backgrounds [14]. GP listed several reasons: lack of time, early symptoms unrecognition, insufficient knowledge about screening methods, discomfort between physician, patients and their family caused by coping with this issue, lack of disease-specific biomarker and limitation of treatment options [15]. Although the physicians generally do not ignore MCI symptoms, they rarely proceed to perform regular cognitive functional tests. Physicians responded that in most of the cases they perform CI functional tests in less than 25% of patients suspected to have MCI. The reasons could be patient-related, as shown in a study by Judge et al. [15], stating that patients frequently avoided disclosing symptoms, assuming that they

are part of normal aging; long waiting list and insufficient time for patients' examination hinder GPs ability to act and cause patients miss further checkups. Higher percent of physicians (38%) referred more patients to neurologist for specialized diagnostics and therapy (Figure 4), and this, while more encouraging, is still a low percent of intervention for MCI suspected patients. According to the Serbian National Guideline for Good Clinical Practice – Alzheimer's disease [16], every patient with cognitive symptoms and suspicion of CI must be tested and referred to a neurologist. GP estimated DM as the most important RF, then hypercholesterolemia, smoking, and physical inactivity. This part of investigation could explain general position of these CVD RF as significant predictors of universal cognitive health determinant (Figure 3). DM, smoking and physical activity were rated as equally important as CI predisposing RF by 50% or more GP. Physicians rated diabetes as important RF are more likely to cooperate with neurologist. Hypertension and especially obesity were rated as less important RF, which is surprising finding of this study. It is worrisome, that arterial hypertension so as hypercholesterolemia are perceived as important RF only for older people, by significantly large percent of GPs (31%, 37%, respectively). CVD-related pathology alters brain structure, leading to gray matter atrophy, white matter lesions, and damage of subcortical white matter pathways [17]. Current neurophysiological literature reports significance of hypertension directed to memory, attention, complex activities and meaning, appropriate behavior [18]. Regular testing of cognitive abilities, even from middle age, and CVD RF screening is essential, because adequate control of these factors could prevent CI progression to dementia [12]. The results we obtained have shown urgent need for increasing the awareness of GPs on the connection between CVD RF, especially hypertension, and CI and the basic CI testing recommendations and relevant therapeutic approaches for this condition. In the light of the current COVID-19 pandemic situation, we must pose a question – what will be the consequences of subordinating all domains of healthcare system to this infectious disease and what will be the destiny of MCI patients if left undiagnosed and without therapeutic and/or lifestyle interventions?

GPs and general medicine specialists' comparison, underlined more experience and broader view of later study participants. The main results refer to more cautious stance of specialists towards CI phenomenon among patients (Table 3). Also, four important CVD RF were estimated as more important by specialists compared to GPs. The similar result was evident when comparing GPs experience: physicians with more than 30 years of service rated hypercholesterolemia, obesity, and physical inactivity as more important RF for MCI than participants with less than 30 years of service. This is surplus evidence that the amount of knowledge and experience is crucial in forming the physicians' attitude towards CI and their clinical decisions. Physicians with higher workload are more diligent towards CI test performance. We suppose that GPs with more patients are in fact "popular" physicians, maybe because of their professionalism and assiduity (Serbian general practice is based on a "chosen physician" model). There are attempts to develop simple diagnostic screening tool rely on verbal fluency, which is recently confirmed by McDonnell et al. [19] and Nguyen and Lee [20], but Abdivalievna et al. [21] emphasized the significance of patient's emotional state for the proper diagnosis of MCI.

One of the discouraging reasons for GP s' hesitation apropos MCI diagnosis is the lack of appropriate therapy options, the data showed in the study by Judge et al. [15]. Therapy for MCI is Alzheimer's indicated therapy, like donepezil (acetyl-choline esterase inhibitor), with proofed ability to postpone dementia [22]. Majority of GPs suggest different antioxidants as therapeutic choice. *Ginkgo biloba* supplements alone, or in combination with antioxidants are the most frequently used, while a small percent of GPs prescribe pentoxifylline or vinpocetine as the only two substances categorized as medicinal products approved for the circulatory disorders in Serbia. GPs' prescribed combination of pentoxifylline or vinpocetine with antioxidants or *Ginkgo biloba* supplements in low percent of cases. Physicians are more prone to providing therapy if they perceive arterial hypertension and/or hyperlipidemia as highly important RF for all patients, regardless of age. Physicians who estimate DM and/or hyperlipidemia as important RF for MCI development are likely to prescribe pentoxifylline and/or vinpocetine (Figure 3).

Pentoxifylline activity depends on its positive hemorheological characteristics that affects microcirculation, its main indication is in the treatment of intermittent claudication [23]. McCarty et al. [24] revealed positive pentoxifylline influence at reducing dementia progress in patients with documented cerebrovascular disease, which was confirmed in studies by Rasyid et al. [25], Khan et al. [26] and Sha and Callahan [27]. Vinpocetine is the active ingredient of a drug registered for the treatment of symptoms of chronic cerebrovascular disorders. Vinpocetine has complex mechanism of action, involving brain circulation augmentation, oxygen utilization, increasing tolerance of neural tissue towards hypoxia/ischemia, anticonvulsive activity, inhibition of the phosphodiesterase enzyme, enhancement of blood rheological properties and anti-aggregatory activity [28]. Study dealt with geroprotectors development presented vinpocetine as potential anti-aging agent, even its activity in Alzheimer's has not been confirmed [29]. Multiple regression analysis of predictors model of factors which are connected with CV RF recognition so as commitment to symptomatic therapy for MCI prescription stressed the importance of more years of work experience and more specialized medical education. Experienced clinicians use sophisticated diagnostic techniques, because of awareness about different therapeutic possibilities. Lee et al. documented the need for continuing medical education, which is in line with our own results [30]. We want to emphasize that the answer about prescribing practice could have been significantly influenced by the fact that GPs were asked about prescribing drugs for symptoms of small vessel disease in the brain, which affects not only cognitive functions, but also other brain functions.

CONCLUSION

Results of this study revealed physicians' working experience and specialization as main factors for MCI diagnosis and treatment. General medicine specialists showed better recognition of MCI, so as CVD RF appreciation. The target group for education are younger physicians with less experience/without specialization. New age demands about faster and more focused education revealed short courses or educational workshops devoted to MCI diagnostics and therapy as acceptable option. Therapeutic approach should be grounded on evidence-based prescription treatment, instead of *per inertia* dietary supplements use, due to the lack of valid clinical evidence for the latter. Current recommendations include different cognitive exercises and physical activity for older people. Although we do not have a direct question about this kind of practice among Serbian GPs, according to our experience, this is a real-life practice in Serbia, as a potential measure for dementia prevention. The implementation of these interventions has a proven beneficial effect in slowing down MCI progression and when combined with adequate control of RF, even leading to its reversal in certain number of affected subjects. Future investigation should be more patients-oriented in order to estimate their real-life behavior and to suggest these simple, but potent life-style measures.

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Препознавање и лечење благог когнитивног поремећаја у пракси лекара опште медицине Србије

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САЖЕТАК

Увод/Циљ Благо когнитивно оштећење (БКО) стање је прогресивног когнитивног опадања, које лекари опште медицине ретко препознају, што је разлог касног лечења и брзог напредовања ка озбиљнијем стању. Главне сметње правовременом лечењу БКО су недостатак дијагностичких протокола и клиничких смерница, као и недостатак знања и неповерење у фармаколошке терапијске могућности.
Циљ овог истраживања био је да се процени ниво препознавања симптома БКО од стране лекара опште медицине и њихово опажање значајних фактора ризика за развој овог обољења.

Методе Учесници конференције „Дани опште медицине", лекари опште медицине (Србија, март 2018), $n = 340$, попунили су упитник са 12 питања о препознавању и лечењу болесника са БКО. За приказ резултата коришћени су дескриптивна статистика, χ^2, Ман–Витнијев U тест, бинарна логистичка регресиона анализа, да би се проценили предиктори избора терапије лековима.

Резултати Показано је да лекари опште медицине препознају дијабетес као најважнији фактор за БКО, затим хиперхолестеролемију, пушење и седентарно понашање, док се хипертензија и гојазност сматрају мање важним. Они лекари опште медицине који су дијабетес и хиперхолестеролемију проценили као важније за све болеснике знатно су склонији прописивању симптоматских лекова (пентоксифилин и винпоцетин), $p < 0,05$ према χ^2 тесту. Логистичка регресиона анализа у вези са предвиђањима примене симптоматске терапије показала је да су године искуства лекара најважнији предиктор избора терапије лековима ($p < 0,01$).

Закључак Резултати овог истраживања указали су на потребу едукације младих лекара о БКО, како би се побољшали дијагноза и лечење ових болесника.

Кључне речи: благо когнитивно оштећење; дијагноза; фармаколошка терапија; лекари опште медицине

Supplementary Table S1. Comparison of attitudes towards mild cognitive impairment risk factors importance in subgroups of general practitioners according to years of experience

Experience – years of service			
Variable	< 30 years	> 30 years	p
Risk factors for cognitive impairment – physicians' perception			
Hypercholesterolemia unimportant / important only for older patients / important for all patients	60/96/84 (25/40/35%)	16/25/44 (18.8/29.4/51.8%)	0.025
Obesity unimportant / important only for older patients / important for all patients	117/52/67 (49.6/22/28.4%)	27/17/41 (31.8/20/48.2)	0.003
Physical inactivity unimportant / important only for older patients / important for all patients	65/60/109 (27.8/25.6/46.6%)	13/20/53 (15.1/23.3/61.6%)	0.029

Supplementary Table S2. List of different drugs, supplements or its combination prescribed by physicians involved in this study

Cognitive impairment therapy	Number of physicians	Percent
Without any drug / supplement	17	5
Antioxidants	24	7.1
Ginkgo biloba preparation	70	20.6
Vinpocetine	16	4.7
Pentoxifylline drug	7	2
Other	2	0.6
Combination		
Antioxidant + *Ginkgo biloba* supplements	113	33.2
Antioxidant + vinpocetine	3	0.9
Antioxidant + pentoxifylline	6	1.8
Ginkgo biloba supplements + vinpocetine	3	0.9
Ginkgo biloba supplements + pentoxifylline	8	2.4
Ginkgo biloba supplements + other	1	0.3
Vinpocetine + pentoxifylline	1	0.3
Vinpocetine + other	2	0.6
Pentoxifylline + other	1	0.3
Antioxidant + *Ginkgo biloba* supplements + vinpocetine	15	4.4
Antioxidant + *Ginkgo biloba* supplements + pentoxifylline	24	7.1
Antioxidant + *Ginkgo biloba* supplements + other	4	1.2
Antioxidant + vinpocetine + pentoxifyllin	1	0.3
Ginkgo biloba + vinpocetine + pentoxifylline	4	1.2
Ginkgo biloba + vinpocetine + other	1	0.3
Antioxidant + *Ginkgo biloba* supplements + vinpocetine + pentoxifylline	6	1.8
Antioxidant + *Ginkgo biloba* supplements + vinpocetine + other	2	0.6
Antioxidant + *Ginkgo biloba* supplements + pentoxifylline + other	1	0.3
Antioxidant + *Ginkgo biloba* supplements + vinpocetine + pentoxifylline + other	3	0.9
Missing data	5	1.5
Total	340	100

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Is there a difference between patients with functional dyspepsia and irritable bowel syndrome in headache manifestation?

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SUMMARY
Introduction/Objective The objective was to explore whether there is a difference in headache manifestation and level of its intensity in patients with functional dyspepsia and irritable bowel syndrome.
Methods We assessed a cohort of 420 participants out of which 300 satisfied the recruiting criterion of the presence of irritable bowel syndrome (148) or functional dyspepsia (152). Diagnoses of irritable bowel syndrome and functional dyspepsia were made according to Rome IV criteria. Intensity of headaches was estimated in irritable bowel syndrome and functional dyspepsia participants using visual analog scale. All the patients underwent subsequent testing by Hamilton's Depression Inventory and anxiety scale.
Results Our results showed that males with headaches are more susceptible to functional dyspepsia, statistical significance in the group of patients with irritable bowel syndrome with high scores on the visual analog scales, in relation to Hamilton's anxiety scores in the group of patients with irritable bowel syndrome. Gender and visual analogue scale scores were determinants to show whether the patient falls within the group of functional dyspepsia or irritable bowel syndrome. Scores of visual analogue scale where the patient felt the best was statistically borderline (p = 0.061) and its higher values pinpointed which of those patients fall into irritable bowel syndrome group.
Conclusion Gender and level of headache intensity as a extraintestinal manifestation showed to be the main variables to make a difference between patients with functional dyspepsia and irritable bowel syndrome where irritable bowel syndrome had higher scores and greater dominance in differential diagnosis if the headache was determining variable.
Keywords: headaches; functional dyspepsia; irritable bowel syndrome

INTRODUCTION

Migraine is a primary headache typically characterized by unilateral pulsating head pain that is aggravated by routine physical activity and may be accompanied by a variety of autonomic, cognitive, and emotional disturbances [1]. Headaches are reported to be evaluated as one of the top rated self-reported physical disorders [2]. Estimated one-year prevalence of migraine is approximately 14% in the general population and the association between headache and gastrointestinal complaints increased with increasing headache frequencies. Chronic migraine-like headache was reported in about 30% patients with functional dyspepsia (FD), but the pathophysiology is still not fully understood [3, 4]. Functional gastrointestinal and motility disorders are a group of disorders of gut–brain interaction, which are categorized by Rome diagnostic criteria as symptom-based diagnostic criteria for each category [5]. Due to the fact that the prevalence of functional digestive disorders and irritable bowel syndrome (IBS) are still underestimated with the currently applied diagnostic tools, some other improved criteria or point of view are needed as the treatment is still not very efficient and satisfactory. IBS presents a neurogastroenterological functional disorder that shares some environmental risk factors with migraine (predominately affecting the female sex and younger individuals). It is a group of bowel disorders with specific abdominal discomfort or pain correlated with bowel habit irregularities. FD refers to pain or specific discomfort in the topographic region of the upper abdomen. IBS and FD share many somatic and psychiatric comorbidities [6]. Except for the headaches as one of the most prominent extraintestinal neurological manifestation, GH presents one of esophageal disorders manifesting as a sensation of a lump or tightness in the throat, which also can be attributed to psychogenic cause i.e., somatoform or anxiety disorder [7].

The objective was to explore whether there is a difference in headache manifestation and to evaluate the level of its intensity in patients with FD and IBS.

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METHODS

We assessed a cohort of 420 participants, out of which 300 (174 females and 126 males) satisfied a recruiting criterion of the presence of IBS (148) or FD (152). The participants were 18–80 years old and were referred to the gastroenterology unit of the Dr Dragiša Mišović – Dedinje Clinical and Hospital Center from January to December of 2019. Diagnoses of IBS and FD were made according to Rome IV criteria [5]. The participants satisfied the following inclusion criteria: 1) older than 18 years; 2) no evidence of organic disease on the upper and lower endoscopy examination; 3) normal findings on abdominal ultrasonography; 4) no history of abdominal surgery; 5) absence of any cardiovascular or metabolic disease to avoid vasculoprive or headaches related to the impaired metabolism or endocrine function; and 6) no evidence about prior neuropsychiatric treatment.

Participants underwent a clinical interview and physical and neurological examinations by experienced neurologists in order to exclude headaches associated with neurological disorders and to assess for presence of migraine-like migraine. A migraine has been diagnosed according to International Classification of Headache Disorders 3rd edition [8].

The intensity of headaches has been estimated in IBS and FD participants using visual analogue scale (VAS), where 0 is the absence of pain and 10 is the worst possible pain. VAS scale was used to assess pain in three states: pain when the patient was at his/her best (VAS best), baseline pain (VAS typical), and pain when the patient was at his/her worst (VAS worst). It is important to note that we reanalyzed the data from our two groups (IBS, n = 148, and FD, n = 152) to determine the mean of VAS pain intensity rating and changes scores on 10-cm-rating scale, 0–0.4 cm signified no pain; 0.5–4.4 cm signified mild pain, 4.5–7.4 cm moderate pain, and 7.5–10 cm severe pain.

Participants underwent psychiatric examination including psychiatric interview/evaluation by the specialist of psychiatry in order to assess the presence of depressive or anxiety disorder and to exclude other psychiatric comorbidities. The patients underwent subsequent testing by Hamilton's anxiety (HA) and depression (HD) 21-item inventory, Serbian version [9]. Typically, Hamilton Depression Inventory contains items related to gastrointestinal symptoms and weight loss. Please note that these were omitted because the mentioned symptomatology is part of the illness. The diagnosis of globus hystericus (GH) has been made according to the 10th revision of the International Classification of Diseases (ICD-10) criteria for the diagnosis code F 45.8 [7]. The presence of GH was assessed by routine questionnaire used during the first visit to the gastroenterologist.

Statistical analysis

We used Pearson's χ^2 test with likelihood ratio correction to compare groups among categorical data when necessary. For those variables expressed with the scores, testing was performed to verify if the normal distribution exists and, in that case, we used the Kolmogorov–Smirnov test. Non-parametric test methods were used for further analysis. The Mann–Whitney test was used to compare the parameters on the scale to determine the difference. A binomial logistic regression analysis, the stepwise backward method, was used to define the determining variables that may be influencing the prediction of group affiliation. We used the software program IBM SPSS Statistics, Version 27.0 (IBM Corp., Armonk, NY, USA) with a significance threshold of $p = 0.05$.

The protocol involving human data was in accordance with national and institutional guidelines and the Declaration of Helsinki. All the participants were informed about the study protocol and they provided written consent. The study was approved by the Ethics Committee of Dr. Dragiša Mišović – Dedinje Clinical and Hospital Center (18-6685/2019).

RESULTS

Demographic data imply that examined groups were of similar size ($p = 0.808$), gender-balanced with slightly more women within examined groups ($p = 0.122$). The manifestation and occurrence of headaches is less pronounced according to our results but not statistically significant ($p = 0.073$). Manifestation of GH and scores of HA were almost completely uniform within the observed categories ($p = 0.755$ and $p = 0.949$, respectively). The HD scores were mostly uniform and did not show a statistically significant difference ($p = 0.271$). The scores of HD, HA, VAS, as well as the ages of the examined patients did not have a normal distribution, therefore we used non-parametric tests and based our results on the Mann–Whitney test. In all cases, the groups were uniform ($p > 0.05$) and at the very beginning did not differ according to the observed parameters (Tables 1 and 2).

Demographic data showed no statistical difference between FD and IBS groups ($p > 0.05$) (Table 1).

VAS score and Hamilton's scales showed no difference between the examined groups when Mann–Whitney test was done, but when we made a separation into groups of those who did experience headaches and those who did not, the statistical significance was shown in male patients with FD, which is shown in Table 2.

Since headache was found as one of the dominant determinant variables in logistic regression analysis, the influence of the determining variable between the examined groups and observed variables was measured (Table 3).

Sex (gender) had an impact on FD and IBS when related to headache, as seen in Table 4. Males with headaches were more susceptible to FD, HR = 1.829 (1.043–3.206).

GH, HD, HA show no statistical difference between the groups if headache is observed as a determining variable ($p > 0.05$).

Table 1. Group comparisons by category parameters

Parameters			Group		Total	p
			FD	IBS		
Sex	Male	n (%)	49 (56.3)	38 (43.7)	87 (100)	0.122
	Female	n (%)	99 (46.5)	114 (53.5)	213 (100)	
Headache	Yes	n (%)	51 (57.3)	38 (42.7)	89 (100)	0.073
	No	n (%)	97 (46)	114 (54)	211 (100)	
Globus	Yes	n (%)	63 (50.4)	62 (49.6)	125 (100)	0.755
	No	n (%)	85 (48.6)	90 (51.4)	175 (100)	
HD	None	n (%)	7 (43.8)	9 (56.3)	16 (100)	0.271
	Mild	n (%)	39 (47.6)	43 (52.4)	82 (100)	
	Moderate	n (%)	52 (44.8)	64 (55.2)	116 (100)	
	Heavy	n (%)	50 (58.1)	36 (41.9)	86 (100)	
HA	None	n (%)	9 (56.3)	7 (43.8)	16 (100)	0.949
	Mild	n (%)	75 (49.3)	77 (50.7)	152 (100)	
	Moderate	n (%)	22 (47.8)	24 (52.2)	46 (100)	
	Heavy	n (%)	42 (48.8)	44 (51.2)	86 (100)	
Total		n (%)	148 (49.3)	152 (50.7)	300 (100)	

FD – functional dyspepsia; IBS – irritable bowel syndrome; HA – Hamilton's anxiety; HD – Hamilton's depression

Table 2. Group comparisons by score parameters

Parameters	Group				p
	FD		IBS		
	Median	IQR	Median	IQR	
Age	42.5	25	45	22	0.333
VAS now	0	4	0	2	0.815
VAS best	0	0	0	0	0.168
VAS typical	0	5	0	4	0.170
VAS worst	0	8	0	8	0.430
HD	21	13	21	9	0.242
HA	15.50	12	16	12	0.391

FD – functional dyspepsia; IBS – irritable bowel syndrome; HA – Hamilton's anxiety; HD – Hamilton's depression; IQR – interquartile range; VAS – visual analogue scale

Table 3. Logistic regression, stepwise backward method, for Group predictions and determined parameters

Parameters	HR	95% CI LL	95% CI UL	p
VAS Best	1.438	1.042	1.985	**0.027**
HA	1.040	1.001	1.080	**0.043**
Headache (No)	3.307	1.599	6.839	**0.001**
Constant	0.186			0.005

HA – Hamilton's anxiety; VAS – visual analogue scale

Table 4. Determined Headache parameter and comparison of the parameters Sex and Groups

Headache				Group		Total	p
				FD	IBS		
Yes	Sex	Male	n (%)	27 (71.1)	11 (28.9)	38 (100)	**0.024**
		Female	n (%)	24 (47.1)	27 (52.9)	51 (100)	
	Total		n (%)	51 (57.3)	38 (42.7)	89 (100)	
No	Sex	Male	n (%)	22 (44.9)	27 (55.1)	49 (100)	0.863
		Female	n (%)	75 (46.3)	87 (53.7)	162 (100)	
	Total		n (%)	97 (46%	114 (54)	211 (100)	

FD – functional dyspepsia; IBS – irritable bowel syndrome

In VAS scores (worst and best) there was a statistical significance between FD and IBS where IBS had higher scores if the headache is the determining variable. In contrast to this, in a situation without headache only HA scale showed some upper limits in IBS group of patients as statistically significant ($p < 0.05$) (Table 5.)

In the group of those who had headache, logistic regression showed determining variable within each examined group and sex (gender), VAS best, VAS typical, and VAS worst determined whether patient falls within the group of FD or IBS. VAS best was statistically borderline ($p = 0.061$). Higher VAS best score shows HR = 1.410 (0.984–2.020), which pinpoints that those patients fall into the IBS group. VAS typical shows less hazard to be IBS if scores are higher HR = 0.577 (0.377–0.884). VAS worst shows a more important role to determine the IBS group with HR = 2.191 (1.273–3.771).

In situations without headache, the only important variable is HA, where HR score shows to fall within the scope of IBS with higher values HR = 1.092 (1.022–1.166) (Table 6)

DISCUSSION

There is a significant overlap between FD and IBS clinical manifestations. Headaches, especially migraines, present one of the most important and disabling manifestations in above mentioned gastrointestinal disorders, proving a very important and powerful role of the brain–gut axis [4, 10].

In our study, we used the presence of headaches and relation to their specific intensity (VAS scale scores) based on which we made a separation between the patients with FD and those with IBS.

Migraine-like headaches present a very disabling condition, often recurrent and severe with concomitant gastrointestinal features and affect women more frequently than men [11]. It was also shown that FD affects women more than men in daily life [12].

Our results showed that gender had an impact on FD and IBS when related to headache, showing that males with headaches are more susceptible to FD. When we made a separation into groups of those who did experience headaches and those who did not, the statistical significance was shown in the group of male patients with FD. In previous studies conducted related to gender differences in migraines it was shown that man tend to have longer remission periods than women and that headache attack frequency and their intensity are similar to both genders with severe migraines persisting longer in women [13].

The VAS evaluates the severity of subjective symptoms in patients, especially in measuring pain. Our results showed that there is statistical significance in the group of patients with IBS who had high scores on the VAS, which correlates with previous studies.

Migraine headaches have a higher prevalence in patients with IBS compared to the general population. Li et al. [14]

Table 5. Determined Headache parameter and comparisons with parameters Score and Groups

Parameter	Group	Headache									
		Yes					No				
		n	Mean	Median	STD	p	n	Mean	Median	STD	p
Age	FD	51	41.41	33	15.478	0.549	97	44.61	43	13.610	0.557
	IBS	38	41.03	41.50	11.554		114	45.63	45	13.415	
VAS Now	FD	51	4.73	5	2.601	0.123	97	0.05	0	0.508	0.142
	IBS	38	5.18	6	2.415		114	0.29	0	1.480	
VAS Best	FD	51	0.63	0	1.183	**0.028**	97	0	0	0	0.191
	IBS	38	1.21	0	1.492		114	0.04	0	0.295	
VAS Typical	FD	51	6.06	6	1.580	0.943	97	0.12	0	0.869	0.351
	IBS	38	5.97	6	1.602		114	0.25	0	1.209	
VAS Worse	FD	51	8.47	8	1.206	**0.023**	97	0.27	0	1.517	0.423
	IBS	38	9.03	9	1.150		114	0.50	0	2.138	
HD	FD	51	28.61	28	4.976	0.656	97	16.74	17	5.553	0.748
	IBS	38	28.53	27	4.607		114	16.82	18	5.508	
HA	FD	51	26.61	30	8.139	0.403	97	15.70	15	4.895	**0.035**
	IBS	38	27.82	30	7.665		114	17.31	15	6.046	

FD – functional dyspepsia; IBS – irritable bowel syndrome; HA – Hamilton's anxiety; HD – Hamilton's depression; VAS – visual analogue scale

Table 6. Logistic regression, stepwise backward method, by determining parameter Headache to identify prediction variables to identify the groups

Headache	Parameters	HR	95% CI LL	95% CI UL	p
Yes	Sex (male)	0.253	0.089	0.718	**0.010**
	VAS Best	1.410	0.984	2.020	0.061
	VAS Typical	0.577	0.377	0.884	**0.011**
	VAS Worse	2.191	1.273	3.771	**0.005**
	Constant	0.028			0.055
No	HA	1.092	1.022	1.166	**0.009**
	Constant	0.613			0.402

HA – Hamilton's anxiety; VAS – visual analogue scale

showed that patients with reported chronic headaches are more likely to have IBS.

Our results also showed statistical significance in relation to HA scores in the group of patients with IBS. Anxiety presents a psychiatric disorder which attacks individuals with IBS and therefore might worsen their condition. The reason lies in the fact that colon as an anatomical substrate is under control of nervous system responding to stress. Affected hypothalamic-pituitary axis (HPA) activates a stress biochemical cascade, which triggers the immune system as well, playing a significant role. Although anxiety itself mostly does not cause a gastrointestinal disorder, these patients are more emotional to everyday life stressors.

When we analyzed all significant variables in the group of patients with headaches, our results showed that gender, VAS best, VAS typical, and VAS worst were determinants whether a patient falls within the group of FD or IBS. VAS best was statistically borderline and higher VAS best score pinpointed which of those patients fall into the IBS group. Generalized inflammatory response rather than isolated bowel inflammation may play the key role in the pathogenesis of the extra-intestinal manifestations of IBS.

The activation of HPA was associated with stress and the increase of IL-6 in the peripheral blood. There is also a link between inflammation and mental disorders in patients with anxiety and depression that had immune response correlated to increased levels of serum C-reactive protein and other inflammatory mediators [15, 16, 17].

Patients with overlapping IBS and FD symptoms had more severe psychological problems and problems with anxiety and depression as an independent factor [18].

It has been hypothesized that the underlying pathophysiology for both IBS and migraine is a genetically established hypersensitive or hyperexcitable brain [19]. Environmental, psychological, and immunological factors may increase sensitization in the enteric nervous system and brain gut axis in IBS. Increased amygdala activity, demonstrated in IBS, could also be linked with the conversion dysphagia, also known as GH and subsequent influence to the emotional zones [20]. Abnormalities in emotion regulation and connectivity have been identified in non-symptom studies about conversion disorders, potentially pointing to a diathesis or vulnerability: two studies found an abnormal emotion–motor connectivity, and a failure of normal habituation [21].

Many researchers debated about precise pathophysiological mechanisms of migraines and one of them is vascular due to the vasodilatation of the middle meningeal artery and middle cerebral artery on the side of the brain where the pain occurs, or bilaterally if the pain attacks from both sides. It is widely considered that the inflammation is the core mechanism and that the inflammatory mediators play the main role. Among the others the most important and the oldest are histamine and tumor necrosis factor alpha [17].

The link between depressive and anxiety symptomatology with functional gastrointestinal disorders' clinical symptoms may refer to a low concentration of serotonin (5-HT), which correlates to greater nociception of trigeminal neurons, which also produces a clinical correlation with different migraine intensities in pain [22]. Serotonin 5-HT1F receptor agonists are on the list of prophylactic drugs for migraine, implying that a lower concentration of serotonin decreases the stimulation of the mentioned receptors which are hypothesized to have an important role in migraine genesis [23, 24]. Moreover, probiotics are

believed to be of potential benefit in the treatment of migraine, as well as IBS and FD [25].

The results from a double-blind randomized controlled experimental investigation showed based evidence of correlation between IBS and migraine showing expressed immunoglobulin G antibodies reduced the frequency and the level of migraine attacks after specific food deprivation and reduction, which pinpoints the growing significance of the gut–brain axis [26].

There is also evidence showing a correlation between pain-related functional gastrointestinal disorders and migraine in pediatric population, as well as much evidence based on association of anxiety, depression, and FD [27].

Finally, migraine in functional disorders of the gastrointestinal tract is interpreted as disrupted balance of microbiota in the gut and its influence to pain sensations and impaired brain–gut axis [15]. The concept of microbiota gut–brain axis refers to a significant role of the modulated enteric and central nervous system function disrupting mood and affection by modifying serotonin, which plays a key role in both gastrointestinal tract and in the brain [28, 29].

Gut microbiota in correlation with the gut–brain axis defines itself as the main new to-be-defined axioma in functional sense of evidence-based functional substrate on precise explanation of neuropsychiatric and functional gastrointestinal disorders interaction. Management options of headaches which are typically diagnosed very late impact the quality of life of a patient and therefore the development of treatment regime with less potentional side effects correlated with patients with functional gastrointestinal disorders is of huge importance. Defining morphological anatomical substrate is the main step in defining an illness or disorder, but in this case we must be aware of the fact that the systems and their interaction understanding present the main step in defining functional gastrointestinal disorders and migraine attacks [30].

Study strengths

The study largely contributes to the development and improvement of differential diagnosis and treatment of patients diagnosed with neuropsychiatric intestinal problems.

Study limitations

An important limitation is that this is a referred sample. Physicians referred patients to participate in the study. This may be justified by the fact that these are hard-to-reach groups due to social cultural stigma. As these patients were 'referred,' we acknowledge that there is a significant risk of selection bias (choosing a large number of people with similar characteristics or views to the initial individual identified). Data about pain intensity in migraine are depending on sincerity of the patients. GH is a symptom, thus it is a subjective feeling and might be interpreted differently by patients and physicians. Nonetheless, the criteria from ICD-10 are attenuating, and not eliminating, the subjectivity. Psychiatrists were not blinded to the patients' diagnosis, because psychiatric evaluation is the part of routine treatment of IBS and FD patients.

CONCLUSION

In conclusion, according to our results, headaches and their intensity are more related to males with FD but higher VAS scores showed great significance in diferentiating between patients with FD and IBS, where IBS had higher scores if the headache was a determining variable. Both functional gastrointestinal disorders probably induce morphological and functional brain alterations due to impaired metabolism of serotonin with extraintestinal manifestations, but more different tests should be performed in this field of investigation.

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Постоји ли разлика у манифестацији главобоље између болесника са функционалном диспепсијом и синдромом иритабилног црева?

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САЖЕТАК

Увод/Циљ Циљ овог истраживања је да утврди да ли постоји разлика у манифестацији главобоље и степену њеног интензитета код болесника са функционалном диспепсијом и синдромом иритабилног црева.

Методе Група испитаника сачињавала је 420 болесника од којих је 300 задовољило укључујуће критеријуме у виду присуства синдрома иритабилног црева (148) или функционалне диспепсије (152). Дијагнозе синдрома иритабилног црева и функционалне диспепсије постављене су у складу са критеријумима Рома IV. Интензитет главобоља процењен је у групама болесника са синдромом иритабилног црева и функционалном диспепсијом помоћу визуелно-аналогне скале. Сви болесници подвргнути су тестирању помоћу Хамилтонове скале депресије и скале анксиозности.

Резултати Наши резултати показују да су мушкарци са главобољом подложнији функционалној диспепсији, као и да постоји статистички значајна разлика у групи болесника са иритабилним синдромом црева који су имали веће резултате на визуелно-аналогној скали и статистички значајна разлика у погледу резултата скале анксиозности у групи болесника са синдромом иритабилног црева. Пол и резултати на визуелно-аналогној скали били су детерминанте одређивања да ли болесник припада групи функционалне диспепсије или синдрома иритабилног црева. Резултати на визуелно-аналогној скали где су болесници навели да се најбоље осећају били су гранично статистики значајни ($p = 0,061$) и њихова већа вредност истакла је оне болеснике који припадају групи са синдромом иритабилног црева.

Закључак Пол и ниво интензитета главобоље као екстраинтестиналне манифестације представљају главне варијабле за утврђивање разлике између болесника са функционалном диспепсијом и синдромом иритабилног црева, где синдром иритабилног црева има веће резултате и доминацију у диференцијалној дијагнози уколико је главобоља детерминишућа варијабла.

Кључне речи: главобоље; функционална диспепсија; синдром иритабилног црева

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of PD-L1 expression in recurrent nonmetastatic sacral chordomas – a retrospective study

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SUMMARY

Introduction/Objective Chordomas are rare tumors of a notochordal origin. Wide surgical resection is recommended for treatment. However, it is associated with a high risk of morbidity and mortality. Additionally, these tumors are resistant to chemotherapy. Thus, targeted therapy is needed for the treatment of chordomas. Programmed death ligand 1 (PD-L1) is a promising target for cancer treatment. Here, we investigated PD-L1 expression in patients with chordoma in a single-center study.

Methods Formalin-fixed paraffin-embedded blocks were evaluated for immunohistochemical analysis to evaluate PD-L1 expression. Clinicopathological variables, such as sex, age, and follow-up data (recurrence and outcome), were retrospectively collected from the patients' medical records.

Results Ten patients diagnosed with sacral chordoma in a single institution between December 2015 and November 2021 were included in this study. The median patient age was 57 years and the median follow-up period was 40 months. The surgical margins were negative in all cases, without any preoperative medical treatment. Four of the ten patients showed PD-L1 positivity on immune cells. These patients showed local recurrence, without metastasis. In these cases, the median time to local recurrence was 15 months. All the patients with the disease were alive.

Conclusion This study demonstrated that PD-L1 positivity in immune cells can be used as a predictive marker for local recurrence at the time of surgical treatment. This can potentially be used to determine the necessity to administer immunotherapy.

Keywords: chordoma; wide surgical resection; PD-L1; immunotherapy

INTRODUCTION

Chordomas are locally destructive, slowly enlarging tumors. The incidence of chordoma is estimated to be one per million per year in the United States and Europe [1]. These rare, primary tumors are derived from neural crest cells. They are expansile lytic lesions located in the midline of vertebral bodies.

These tumors are difficult to treat, with a very low rate of complete recovery. In advanced cases, chordomas invade the normal fascial barriers. Wide surgical resection is accepted as the primary line of treatment; however, it can result in high morbidity [2]. Neurological sacrifice causes bowel, bladder, and sexual dysfunction. Metastasis is rare, but recurrences have a poor prognosis, which makes the treatment more complicated.

Several multidisciplinary treatment strategies can be applied. This is particularly crucial when wide resection is not feasible. Therefore, the treatment strategies for chordomas should be formulated on a case-by-case basis. With advances in diagnostic and treatment options, promising new treatment strategies are being developed for chordoma. These include chemotherapy, imatinib treatment, adjuvant radiotherapy, and proton-beam therapy [3]. No standard effective adjuvant therapy regimens are currently available for chordomas treatment. In addition, the effect of these treatments on survival remains unclear. Such treatments, if effective, can avoid marginal excision, thus evading the morbidity risk.

Chordomas are resistant to radiation and chemotherapy [4]. New therapeutic options include the use of imatinib, which is an inhibitor of the platelet-driven growth factor receptor beta. It has been found to be effective in treating chordomas. In future, targeted therapy and immunotherapy for treating bone and soft tissue tumors have the potential to further improve prognosis.

Programmed cell death protein 1 (PD-1) is a candidate biomarker for chordomas [5]. PD-1 and programmed death ligand 1 (PD-L1) were discovered in 1992. It plays an important role in immune surveillance during cancer. Along with the activation of cytotoxic T lymphocytes, the balance of positive and negative signals is also important for the development

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of antitumor immunity. Similar to surface molecules, cytotoxic T-lymphocyte antigen-4 and PD-1 show negative signals. PD-1 is an inhibitory receptor, which belongs to the CD28 family and plays an important role in tumor immune escape. PD-1 and PD-L1 are the targets of new-generation drugs and can potentially be used for the treatment of many types of cancers. However, only two studies have evaluated the PDL1 receptor status in chordoma, thus requiring further research to prove its therapeutic efficacy. This study aimed to assess PD-L1 expression in patients with sacral chordomas.

METHODS

A retrospective review was conducted between 2016 and 2020 for all cases with histologically proven chordoma. Ten cases of sacral chordoma were identified. The diagnosis was made based on typical morphological features, S100/cytokeratin, and brachyury expression (Figure 1). Resected materials, including whole-tumor sections, were analyzed. The patients had not received treatment earlier. Adjuvant treatment was administered in patients with recurrence.

Clinicopathological characteristics, such as sex, age, and follow-up data (recurrence and outcome), were retrospectively collected from the patients' medical records.

Tissue samples obtained from resected specimens were used to prepare formalin-fixed paraffin-embedded blocks. Four-micron thick sections were obtained from these blocks for immunohistochemical analysis. General evaluation was performed using hematoxylin-eosin-stained slides. Necrosis, muscle invasion, surgical margins, and extracellular myxoid matrix were evaluated. Slides were stained with PD-L1 antibody (Cell Signaling / E1L3N). Immunohistochemical staining was performed as per the protocol provided by Cell Signaling Technology. PD-L1 expression in tumors and immune cells was also evaluated. Immunoreactivity for PD-L1 expression was evaluated in tumor cell membranes and immune cells by two blinded authors (ANY and TZ). PD-L1 results were separately evaluated in both tumor and immune cells in the microenvironment for each tumor component. PD-L1 expression was scored as positive when membranous staining was present in ≥ 5% of the population. Ethical committee approval was not necessary for discussion with the board. This study was conducted on human tissue samples preserved in the archives of the pathology department. Statistical analysis was not performed due to small sample size.

The study was conducted at the Istanbul Medeniyet University Medical School, Goztepe Training and Research Hospital according to the institutional ethical standards of the Helsinki Declaration.

RESULTS

Patient data are detailed in Tables 1 and 2. All cases had conventional chordomas. The median patient age was 57 years (range: 31–67 years). The median follow-up period was 40 months (range: 24–110 months). The surgical margins were negative in all cases and adjuvant therapy was not administered. Among the 10 cases considered in this study, PD-L1 on immune cells was positive in three patients. These patients experienced local recurrence with a median time of 15 months (range: 11–18 months). All the patients with the disease were alive. No preoperative chemotherapy or radiotherapy was administered to any of the patients. Chemotherapy and radiotherapy were administered in cases of metastasis and recurrence.

All cases showed muscle invasion. The resection margin was negative in all cases. Lymphocyte infiltrates were present in all the cases. The expression of PD-L1 by tumor cells was negative in all the cases (Figures 1 and 2). However, the expression of PD-L1 by immune cells varied in different cases. Positive expression in immune cells was observed in cases with local recurrence**.** Intratumoral lymphocytes were present in all specimens; however, only four positively stained for PD-L1 (Figure 3). Tumor margins were negative in all cases.

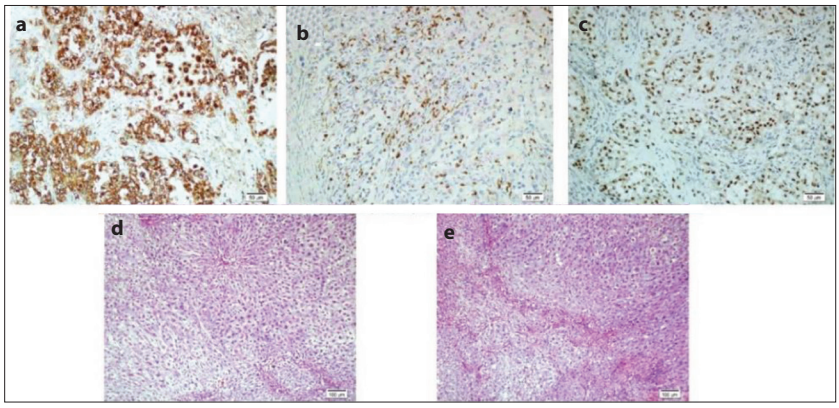

Figure 1. Immunohistochemical results of the tissue sample from the patient (Case 1); a – epithelial membrane antigen; b – S100 are expressed in conventional chordoma; however, they are usually negative in poorly differentiated chordoma; c – brachury is a nuclear stain, which is highly specific for chordoma; it is also positive in poorly differentiated chordoma; d–e – on hematoxylin and eosin-stained sections, tumor cells consist of dense epithelioid sheets in an extracellular myxoid matrix; cells are epithelioid with abundant clear (glycogen) to eosinophilic cytoplasm which may be have a bubbly/vacuolated appearance (physaliphorous cells)

DISCUSSION

Chordomas were first described by Virchow [6]. These slow-growing tumors present with pain and neurological dysfunction, secondary to obstruction of the lesion. Chordomas can occur at any location in the axial skeleton. Those involved in the sacral region can cause pain, urinary and/or bowel dysfunctions, and neuropathy. Chordomas are poor responders to chemotherapy; *en bloc* resection is the most effective method for local control.

Table 1. Clinicopathologic findings of all cases

Cases	Age (years), gender	Localization	Histologic subtype	Grade (high/low)	Metastasis	Recurrence	Time to recurrence (months)	Follow-up (months)	Tumor size (cm)
Case 1	63, Male	Sacrum	Conventional	Low	Negative	Negative		44	> 5 cm
Case 2	67, Male	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm
Case 3	31, Male	Sacrum	Conventional	High	Negative	Positive	11	36	> 5 cm
Case 4	52, Male	Sacrum	Conventional	High	Negative	Positive	15	63	> 5 cm
Case 5	62, Male	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm
Case 6	57, Male	Sacrum	Conventional	High	Negative	Negative		25	> 5 cm
Case 7	41, Male	Sacrum	Conventional	Low	Negative	Negative		73	> 5 cm
Case 8	57, Male	Sacrum	Conventional	High	Negative	Positive	18	110	> 5 cm
Case 9	65, Male	Sacrum	Conventional	High	Negative	Positive	16	82	> 5 cm
Case 10	47, Female	Sacrum	Conventional	Low	Negative	Negative		24	> 5 cm

Table 2. Pathologic findings of all cases

Cases	Age (years), gender	Tumor necrosis	Muscle invasion	Surgical margin	EMA	S100 protein	Brachyuria	PD-L1 expression		Status
								Tumor cells	Immun cells	
Case 1	63, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 2	67, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 3	31, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 4	52, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 5	62, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 6	57, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 7	41, Male		Positive	Negative	Positive	Positive	Positive	–	–	AWD
Case 8	57, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 9	65, Male	Positive	Positive	Negative	Positive	Positive	Positive	–	+	AWD
Case 10	47, Female		Positive	Negative	Positive	Positive	Positive	–	–	AWD

EMA – epithelial membrane antigen; PD-L1 – programmed death ligand 1; AWD – alive with disease

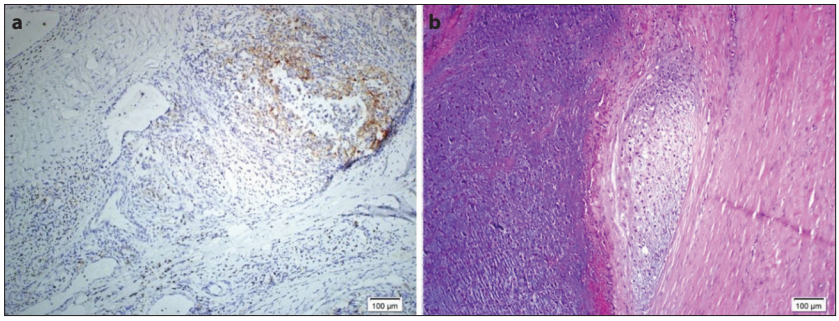

Figure 2. Immunohistochemical results of the tissue sample from the patient (case 2); this case had no recurrence; note that tumor cells and immune cells do not stain; soft tissue infiltration is also observed

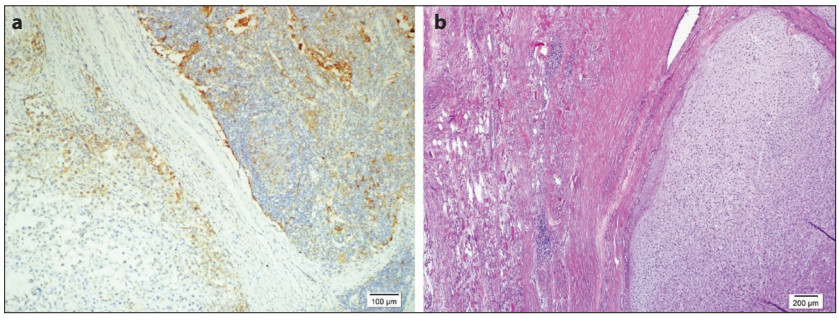

Figure 3. Immunohistochemical results of the tissue sample from the patient (case 3); this case had recurrence; tumor cells do not stain; however, tumor-infiltrating lymphocytes express more than 5 % programmed death ligand 1

There could be a delay in diagnosis, ranging from months to years. In our study, magnetic resonance imaging was used as the standard radiological tool for the evaluation of the tumor. Chest imaging revealed pulmonary metastasis. A biopsy confirmed the histological diagnosis. Magnetic resonance imaging resonance imaging revealed T1 isointense and T2 hyperintense enhancements. Despite aggressive surgery, cancer can metastasize after many years or recur, similar to that observed in the present study. In this clinical setting, subsequent surgery can result in higher morbidity. Therefore, there are limited options for medical therapy, with minimal efficacy [7].

The role of immunotherapy in soft tissue sarcomas is well known [8]. PD-L1 is one of the primary targets for immunotherapy. This study demonstrated that chordomas do express PD-Ll. Several studies have investigated the genetic basis of chordomas, and investigations on prognostic survival factors is ongoing, as demonstrated by a recent review of 78 genetic studies [9]. However, few studies have evaluated the role of PD-L1 in the pathogenesis of chordoma.

Feng et al. [10] investigated the expression score of PD-L1. Expression was higher in metastatic chordomas, with elevated levels of tumor-infiltrating

lymphocytes (TILs). Based on clinical trial data on chordoma treatment, they concluded that PD-L1 inhibition could be a possible immunotherapeutic strategy. Zou et al. [11] found that PD-L1 expression in TILs is associated with local recurrence and overall survival in spinal chordoma. In a more recent study, Zou et al. [12] developed an immunologic score, including CD3+, CD4+, CD8+, CD20+, Foxp3+, PD-1+, and PD-L1+ T cells. A significant correlation has been found between overall survival and the number of PD-1+ and PD-L1+ cells.

Chordoma, a rare bone tumor arising from the notochord, is resistant to conventional cancer treatments. The anti-PD-L1 drug, avelumab, was investigated as a potential treatment for chordoma. Fujii et al. [13] demonstrated killing of chordoma cells by natural killer-cell through antibody-dependent cell-mediated cytotoxicity of PD-L1-expressing tumor cells. Thus, they stated that the PD-1/PD-L1 pathway may be a novel therapeutic target for chordoma immunotherapy. Migliorini et al. [14] reported three cases of metastatic and locally advanced chordoma treated with different immunotherapeutic drugs. Two of them were administered with anti-PD-1 antibodies. Good clinical and radiological responses were observed. These findings suggest an immunogenic nature of chordomas.

Similar to our study, Mathios et al. [15] found that chordoma cells do not demonstrate significant PD-L1 expression; however, PD-L1 expression is evident in tumor-infiltrating macrophages and lymphocytes. Clinically, the role PD-L1 targeted therapy in recurrent chordomas was confirmed by Bishop et al. [16]. In these series, 15 out of 17 patients receiving immune checkpoint inhibitors had clinical benefit. This study supports the application of PD-L1 inhibition therapy in chordoma patients.

In literature, different staining rates have been accepted as positive expressions. This makes it difficult to interpret the results in terms of clinicopathological correlations. In our study, the cases with increased expression were > 5%. Therefore, we accepted 5% as the threshold. We believe that a linear correlation without using a threshold value is needed for the association between PD-L1 expression and clinicopathologic variables.

Study limitation

The main limitation of this study was the small sample size. Although recurrent cases have been found to have positive expression, the correlation of PD-L1 expression with recurrence, metastasis, and survival is needed from a larger population. Furthermore, it is not known how immunotherapy affects PD-L1 expression. Future research should evaluate the response rate of different therapeutic agents in PD-L1 positive cases.

CONCLUSION

In conclusion, this study demonstrated that PD-L1 expression is negative in nonrecurrent chordomas. These findings demonstrate less aggressive forms of chordomas, which do not require immunotherapy. Limitations arise from the limited number of chordoma cases considered in this study. Multicenter studies evaluating PD-L1 expression will have future implications for anti PD-L1 therapy, similar to other nonsurgical treatments.

Conflict of interest: None declared.

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Процена експресије *PD-L1* у рекурентним неметастатским сакралним хордомима – ретроспективна студија

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САЖЕТАК

Увод/Циљ Хордоми су ретки тумори нотохордалног порекла. За лечење се препоручује широка хируршка ресекција. Међутим, постоји високи ризик од морбидитета и морталитета. Поред тога, ови тумори су отпорни на хемотерапију. Дакле, за лечење хордома је потребна циљана терапија. Лиганд програмиране смрти 1 (*PD-L1*) обећавајући је избор за лечење рака. Истраживали смо експресију *PD-L1* код болесника са хордомом у студији једног центра.

Методе Блокови уграђени у парафин фиксирани у формалину процењени су за имунохистохемијску анализу да би се проценила експресија *PD-L1*. Клиничко-патолошке варијабле, као што су пол, старост и подаци о праћењу (понављање и исход), ретроспективно су прикупљени из медицинске документације болесника.

Резултати У ову студију је укључено десет болесника са дијагнозом сакралног хордома у једној установи између децембра 2015. и новембра 2021. године. Средња старост болесника је била 57 година, а средњи период праћења био је 40 месеци. Хируршке маргине су биле негативне у свим случајевима, без икаквог преоперативног медицинског третмана. Три од 10 болесника показала су позитивност *PD-L1* на имуним ћелијама. Ови болесници су показали локални рецидив, без метастаза. Средње време до локалног рецидива било је 15 месеци. Сви болесници су били живи на крају студије.

Закључак Ова студија је показала да се позитивност *PD-L1* у имуним ћелијама може користити као предиктивни маркер за локални рецидив у време хируршког лечења, што се може користити за утврђивање неопходности давања имунотерапије.

Кључне речи: хордом; широка хируршка ресекција; *PD-L1*; имунотерапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Microanatomical study of the posterior medial choroidal artery

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SUMMARY

Introduction/Objective The aim of this study was a detailed examination of microanatomy of the medial posterior choroidal artery (MPChA).

Methods The microanatomical characteristics of the MPChA were studied in 30 formalin fixed brain hemispheres using 6.3–10 × magnification of the stereoscopic microscope. The arteries were injected with the mixture of 10% India ink and gelatin. The second group of 10 hemispheres consisted of specimens injected with methyl methacrylate fluid into the cerebral arterial vessels, for the preparation of corrosion casts.

Results The MPChA was present in all 30 hemispheres, always as the single artery. The MPChA were divided into proximal and distal types of vessels. We distinguished two segments of the MPChA: a cisternal and plexal. Proximal MPChA was present in 53.3% of cases, with the caliber of 0.6–1 mm (mean 0.8 mm). The point of its origin from the posterior cerebral artery was always before the origin of the first temporal cortical branch. Distal MPChA existed in 46.7% of cases, with the diameter of 0.4–1 (mean 0.74 mm). The cisternal segment the most frequently gave of the origin of fine branches to the cerebral crus, medial geniculate body and thalamus. The plexal segment gave rise arteries to the thalamus, and choroid branches for the supply of the choroid plexus of the third ventricle. Anastomoses in the region of the MPChA were found in all of 20 examined brains, most often among the plexal branches.

Conclusion The results describing the microanatomical characteristics of the MPChA may have diagnostic and microsurgical significance.

Keywords: medial posterior choroidal artery; choroid plexus; thalamus; cerebral crus

INTRODUCTION

The morphology and topography of the medial posterior choroidal arteries (MPChA) is extremely complex and so little known. In most anatomical textbooks no mention is made of the MPChA. The MPChA, as a branch of the posterior cerebral artery (PCA), has been neglected in the anatomical, neuroradiological, and the neurosurgical literature [1, 2, 3]. Several microanatomical, microneurosurgical and angiographic studies described deep arteries of the brain and their importance, but choroidal arteries received very modest attention [4–7]. The choroidal arteries are positioned in the close relation with the pineal gland, and the posterior and medial surfaces of thalamus. Displacements of the choroidal arteries from the correct anatomical position are sign of pineal or thalamic tumors [8, 9]. The microsurgical procedures with use of sophisticated optical equipment are frequently performed for endovascular treatment of the cerebral arteriovenous malformations (AVM). Correct and detailed knowledge of the microanatomical characteristics of the choroidal arteries is essential for a safe and precise intervention [10, 11].

The aim of this microanatomical research investigating the choroidal arteries is to provide necessary anatomical support for enhancing diagnostic procedures, and the quality of microsurgical interventions in this region.

METHODS

For this microanatomical study of the medial posterior choroidal artery (MPChA) we used thirty human hemispheres (15 right and 15 left) with no pathological changes, from the collection of the Laboratory for Vascular Anatomy. The adult brains (18 males and 12 females), belonged to persons with an average age of 56.4 years (from 42–71 years). We perfused the cerebral arterial system with worm water mixed with a 4% formalin solution, and we finally intraarterially injected a 10% mixture of India ink and melted gelatin through the basilar artery.

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After the period of three weeks necessary for fixation, the brain specimens were meticulously dissected.

We used another ten hemispheres for preparing the corrosion casts, important for a precise analysis of topographical relationships between specific branches. The fluid of methyl methacrylate, with added hardener and color pigment, was injected into the basilar artery. The process of hardening of methyl methacrylate was completed in four hours, and for the next 14 days the injected specimens were immersed in 40% solution of potassium hydroxide necessary for corrosion of the soft tissue. The hot water was used for the final cleaning of digested specimens. The corrosion cast specimens of cerebral arteries were analyzed under the zoom microscope (Leica MZ6, Leica Camera, Wetzlar, Germany), and photographed by a digital photo camera (Leica DFC295, Leica). We engaged the specific software (Leica Interactive Measurements, Leica) for realizing different kind of measurements. The obtained data were introduced into the schematic drawings of every specimen. The statistical analysis that we used were mean values with standard deviation (SD). The research method of using the corrosion casts in analyses provided a precise 3D spatial distinction between the arteries, offering much better specimens than traditional dissections. On the other side the length of time required for the preparation and examination in detail for every specimen is incomparable with any other method of research used in morphological studies. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia (No. 1322/V-10, Date 20-05-2021).

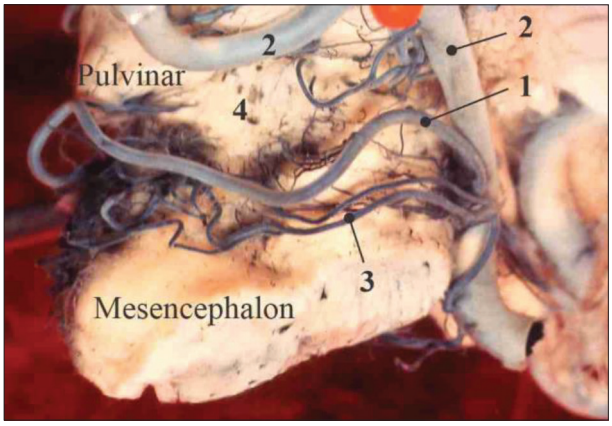

Figure 1. 1 – long proximal medial posterior choroidal artery; 2 – originating from the prominent posterior cerebral artery; 3 – curving around the mesencephalic crus above the collicular artery; 4 – and below the medial geniculate body; (lateral view, dissection of specimen injected with India ink)

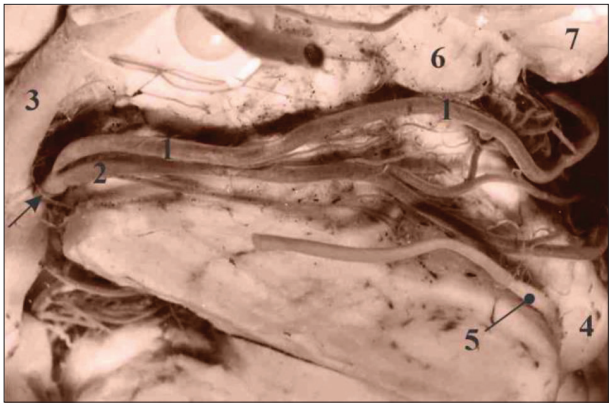

Figure 2. 1 – the proximal medial posterior choroidal artery with a common origin (arrow) with the 2 – collicular artery from the 3 – posterior cerebral artery; 4 – note the inferior colliculus; 5 – the trochlear nerve; 6 – the medial geniculate body; 7– the pulvinar (lateral view, dissection of specimen injected with India ink)

RESULTS

The PCA gave off the MPChA in all 30 (100%) studied hemispheres, always one artery. According to the level of beginning from the PCA we distinguished between an a) proximal MPChA (MPChAprox), and b) distal MPChA (MPChAdist). We described two segments of the MPChA: a cisternal and plexal segment. The cisternal segment extended from the point of origin of the artery to the choroid plexus of the third ventricle where the plexal segment began.

a) The MPChAprox, found in 16 (53.3%) cases, bilaterally present in three (20%) brains, began in the anterior peduncular mesencephalic region from the posterior side of the PCA. It was characterized by the point of its beginning from the PCA before the origin of the first temporal cortical branch. The MPChAprox originated from the precommunicating (P1) segment of the PCA in four (25%) cases, and from the postcommunicating (P2) segment of the PCA in 12 (75%) hemispheres (Figure 1). In two (12.5%) cases the MPChAprox has had a common origin with the collicular artery form the PCA (Figure 2). The mean caliber of the MPChAprox at the level of its beginning was 0.8 ± 0.11 mm (from 0.6 to 1.0 mm) (Table 1).

Curving around the mesencephalic crus the cisternal segment of the artery was in close relationship with the inferior side of its parent vessel, superior cerebellar artery, basal vein of Rosenthal and the trochlear nerve in the ambient cistern. The MPChAprox coursed backwards following the lateral side of the mesencephalon, immediately bellow the medial geniculate body, and extended into the quadrigeminal (collicular) cistern (Figures 1 and 2). After making a sharp bend it formed a loop and entered the choroid plexus of the third ventricle and continued by the plexal segment. This tortuous retrothalamic segment is in close proximity to the lateral surface of the pineal gland. It extended throughout the length of the choroid plexus of the third ventricle, adjacent to the internal cerebral vein and the opposite MPChA, to the anterior pole of the thalamus where the artery passed through the interventricular foramen and entered the choroid plexus of the lateral ventricle (Figures 3 and 4).

b) The MPChAdist, found in 14 (46.7%) cases, originated from the postcommunicating (P2) segment of the PCA in the region of the bifurcation or ramification of the PCA in temporal cortical branches, below or behind the pulvinar of the thalamus. Of the 14 MPChAdist, 11 (78.6%) originated directly from the PCA, two (14.3%) from the parietooccipital artery, and one (7.1%) from the calcarine artery (Figure 3). The average caliber of the MPChAdist at the level of its beginning was 0.74 ± 0.17 mm (0.4–1.0 mm) (Table 1)

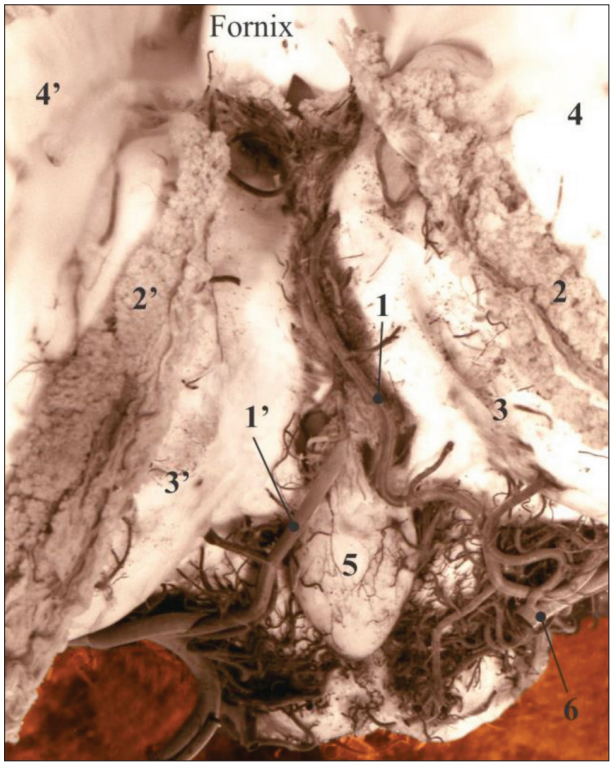

Figure 3. 1 – the roof of the third ventricle; plexal segments of the right proximal medial posterior choroidal artery; 1′ – the left distal medial posterior choroidal artery extending throughout the length of the roof; 2, 2′ – right and left choroid plexus; 3, 3′ – the lateral ventricle right and left thalamus; 4, 4′ – right and left caudate nucleus; 5 – the pineal gland; 6 – right posterior cerebral artery (dorsal view, dissection of specimen injected with India ink)

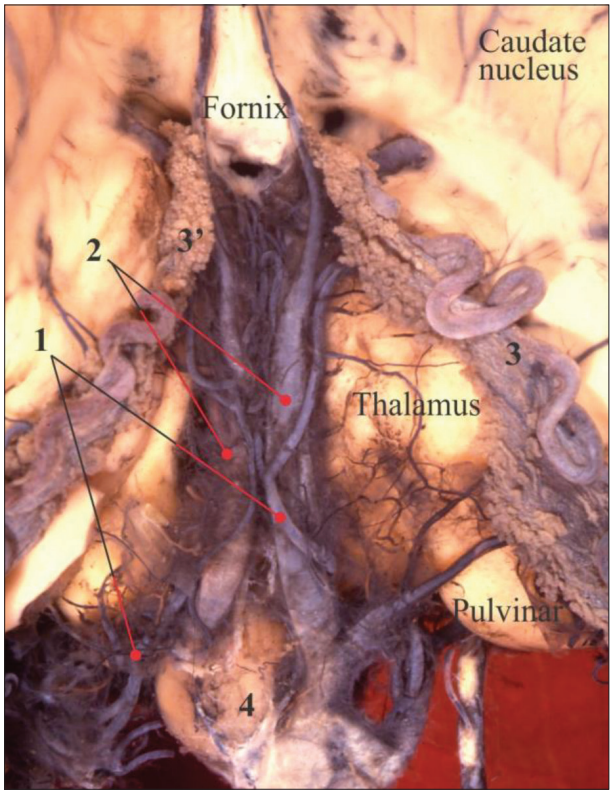

Figure 4. 1 – the roof of the third ventricle; plexal segments of the right and the left medial posterior choroidal artery; 2 – adjacent to the internal cerebral veins; 3, 3′ – right and left choroid plexus of the lateral ventricle; 4 – the pineal gland (dorsal view, dissection of specimen injected with India ink)

Table 1. Morphometric characteristics of the medial posterior choroidal artery

Artery	Frequency (%)	Diameter (mm) Range (Mean±SD)
MPChAprox	16 (53.3%)	0.6–1 (0.8 ± 0.11)
MPChAdist	14 (46.7%)	0.4–1 (0.74 ± 0.17)

MPChAprox – proximal medial posterior choroidal artery; MPChAdist – distal medial posterior choroidal artery

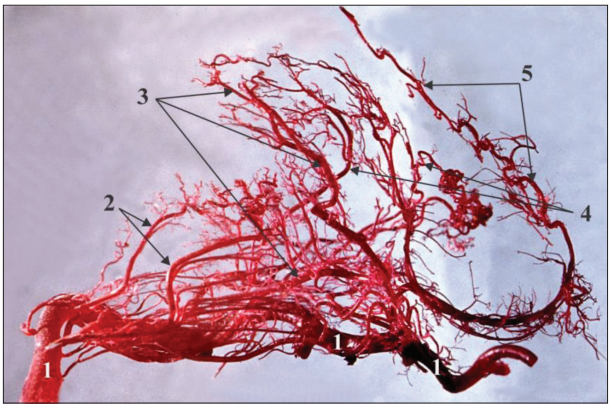

Figure 5. 1 – corrosion cast of the right posterior cerebral artery, medial view; 2 – the thalamoperforating arteries; 3 – right medial posterior choroidal artery; 4 – right lateral posterior choroidal artery; 5 – the splenial branch of posterior cerebral artery over the dorsal side of splenium

The MPChA coursed from the level of its subsplenial beginning inward, superiorly and anteriorly. In a lateral projection the artery run upward at an angle of 45°, with the concave middle part in a form of number 3. The lower curved part belongs to the parapineal segment, and the upper part after the midpoint is the plexal segment of MPChA. Projection of the lateral posterior choroidal arteries (LPChA) is immediately caudally to the previous, and finally the most caudal vessel is splenial artery encircling the caudal and dorsal surfaces of the splenium of the corpus callosum (Figure 5).

The postero-medial choroidal system gives off various kinds of branches. Ventrally, the first group of cisternal branches are arteries for the supply of the cerebral crus. A second group is constituted with branches which supply the medial geniculate body. Another group is composed of arteries for the caudal part of pulvinar. Posteriorly, and superiorly, various plexal branches irrigated the superomedial part of the pulvinar, the superomedial part of the thalamus and the choroid plexus in the roof of the third ventricle.

DISCUSSION

Most frequently only one MPChA was noticed in our specimens. However, other authors described a range of 1–3 arteries (average 1.7) [4, 5, 12]. In this study, we noticed this artery to average 0.8 and 0.74 mm in diameter, for the MPChAprox and MPChAdist respectively, which is similar to 0.8 mm reported by Fujii et al. [4], and larger than the value of 0.4 mm described in the article by Garcia et al. [5]. The origin of the MPChAprox (75%) from the P2 segment of the PCA was the most frequent in the present study, in

agreement with 85% reported by Fujii et al. [4], and not consistent with the findings (5.1%) of Garcia et al. [5]. In this series, proximal and distal type of the MPChA was described according to the different point of its origin, a characteristic described only by our group of authors. We did not notice the presence of duplication of the MPChA reported only by Garcia et al. [5].

The MPChA supplies tumors, AVM, and aneurysms arising in and adjacent to the choroid plexus and the third ventricle [4].

Neoplastic masses in the pineal gland are uncommon and a rare clinical entity, with less than 10% of pediatric tumors, and less than 1% of all intracranial tumors. Patients with pineal tumor may present symptoms connected to elevated intracranial pressure, such as headache, nausea, vomiting, and somnolence [13]. The pineal gland is centrally positioned with a very complex relationships, below the splenium of corpus callosum, posterior the third ventricle, above and behind the mesencephalic tectum and medially to the left and right thalamus [4, 8]. Different microscopical, macroscopical and radiological appearances are characteristics of pineal tumors. The MPChA has a close relationship with lateral borders of the pineal gland, and local changes in its shape are visible in case of pineal neoplasm. The tumors originating in the pineal gland make a pressure and displace the MPChA superiorly and posteriorly. The further enlargement of the tumor makes additional displacement of the MPChA posteriorly. Meningioma arising from the tentorium behind the pineal gland grow slowly, push and displace the MPChA anteriorly [4, 8, 9].

The MPChA territory infarctions are rare events with sparse data about the vascular territories within the thalamus. Medial parts of the thalamus and the pulvinar, related to the roof of the third ventricle and mainly supplied by branches of the MPChA are the most frequently affected, but with no evident specific neurologic dysfunctions [14]. The MPChA territory infarcts may be lacunar, with a small ischemic area within the thalamus, and they can be asymptomatic and silent in long-term follow-up [15]. Another MPChA branches, originating more distally may supply the anterior thalamic nuclei, but disabilities are usually absent or slight. The anatomical explanation is existence of plexiform anastomotic supply from branches of the anterior choroidal artery, from the choroid plexus of the lateral ventricle, entering through the interventricular foramen [14]. The existence of the peduncular branches originating from the collicular, from the superior cerebellar artery, and form the PCA are the explanation why the eye movement disorders and sensory-motor dysfunction were uncommon for the selective MPChA territory infarct [4, 16].

AVM of the brain is a congenital vascular disease, an anomaly characterized by direct connection between arteries, the nidus (the arteriovenous shunt) and draining veins, characterized by an intranidal network of maldeveloped vessels [17]. An AVM perfused by the PCA is located near the posterior part of the corpus callosum and the deep cerebral veins to form a callosal circle. The feeding arteries may involve the MPChA and the treatment was mostly via this small artery. Surgical resection, radiotherapy, and endovascular treatment are available therapeutic methods for AVM [18]. Embolization with the microcatheter performed as a curative procedure through the MPChA should be taken with the main aim to secure the weak structures [18, 19].

Aneurysm of the MPChA is very rare reported condition. Because of the specific position the MPChA curving lateraly to the mesencephalon and entering the roof of the fourth ventricle, the rupture of the aneurysm of its cisternal segment is manifested as subarachnoid hemorrhage in the ambient cistern, and spasm affecting the PCA, comparing to the rupture of the aneurysm of its plexal segment causing the ventricular and thalamic hemorrhage. Because of the small size of the MPChA, preferable endovascular coiling with parent artery protection is unfeasible, and aneurisms or parent vessels are isolated and clipped by open surgery [20].

Numerous anastomoses interconecting the choroidal arteries, coming from the internal carotid artery and PCA, exist in the structure of choroid plexus. If the occlusion of the proximal part of the PCA happens, the part of PCA distally to the blockade receives blood in the opposite direction from capillary choroidal network of the lateral and third ventricles respectively, supplied by the anterior choroidal artery, branch of the internal carotid artery, then via the MPChA entering the PCA distally to the obstruction [1]. The existence of abnormal periventricular collaterals from the choroidal vessels is typically connected to Moyamoya disease. The existence of enlarged pathological interconnections between the deep parts of the medullary and choroidal arteries results in reversed blood flow to the cortical area [21]. The occurrence of periventricular subependymal hemorrhage is a common manifestation of Moyamoya disease in adults. The enlarged choroidal collaterals are responsible for bleeding in the area of atrium of the lateral ventricle [22]. The MPChA may play an important role in supply of subependymal arteries involved in repeated hemorrhage in patients with Moyamoya disease. The potential trigger of recurrent hemorrhage is the existence of ventricular microaneurysms in the choroidal arteries [23]. Reduction of choroidal anastomoses, after the superficial temporal-middle cerebral artery bypass surgery, was confirmed in 85% of hemispheres [21].

CONCLUSION

The MPChA was the most common point of origin of neural arteries to the cerebral crus, medial geniculate body, thalamus and choroid plexus of the third ventricle. The data obtained on the microanatomical characteristics of the MPChA may have diagnostic and microsurgical implications.

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Микроанатомска студија задње унутрашње хороидне артерије

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САЖЕТАК

Увод/Циљ Циљ ове студије био је детаљна анализа микроанатомских карактеристика унутрашње задње хороидне артерије (УЗХА).

Методе Микроанатомске карактеристике УЗХА проучаване су на 30 мождaних хемисфера фиксираних формалином коришћењем стерео-микроскопа са увеличањима од × 6,3 до × 10. У артерије је убризгана мешавина 10% раствора туша и растопљеног желатина. Користили смо и другу групу, од 10 хемисфера одраслих особа, где смо у артерије убризгали течни метилметакрилат, да бисмо припремили корозионе препарате.

Резултати УЗХА је била присутна на свих 30 хемисфера, увек по једна артерија. Описали смо два типа ове артерије, проксимални и дистални. Свака УЗХА је подељена на цистернални и плексусни сегмент. Проксимални тип УЗХА је постојао на 53,3% хемисфера, са измереним пречником од 0,6–1,0 mm (просечно 0,8 mm). Одвајао се од стабла задње мождане артерије пре места одвајања прве кортикалне слепоочне гране. Дистални тип УЗХА је постојао на 46,7% хемисфера, са измереним пречником од 0,4–1 mm (просечно 0,74 mm). Цистернални сегмент је најчешће био део артерије који је давао неуралне гране за крус церебри, унутрашње коленасто тело и таламус. Плексусни сегмент је давао гране за таламус и хороидни сплет на крову треће мождане коморе. Анастомозе у сливу УЗХА су биле присутне на свим препаратима, најчешће између плексусних грана.

Закључак Добијени подаци о микроанатомским карактеристикама УЗХА могу имати дијагностички и микрохируршки значај.

Кључне речи: унутрашња задња хороидна артерија; хороидни сплет; таламус; мождани крак

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Nodular amyloidosis of the lung presenting as lung malignancy

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SUMMARY

Introduction Amyloidosis is a disease associated with the extracellular deposition of insoluble protein material called amyloid. It can be acquired or hereditary, systemic or organ-limited. Nodular pulmonary amyloidosis is defined as one or more tumefactive amyloid deposits in the lungs.

Outlines of cases This study presents two cases that were hospitalized at the Institute for Pulmonary Diseases to clarify the origin of lesions detected on computed tomography (CT) scans of lung parenchyma. In the first case, in a 78-year-old woman, numerous non-calcified nodules were described on the chest CT. The patient died during hospitalization, and the autopsy revealed diffusely distributed greyish-yellow nodular lesions in the upper and middle parts of the right lung, as well as lesions in the form of partially calcified nodules in both lungs. Histological analysis of samples from macroscopically described nodules confirmed nodular amyloidosis. The second patient is male, 58 years old, who was operated on for rectal adenocarcinoma three years ago. A CT scan of the lung parenchyma shows a tumor nodule localized in the lower lobe and a nodular lesion localized in the upper lobe of the right lung. Histological analysis confirmed that the lesion from the lower lobe corresponds to the metastasis of colorectal cancer, while in the lesion from the upper lobe amyloid deposits were found.

Conclusion Pulmonary nodular amyloidosis is a rare condition, and because of the imaging similarities it is difficult to distinguish it from malignant nodules in the lung parenchyma. Therefore, as a part of routine practice, a definitive diagnosis of amyloidosis needs to be confirmed by tissue biopsy.

Keywords: amyloidosis; amyloid; lung malignancy; autopsy; Congo red

INTRODUCTION

Amyloidosis is a disease associated with the extracellular deposition of insoluble protein material called amyloid in various tissues and organs. This condition can be found in humans as well as in other vertebrates. The term amyloid was used in medicine for the first time by Rudolf Virchow in 1854. Amyloid deposits are composed of fibril proteins (95%), and the remaining part is amyloid P and glycoproteins. Up to now, 36 amyloid fibril proteins and their precursors have been identified in humans, and ten in other vertebrates [1, 2].

Amyloidosis can be acquired or hereditary, systemic or organ-limited. Pulmonary amyloidosis can be seen in three distinct forms: diffuse alveolar-septal amyloidosis, nodular pulmonary amyloidosis, and tracheobronchial amyloidosis [3].

Nodular pulmonary amyloidosis is defined as one or more tumefactive amyloid deposits in the lungs and usually represents incidental findings on chest imaging [4]. The imaging similarities of multiple pulmonary nodules (with calcification and cavitation) with malignant lung tumors make differential diagnosis particularly difficult. For that reason, histological confirmation should be mandatory. In this case study, we report two cases of pulmonary nodular amyloidosis that were suspected of lung malignancy.

Case No. 1

A 78-year-old woman was admitted to the Institute for Pulmonary Diseases of Vojvodina for cough, fever, hemoptysis and nausea. Upon admittance, at initial examination, there was not any palpable lymphadenopathy. Results of periphery blood showed anemia and discrete leukocytosis, while the analysis of bio-humoral tests showed elevated values of inflammatory markers such as urea and lactate dehydrogenase. In addition, a blood gas test showed hypercapnic respiratory failure.

Computed tomography (CT) scan of the chest has shown multiple non-calcified nodules in the right lung (between segments S2/S6 measuring 30 × 50 mm and S5 38 × 12 mm), and in the upper lobes of both lungs, and in the lower lobe of the left lung there were partly calcified nodules with a diameter of up to 24 mm. Cytological analysis showed that tracheobronchial aspirate contained mucopurulent exudates.

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Despite the treatment, intensive monitoring, and care, on day 12 of hospitalization the patient died. At the autopsy, the weight of the left lung was 670 g, while the weight of the right lung was 990 g. Both lungs were pinkish-colored on the cross-section, and a large amount of pale pink foamy fluid flew from its cut surface. In the upper and middle lobes of the right lung and the upper lobe of the left lung, individual nodules of greyish-yellow color, less than 2 cm in diameter were found. In the lower lobes on both sides of the lungs, there were greyish-yellow nodules, up to 4.6 cm in diameter (Figure 1). Some of them had the appearance of grapes.

Histologically, nodules are composed of homogeneous, densely eosinophilic amorphous material. Deposits are orange-red on Congo red stain (Figure 2) and under polarized light show apple-green birefringence (Figure 2). In the surrounding lung tissue, during the pathohistological examination, hemorrhagic infarcts, chronic catarrhal bronchitis, emphysema, and pulmonary edema (which was established as the cause of death) were revealed. Elements of malignant tissue were not seen.

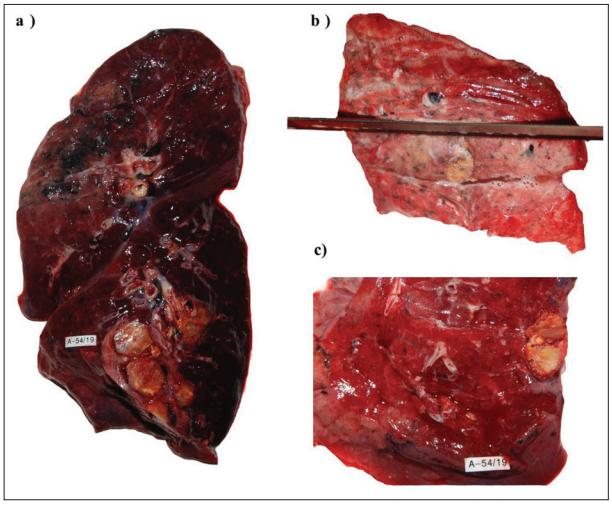

Figure 1. Gross images of lung with amyloid nodules

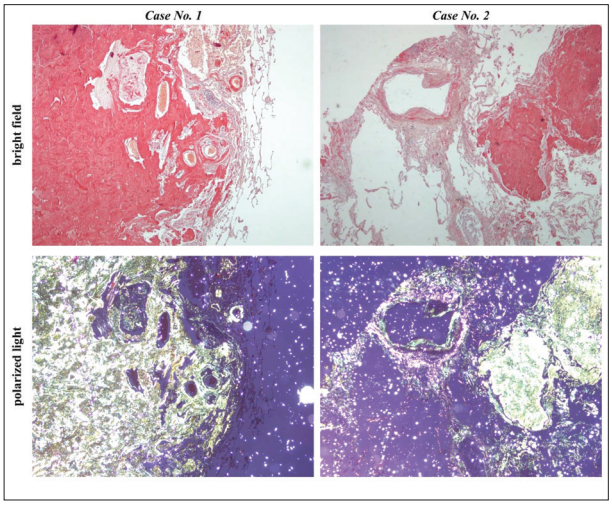

Figure 2. Congo red stained lung parenchyma with amyloid deposit, examined under bright field and polarized light microscopy, 4 ×

Case No. 2

A 58-year-old male patient was admitted to the Institute for Pulmonary Diseases of Vojvodina for identification of etiology of pathologic lesions in lung parenchyma which were found on CT imaging. His past medical history included arterial hypertension and dilated cardiomyopathy, and three years ago, the patient was diagnosed with adenocarcinoma of the rectum, which had been surgically removed.

During the surgery, whitish lesions were noted in the upper lobe of a lung. Tissue fragments from the lower and upper lobe of the right lung were sent for histopathological analysis.

Upon gross examination of lung parenchyma of the lower lobe of the lung, we found a clearly defined whitish tumor nodule, with 3.7 × 3 cm in size. In tissue samples taken from that area, we found a tumor tissue that, due to histological characteristics, corresponds to the metastasis of colorectal cancer.

In the histological samples from the upper lobe of the right lung in alveolar parenchyma there were nodular acellular homogeneous deposits that were eosinophilic by standard histological staining. Around described deposits, a lymphocyte infiltrate as well as giant cells were noted. Upon Congo red histochemical staining, acellular deposits were colored orange, and under polarized light showed apple-green birefringence (Figure 2). Described findings correspond to nodular amyloidosis.

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

DISCUSSION

Amyloidosis is a rare disease of unclear etiology, and its most common clinical forms are amyloid A amyloidosis, immunoglobulin light chain amyloidosis, transthyretin amyloidosis, and beta-2 microglobulin amyloidosis (Aβ2M) [5, 6].

The exact incidence of amyloidosis is unknown, but estimates show that 6–10 cases per million are diagnosed annually [7]. Amyloid deposits commonly affect males (2:1) of the middle age group (50–60 years) [8].

Amyloid can even affect pleura, pulmonary arteries, lymph nodes, and diaphragm [9, 10]. Deposition of amyloid results in destruction of tissue structure and impairment of organ function. The diagnosis of amyloidosis can only be confirmed by the histopathologic section of tissue stained with Congo Red, and the amyloid will show green apple birefringence under polarized light, as in this case. In addition, amyloid can be detected when stained with metachromatic dye, such as crystal violet, or with thioflavin T as a fluorescent marker [11, 12].

Nowadays, the use of immunohistochemistry in combination with clinical testing can help identify precursor proteins in amyloid deposits and their subtyping [13, 14].

Mass spectrometry-based proteomic analysis can be performed on formalin-fixed, paraffin-embedded tissue,

and due to its high sensitivity and specificity, this method can be applied in clinical biopsy specimens and it will be a useful tool in amyloid subtyping [5].

Clinically, nodular pulmonary amyloidosis is asymptomatic and on CT is seen as multiple nodules of varying size with sharp or lobulated margins, usually peripherally located in lower lobes of the lungs [4, 15]. Calcifications are seen in 50% of cases, and one of the characteristics is slow nodule growth with no regression [16, 17]. That is very similar to localization of deposits in our first case, where we had amyloid nodules in lower lobes of both lungs, but the uncommon presentation was finding amyloid deposits in the middle lobe of the right lung.

For pulmonary nodular amyloidosis, differential diagnosis includes malignancy of the lung (primary or metastatic) and granulomatous diseases [18]. Previous study reported that during fludeoxyglucose (18F-FDG) positron emission tomography (PET)/CT imaging of patients with amyloid deposits, most of them showed increased FDG uptake, but no FDG uptake has been mentioned [19]. Therefore, 18F-FDG PET/CT is not a useful diagnostic tool for distinguishing amyloidosis from lung malignancy, but in the future, the use of some more specific tracers such as C-labeled Pittsburgh compound B and 18F-florbetapir might be a better solution [20].

Pathohistological examination is essential for a definitive diagnosis. Differential diagnoses of nodular pulmonary amyloidosis on histological tissue specimens include pulmonary hyalinizing granuloma and amyloid-like nodules in light-chain deposition disease. In light-chain deposition disease, amyloid-like nodules in lung parenchyma are histologically very similar to amyloid. However, they are composed of κ-light chain fragments which are Congo red negative and do not form fibrils, but present as granular material under electron microscope [5].

Pulmonary hyalinizing granuloma is a rare benign disease, and on chest imaging, shows unilateral or bilateral well-defined nodules. In histological slides, hyalinizing granulomas are different from amyloid deposits. They are presented as fibrotic nodules composed of hyalinized collagen bundles with lamellar arrangement, so they are not homogeneous and the Congo red staining is negative [21]. Chronic inflammation is localized both around and within them. The inflammatory infiltrate predominantly contains lymphocytes and plasma cells, but it can also hold neutrophils and histiocytes [22].

Studies have shown that most cases of nodular pulmonary amyloidosis are associated with B-cell lymphomas [23] and mucosa-associated lymphoid tissue lymphoma [24], and association with pulmonary amyloidosis and lymphomas has been found in Sjögren's disease [25, 26, 27]. In the second case, we reported nodular amyloidosis in a patient with metastatic adenocarcinoma in the same lung.

Pulmonary nodular amyloidosis is a rare condition, but because of imaging similarities it is difficult to distinguish it from malignant nodules in the lung parenchyma. For that reason, it should be added to the differential diagnosis. In routine practice, a definitive diagnosis of amyloidosis needs to be confirmed by tissue biopsy and, in addition to standard H & E staining, histochemical Congo red should be used.

Conflicts of interest: None declared.

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Нодуларна амилоидоза плућа представљена као малигни тумор плућа

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САЖЕТАК

Увод Амилоидоза је болест повезана са ванћелијским таложењем нерастворљивог протеинског материјала који се назива амилоид. Обољење може бити стечено или наследно, системско или ограничено на органе. Нодуларна плућна амилоидоза се дефинише као један или више тумефактивних депозита амилоида у плућном паренхиму.

Прикази болесника У овој студији приказана су два болесника која су хоспитализована на Институту за плућне болести како би се разјаснило порекло патолошких промена у плућном паренхиму детектованих на снимцима компјутеризоване томографије. У првом описаном случају код особе женског пола, старости 78 година, на компјутеризованој томографији грудног коша описују се бројни некалцификовани нодуси, међутим током хоспитализације дошло је до леталног исхода. На аутопсији се на плућима уочавају сивкасто-жућкасте нодуларне промене дифузно распоређене у горњим и средњим партијама десног плућног крила, као и промене у виду делимично калцификованих чворића локализованих у оба плућна крила. Хистолошком анализом узорака из макроскопски описаних нодуса доказана је нодуларна амилоидоза.

Болесник из другог приказаног случаја је мушког пола, старости 58 година, који је три године раније оперисао аденокарцином ректума. На компјутеризованој томографији плућног паренхима описује се туморски нодус који је локализован у доњем делу десног плућног крила, као и нодулирана промена локализована у горњем режњу десног плућног крила. Хистолошком анализом постоперативних узорака утврђено је да нодус из доњег режња одговара метастази колоректалног карцинома, док се у узорку из горњег режња десног плућног крила налазе депозити амилоида.

Закључак Плућна нодуларна амилоидоза је ретко стање, а због сличности на дијагностичком имиџингу тешко ју је разликовати од малигних тумора плућа, па у рутинском раду коначна дијагноза амилоидозе мора бити потврђена биопсијом ткива.

Кључне речи: амилоидоза; амилоид; плућни малигнитети; аутопсија; конго црвено

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Multidisciplinary treatment of massive trichobezoar caused an acute gastric outlet obstruction

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SUMMARY

Introduction Trichobezoars presents a rare form of bezoar made of swallowed hair, with clinical manifestation of gastric or intestinal obstruction, gastric ulceration, bleeding, and perforation. It is predominantly found in emotionally disturbed or mentally retarded youngsters, who eating their own hair which is clinically known as trichophagia. Patients often deny eating their own hair which makes diagnosis difficult.

Case report We present a case of acute gastric outlet obstruction caused by a giant gastric trichobezoar made of a long thin hair, in a 20-year young female. Although patient had a long history of trichophagia, she did not think her behavior was unusual and she had not been treated before. Following the initial diagnostic procedures, exploratory laparotomy was indicated. After anterior gastrotomy was performed, a massive stomach-shaped trichobezoar was removed. Postoperatively, the patient had a psychiatric consult exam. She recovered well and was discharged without complications. She was referred for further psychiatric follow-up.

Conclusion Trichobezoars are non-digestible collections that usually accumulates in stomach and can extend to small bowel, causing mechanical injury such as hollow viscus obstruction. Patients with acute gastric obstruction caused by a giant trichobezoar require urgent removal of the trichobezoar, to preserve the stomach and avoid further, catastrophic consequences.

Keywords: trichobezoar; gastrotomy; trichophagia; emotional disturbance

INTRODUCTION

Bezoars represent a undigested mass within the gastrointestinal tract which enter the stomach by swallowing non-absorbable materials [1, 2]. It could increase in size passing further into the small intestine [1, 2]. Trichobezoars are rare clinical entity, but they have been known for centuries [1–7].

They are consist of a bunch of hairs in the proximal gastrointestinal tract [1–7]. Hairs enter the stomach by compulsive swallowing, the disorder is called trichotillomania [7]. Trichotillomania is classified as an impulse control disorder, with prevalence estimate to 2% [3, 7]. Repetitive hair pulling may be unconsciously or unintentionally done [3]. The inability to control the impulse to pull one's hair out, is followed by an increase in tension which is replaced by a feeling of satisfaction after the act [8]. Trichophagia is hair swallowing occurs in up to 18% of patients with trichotillomania [1–8] Finally about 33% of patients with trichophagia develop stomach trichobezoars [1–8].

Trichobezoars are most often found in young women, in female children and adolescents with underlying behavioral disorder, but it can occasionally affect healthy adult [2, 4, 5]. Piles of hair create a ball in the stomach that grows slowly for years and is asymptomatic for a long time. Over time, first non-specific symptoms occur such as nausea, vomiting, abdominal pain, anorexia, early satiety, or weight loss. Trichobezoars can enlarge and creates a mechanical damage due to their presence in the lumen of the stomach and intestines. This can lead to serious complications such as gastric mucosal erosions, gastric ulceration, gastric outlet obstruction, pneumatosis, perforation and peritonitis [6, 9].

Trichobezoars are foreign bodies that must be removed. After visualization endoscopic extraction of large trichobezoars often fails. Complete removal of the massive trichobezoars and treatment of the intestinal damage caused by its presence is only possible surgically.

We are presenting a case of acute gastric outlet obstruction caused by giant gastric trichobezoar made of a long thin hair, in a 20-year-old female. Consequences of her behavioral disorder in this young woman was conformed on the computed tomography (CT) scan. After surgical removal of the massive trichobezoar, she recovered without adverse events. Patient would continue with psychiatric follow-up.

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CASE REPORT

A 20-year-old female was referred to the surgical examination at the Emergency Center with intermittent epigastric pain and vomiting, which started two weeks prior. Patient's medical history revealed loss of appetite and early satiety. She previously visited the Emergency Center six months prior complaining of the same symptoms when an abdominal ultrasound and upright abdominal radiography showed no specific findings. On general physical examination, abdominal palpation of epigastric region revealed smooth and hard intrabdominal curved lump approximately 10 cm in diameter. Differential diagnosis of gastric trichobezoar and gastric malignancy was made with further diagnostic tests. Abdominal ultrasound showed a large mass in the epigastric region with echogenic anterior margin. An abdominal CT scan showed a distended stomach with a clearly demarcated intraluminal mass extending from the gastric cardia to the duodenal bulb which was described as heterogeneous dense formation with interposed air inclusions and without postcontrast enhancement (Figures 1a, 1b, 1c). A gastric trichobezoar was diagnosed and gastroenterologist endoscopist was consulted.

Urgent esophagogastroduodenoscopy (EGDS) showed small amount of esophageal blood, foreign body regurgitation and stomach filled with hair and impacted food obstructing 80% of the gastric lumen with completely obstructing the antrum, disabling further propagation of the endoscope. Considering the size of the mass and the extent of bezoar on the CT scan it was thought that endoscopy would likely fail to remove the mass completely without large fragmentation and the risk for distal intestinal obstruction or gastric perforation. Thus, the patient was referred for emergency surgical treatment.

After adequate preoperative treatment by the anesthesiologist, a few hours after admission to the hospital the patient underwent surgery. An exploratory laparotomy through an upper midline abdominal incision was performed. During exploration, smoothly contoured mass was found occupying whole stomach and D1 of duodenum Exploration of other visceral organs was uneventful. A longitudinal 7 cm gastrotomy incision several centimeters

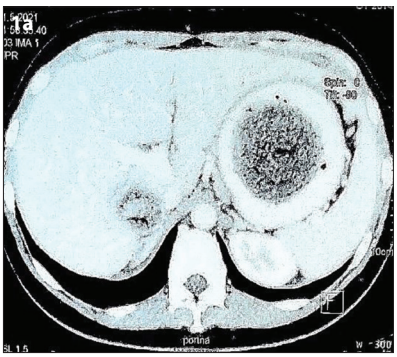

Figure 1a. Abdominal computed tomography scan – axial sections show a trichobezoar as non-enhancing intraluminal mass in stomach

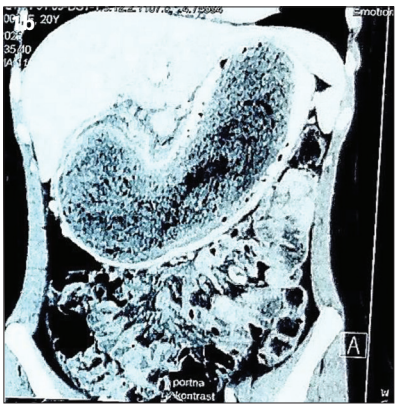

Figure 1b. Abdominal computed tomography scan – coronal section shows a trichobezoar as a non-enhancing intraluminal mass with trapped air bubbles in the interstices which is distending the stomach

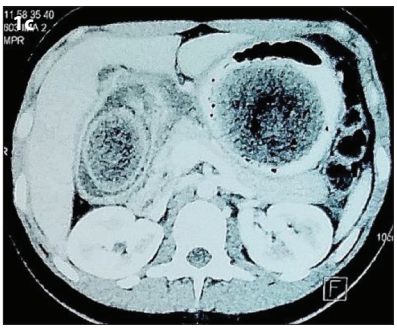

Figure 1c. Abdominal computed tomography – axial sections that shows a trichobezoar as intraluminal mass in stomach and tail in duodenum

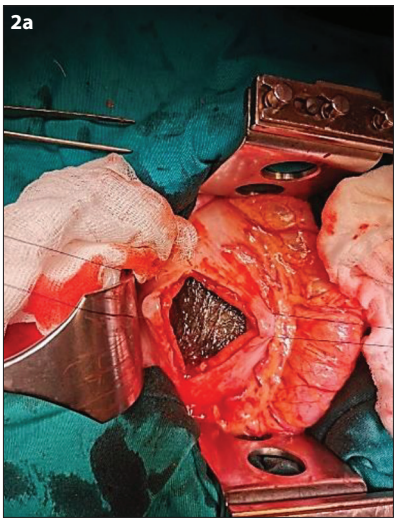

Figure 2a. Gastrotomy, a massive compact foreign body completely occupies the lumen of the stomach

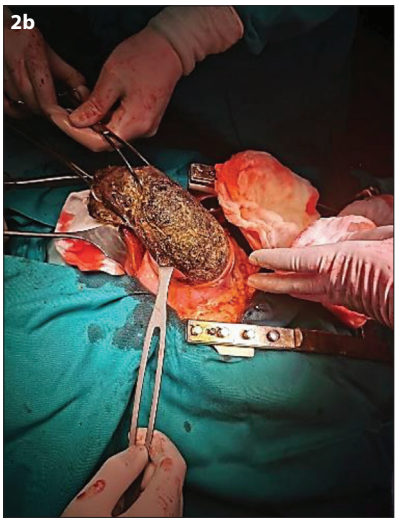

Figure 2b. Intraoperative trichobezoar extraction following a gastrotomy

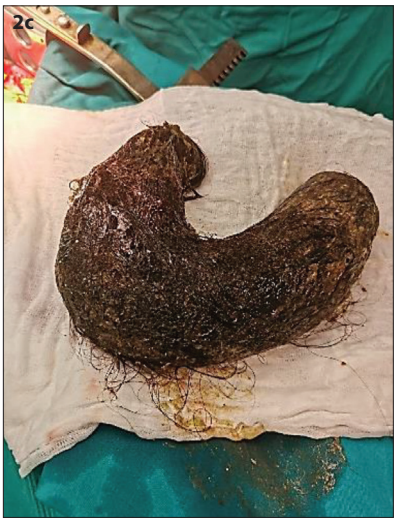

Figure 2c. Trichobezoar forming a cast of the stomach

above pylorus was made on the anterior surface of the corporal region of the stomach (Figures 2a, 2b, and 2c). A massive trichobezoar of the stomach and duodenum was extracted (Figure 2b, and 2c). The gastrotomy was closed in two layers and the abdomen was closed with drainage.

The patient made an excellent post-operative recovery with no complications. Postoperatively the patient was examined by a psychiatrist, this was her first psychiatric exam. According to anamnesis data, she started to bite the ends of her hair and then to pull it out at the root at the age of 13. She denied the existence of any significant stressors in both that period of her life, as well as presently. The diagnosis of trichotillomania was confirmed. During hospitalization oral intake was allowed on the fifth post-op day. She was discharged from Surgery department on the seventh post-op day in stable condition, with advice for psychotherapy and psychiatric follow-up to avoid recurrence.

This study was done in accordance with the institutional standards on Ethics.

DISCUSSION

Trichobezoars are foreign bodies, they are solid masses that cannot be digested. It occurs most often in young people, young women with behavioral disorders and retarded children [4–9]. Trichobezoars are formed when indigestible hairs are retained in the stomach, forming a ball, and instead of being expelled through the peristaltic wave, remains in the lumen, retaining parts of undigested food [1–7, 9]. Over time this solid mass "grows," takes the shape of the stomach and extends distally into the duodenum and small intestine, create the Rapunzel syndrome [4, 10, 11, 12].

If the patient continues to eat the hair, the trichobezoar gets bigger and bigger, this process progresses slowly, and the symptoms are non-specific for years. Sometimes fermentation of the bezoar give the patient's breath a putrid smell which could be a warning clinical sign [6].

Trichobezoars occurs most often in young people, young women with behavioral disorders and retarded children [3, 8].

Trichobezoars, with their mechanical effect on the mucosa of the lumen of the stomach and intestines, can cause minor or major and even catastrophic complications, such as organ perforation [3, 8, 10, 11, 12].

About 6% of all the bezoars presenting with symptoms of gastric outlet obstruction.

We presented a rare case of trichobezoar, which manifested itself as a surgical emergency due to acute gastric obstruction in a young woman who had been swallowing her own hair for years, without the idea of asking for a psychiatric support and without the idea that she could harm herself. She denied the existence of the psychology or external stressors. Considering her first contact with a psychiatrist in the context of the existing conditions this could be understood as an expression of less mature psychological defense mechanisms, as well as dissimulative behavior. In a situation where the first psychiatric examination is performed as part of the treatment of complications of the underlying disease, like in this case of trichotillomania, only detailed psychological and psychiatric exploration, the complete exploration patient's life, as well as examination outside the context of current events can provide sufficient insight into their mental status [3, 8].

The complications of gastric trichobezoar are different, it can rarely detach, and made satellites [11, 12]. Mirza et al. [13] presented 17 cases of trichobezoars with different complications, there was one case that had a satellite small bowel trichobezoar in addition to the gastric trichobezoar. Sometimes the signs of small bowel obstruction with intraluminal mass and the real cause of the intestinal obstruction can recognized late, which is one of the dangers of bezoars. Therefore, it is recommendation that in any case of gastric trichobezoar, during abdominal exploration surgeon should looking for a secondary trichobezoar in small or large bowel.

Trichobezoars most often present with one of three clinical types: acute presentation due to complications made by massive lesions, asymptomatic or atypical picture in small or stable lesions, and incidental lesions. If there is no evidence about behavioral disorder, small trichobezoars can be asymptomatic for a long time, diagnosis is based on clinical suspicion and on the correct selection of diagnostic methods and radiological imaging. Depending on the size, location and mechanical injuries caused by trichobezoar the symptoms could be non-specific (dyspepsia, loss of appetite, nausea, weight loss) or dramatic [9–15]. Most often, trichobezoars are lesions located in the stomach, leading to increased pressure on the mucosa, erosion of the mucosa, malnutrition, gastric ulcerations, bleeding and even gastric perforation [14, 15]. Severe clinical finding is intestinal obstruction in about 25% of the patients and perforation and peritonitis in about 18% cases [7,13–19]. It is important to detect the existence of trichobezoar in time to avoid serious complications. In our case, the patient had an undetected trichobezoar for years, she did not consult a doctor until acute gastric obstruction occurred.

Treatment is multidisciplinary and includes a different specialist, psychiatrists, gastroenterologists, and surgeons in case of complications. Insight into the existence of trichophagia, indicates a detailed clinical, endoscopic, and radiological examination. The presence of hair in the stool and the clinical finding of a palpable mass in the abdomen, especially in the epigastrium, are specific clinical signs. Even abdominal ultrasound and plain radiograph are not sensitive in cases of large gastric trichobezoars, plain radiographs can show a gastric shaped opacified area, which corresponds with heterogenous intragastric mass on ultrasound.

The most sensitive in diagnostic algorithm is abdominal CT shows some distinctive features as well-defined non-attached intraluminal mass with air bubbles retained within the interstices [1, 2, 15]. EGDS is presented as the gold standard because it serves both the diagnosis and the therapeutic evacuation of the trichobezoars but it is not suitable for all patients [16]. The therapeutic approach using EGDS is not suitable for all patients, as was the case with our patient because it was impossible to fragment the

solid impacted gastric mass and there was a risk of unintentional stomach injury. Treatment is complete removal of the foreign body, endoscopically or surgically, by laparoscopic or open surgical approach [17–21]. We performed open midline laparotomy with gastrotomy following by complete bezoar extraction, without any complications. Some studies reported only 5% of successful endoscopic bezoars removals, successful rate of 75% for laparoscopic extraction and 99% successful for conventional laparotomy with gastrotomy [17–21]. Therefore, laparotomy it is considered the treatment of choice. To completely eliminate the lesion from stomach or intestines, there is no clinical evidence of the drugs therapy effectiveness, such as enzyme therapy, and only mechanical removal of the lesion is considered successful [18].

Multidisciplinary treatment is mandatory, and in addition to mechanical removal of bezoars, is psychiatric support and treatment of trichomania as an impulse control disorder. To help the patient it is necessary to discover biopsychosocial specificities in the origin and development of the clinical characteristics of trichotillomania [3, 8]. The success of the treatment in this case can only be attributed to the good teamwork of the surgeon, gastroenterologist, psychiatrist who established the first contact with patient, the initial examination and initial diagnostic evaluation, and further psychiatric supervision. Treating trichotillomania is complex and includes pharmacotherapeutic, socio-therapeutic and psychotherapeutic modalities. In the treatment of these patients the most important results are provided by behavioral or behavioral-cognitive psychotherapy techniques [3, 8]. It is important to emphasize that inadequately treated trichomania is accompanied by relapses when trichophagia occurs, and therefore psychiatric follow-up is necessary [3, 8].

After surgical removal of trichobezoars, clinical and EGDS follow-up is advised during next six, 12 and 24 months due to potential abdominal complications or early detection of recurrence of trichophagia with asymptomatic trichobezoars in the stomach or intestines [19, 20, 21].

This case emphasizes the importance of trichobezoars as a differential diagnosis in early diagnostic and therapeutic procedures in young females with non-specific upper abdominal complaints and an epigastric palpable mass. It is also important to understand that it is a complex clinical problem, primarily a disorder of impulse control which requires psychiatric treatment, while surgery or endoscopy only solve the consequences of this behavior disordered. In patients with trichobezoar and trichotillomania, serious complications may occur due to the presence of a foreign body in the stomach or intestines, and another problem is relapses of the disease. The experience with our patient shows that only the interaction of medical knowledge and skill from different medical disciplines can provide the best understanding and the most successful treatment of this disease.

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Мултидисциплинарно лечење џиновског желудачног трихобезоара – ретког узрока акутне опструкције желуца

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САЖЕТАК

Увод Трихобезоари представљају редак облик безоара насталих од прогутаних длака, са клиничком манифестацијом опструкције желуца или црева, улцерације желуца, крварења и перфорације. Углавном се откривају код емоционално поремећених или ментално ретардираних младих који једу сопствену косу, што је клинички познато као трихофагија. Болесници често поричу да једу сопствену косу, што отежава дијагнозу.

Приказ болесника Представљамо случај акутне опструкције желуца изазване огромним желудачним трихобезоаром сачињеним од дугих танких длака код 20-годишње жене. Иако је болесница имала дугу историју трихофагије, није мислила да је њено понашање необично и раније није лечена. После почетних дијагностичких процедура, индикована је експлоративна лапаротомија. Након учињене предње гастротомије, уклоњен је масивни трихобезоар у облику желуца. Болесница је постоперативно прегледана од стране психијатра. Опоравила се и отпуштена је без компликација. Упућена је на даље психијатријско праћење.

Закључак Трихобезоари су несварљиве колекције које се обично акумулирају у желуцу и могу се проширити до танког црева изазивајући механичка оштећења, као што је опструкција шупљих органа. Болесници са акутном опструкцијом желуца изазваном џиновским трихобезоаром захтевају хитно уклањање трихобезоара, како би се сачувао желудац и избегле даље, катастрофалне последице.

Кључне речи: трихобезоар; гастротомија; трихофагија; емоционални поремећаји

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Implantation metastasis of colorectal cancer following percutaneous biliary drainage

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SUMMARY

Introduction Malignant biliary obstruction represents a poor prognostic sign of metastatic colorectal carcinoma. Percutaneous transhepatic biliary drainage (PTBD) is the procedure of choice for palliative biliary decompression, and this method has both diagnostic and therapeutic values. One of the well-known complications following this procedure is the development of catheter tract metastases that occur in 0.6–6% of cases post-PTBD. In this case report, we present a patient with implantation metastases of colorectal cancer following PTBD.

Case report In the last six years, 89 patients underwent PTBD procedure at the Oncology Institute of Vojvodina. Among these patients, catheter tract implantation metastasis developed in one patient (1.1%). In this report, we present a patient who underwent right hemicolectomy in January 2015 at the Oncology Institute due to colon cancer located in the transverse colon. In January of 2018, a computed tomography scan of the abdomen showed metastatic disease and chemotherapy was initiated. However, 29 months following the start of chemotherapy, the patient developed jaundice, and as a result, PTBD procedure was performed. A control computed tomography scan of the abdomen in March of 2021 showed a *de novo* subcutaneous nodule 20 mm in diameter located at the level of ninth right rib. The nodule had been considered a part of the scar that formed at a place of catheter entry, and was still present eight months after PTBD procedure. Biopsy of the subcutaneous mass and pathohistological analysis confirmed well differentiated colon adenocarcinoma.

Conclusion Catheter tract implantation metastasis is not a rare complication following PTBD for malignant biliary obstruction. It generally has a poor prognosis. Nevertheless, literature review shows that radical surgical excision of the catheter tract tissue with hepatectomy can prolong survival in select group of patients.

Keywords: colorectal cancer; malignant biliary obstruction; implantation metastasis; percutaneous transhepatic biliary drainage

INTRODUCTION

Malignant biliary obstruction represents a poor prognostic sign of metastatic colorectal carcinoma [1]. It usually develops as a consequence of metastatic tissue growth in the liver itself, on the peritoneum at the hilum of the liver, along the extrahepatic portions of the biliary tract, or in the extrahepatic lymph nodes [2]. In these cases, percutaneous transhepatic biliary drainage (PTBD) is the procedure of choice with a main purpose of palliative biliary decompression. In addition, PTBD can also have diagnostic and therapeutic value [3,4]. However, one of the well-known complications following this procedure is the development of catheter tract metastasis. Published reports show that this complication can occur in up to 6% of cases post-PTBD. In this case report, we present a patient with implantation metastases of colorectal cancer following percutaneous biliary drainage.

CASE REPORT

A 68-year-old man was admitted to our department where he had been receiving chemotherapy regularly according to the FOLFIRI protocol (5-Fluorouracil 400 mg/m^2, 5- Fluorouracil 600 mg/m^2 in 22 hours, Leucovorin 200 mg/m^2, and Irinotecan 180 mg/m^2) every two weeks for metastatic colon cancer. During the interview with a physician, the patient complained of painful swelling on his right lower chest wall. On clinical examination, a 5 × 3 cm solid, elastic nodule was palpated in the right anterolateral chest wall over the ninth rib and adjacent intercostal spaces. The mass was fixed to the chest wall. The overlying skin was mobile, but had a scar that corresponded to the previous PTBD procedure (Figure 1).

It is important to note that in January 2015 the patient underwent right hemicolectomy at the Institute due to colon cancer located in the transverse colon. The subsequent pathohistological examination confirmed TNM stage: G2 Adenocarcinoma T3N2(8/23) M0 with perivascular (pV+) and perineural invasion (pN+).

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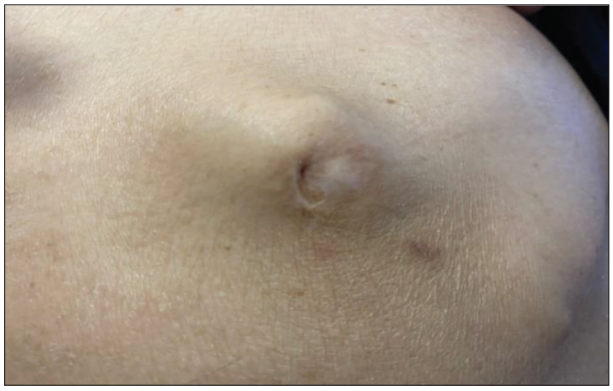

Figure 1. Clinical presentation of implantation metastasis- subcutaneous nodule in the anterior chest wall in the area of scar after percutaneous transhepatic biliary drainage procedure

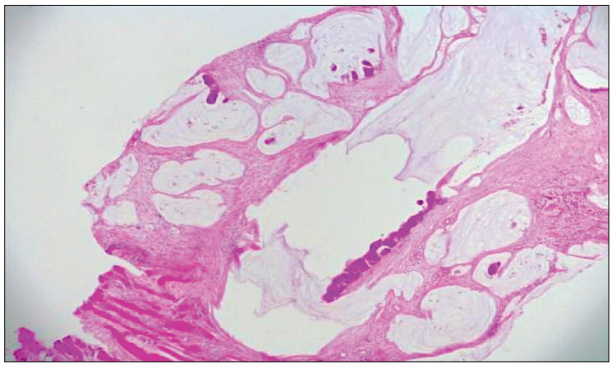

Figure 2. Core biopsy specimen with diagnosis of low-grade mucinous adenocarcinoma; H & E staining, 100 × magnification

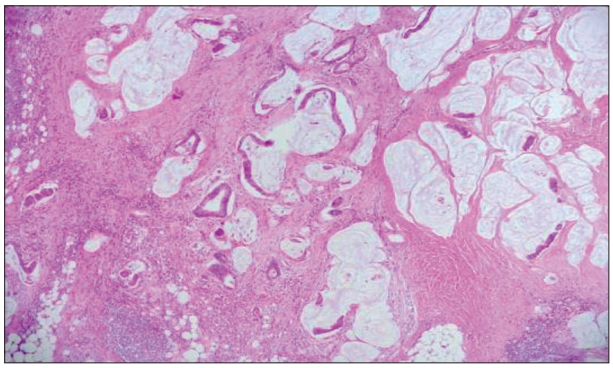

Figure 3. An original sample taken from the right hemicolectomy showing same histologic features of tumor as in the core biopsy; H & E staining, 100 × magnification

In accordance with this, the patient received eight cycles of adjuvant chemotherapy with Capecitabin, and he had regular six-month follow up.

In January 2018, an abdominal computed tomography scan showed enlarged intrabdominal lymph nodes surrounding the celiac plexus and superior mesenteric artery. A multidisciplinary team of physicians recommended the two-week FOLFIRI chemotherapy regimen. In June 2020, after 29 months of stop-and-go chemotherapy regimen, the disease was radiologically stable, but with the apparent clinical onset of jaundice. An abdominal ultrasound showed dilatation of the right and left hepatic duct, as well as the common bile duct. Following this, in July 2020, the PTBD with external and internal biliary drainage was successfully performed, which resulted in decrease of bilirubin levels during the course of the following six weeks. A control computed tomography scan of the abdomen in March 2021 showed stable disease and the presence of a *de novo* subcutaneous nodule 20 mm in diameter at level of the ninth rib on the right in the area considered for a scar at a place of catheter entry during the PTBD procedure performed eight months prior to this. The same treatment regimen (FOLFIRI) was continued, but at each subsequent hospitalization subcutaneous node was growing larger, and the patient started to complain of increasing pain and discomfort in this area. An ultrasound-guided core biopsy of the lesion dimension 4 × 3 cm was performed, and histopathological examination of the standard hematoxylin and eosin stained sections revealed neoplastic infiltration of fibrous tissue in the form of large lakes of extracellular mucin with occasional strips of neoplastic colorectal epithelium (Figure 2). Re-examination of the archived slides of the primary tumor of the transverse colon confirmed that the biopsied subcutaneous tissue had essentially the same morphological features (Figure 3). Moreover, after additional immunohistochemical analysis was performed, immunoreactivity for SATB-2 and CK20, and no staining with anti-CK7 antibody definitely confirmed the colorectal origin of the low-grade metastatic tumor.

This study was done in accordance with the institutional standards on Ethics.

DISCUSSION

Metastases along the catheter tract from PTBD procedure can originate from various primary tumors, but typically originate from metastatic pancreatic and biliary tumors. However, to the best of our knowledge, this is a first case report on implantation metastasis of colon cancer following PTBD and the information regarding the median time to detection post procedure, disease management, median survival, and prognosis specific for this case are lacking.

In cases that originated from the primary tumors of the biliary tract, median time to detection is 14 months post-PTBD, and it has been reported in up to 6% of people who underwent this procedure [5, 6]. Out of 89 patients that had this procedure performed at our institution over the course of six years, only the patient from the present case report developed catheter tract implantation metastasis (1.1% of total number of cases). Although there are several proposed mechanisms that explain pathogenesis of catheter tract metastasis, the precise mechanism has not been completely elucidated. There are reports showing that longer procedure times with multiple catheter insertions and biliary tract manipulations increase probability for tumor cell seeding. In addition, more differentiated tumors, and those with papillary histology are more prone to seeding along the catheter tract [6]. In accordance with this observation, pathohistological report on the presented patient confirmed that implantation subcutaneous metastasis contained well differentiated colorectal adenocarcinoma cells.

Oleaga et al. [7] was the first to report on a case of cutaneous metastasis of hilar cholangiocarcinoma.

Liu et al. [8] reviewed the English literature and found 30 reports on cases of cutaneous metastases in hilar cholangiocarcinoma.

In general, the prognosis for these patients is poor. However, Sakata et al. [5] noted that the surgical removal of solitary implantation metastatic nodules was followed by a survival longer than one year in about 80% of patients. In a study that examined four patients with this complication, patients' survival ranged from 8 to 18 months with post-excision median survival of 10.5 months [9].

PTBD represents an invasive procedure associated with severe complications and significant mortality. Literature review shows that per- and post-PTBD seven-day mortality rate ranges from 2.98% to 5.2%, while 30-day mortality rate ranges from 23.1% to 33% [10, 11, 12]. Lauterio et al. [13] reviewed results of six studies examining management of the patients with metastatic perihilar cholangiocarcinoma who underwent the PTBD procedure. In these studies, the reported mortality ranged between 0% and 12% [13, 14, 15]. The most commonly identified risk factors associated with increased postoperative complications were biliary tract manipulation and subsequent development of cholangitis and sepsis [16, 17].

The two types of interventions that are sometimes used as an alternative to PTBD in treatment of malignant biliary obstruction are endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasound-guided biliary drainage (EUS-BD). A meta-analysis of randomized trials and observational studies that compared technical and clinical success rates and rates of complications for ERCP and EUS-BD, showed they are comparable to PTBD. In addition, in order for EUS-BD to be successfully performed, biliary ducts should be dilated, which is also noted requirement for successful PTBD. In ERCP and EUS-BD, successful biliary drainage is achieved in about 94%, and resolution of jaundice in 91–94% of cases, with no significant difference in procedure duration or the incidence of overall post-procedural complications (overall complications ERCP *vs*. EUS-BD = 22.3% *vs*. 15.2%) [18–21]. Reports confirmed no significant difference in re-interventions because of jaundice in ERCP *vs*. EUS-BD [19, 20]. However, while the EUS-BP was not associated with post-procedural pancreatitis, after ERCP 9.5% of patients developed this severe complication [19].

In conclusion, catheter tract implantation metastasis is not a rare complication following PTBD for malignant biliary obstruction. It is associated with generally poor prognosis. In select group of patients with a solitary node, radical surgery with excision of the catheter tract and hepatectomy allows survival longer than one year.

Conflict of interest: None declared.

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Имплантациона метастаза колоректалног карцинома после перкутане дренаже жучних путева

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САЖЕТАК

Увод Билијарна опструкција је честа компликација метастатског колоректалног карцинома и удружена је са лошом прогнозом код ових болесника. Перкутана трансхепатична билијарна дренажа (ПТБД) широко је распрострањена процедура за билијарну декомпресију узрокована малигнитетом и служи за дијагностичке, терапијске и палијативне сврхе. Појава метастаза на месту увођења катетера јавља се у 0,6–6% случајева.

Циљ рада је приказ болесника са имплантационом метастазом колоректалног карцинома после перкутане билијарне дренаже.

Приказ болесника На Институту за онкологију Војводине у протеклих шест година 89 болесника су подвргнути процедури ПТБД, а појава имплантационе метастазе на месту увођења катетера јавила се код једног болесника (1,1%). Представљамо болесника коме је у нашој установи због карцинома попречног колона у јануару 2015. године учињена десна хемиколектомија. Јануара 2018. године компјутеризована томографија абдомена указала је на појаву метастатске болести, те је започета хемиотерапија, али се 29 месеци касније појавила жутица, те је урађена процедура ПТБД. Контролна компјутеризована томографија абдомена (у марту 2021. године) показала је појаву *de novo* супкутаног чвора 20 *mm* у пределу деветог ребра десно, што је схваћено као место ожиљка на месту увођења катетера осам месеци после процедуре ПТБД. Биопсијом поткожне метастазе патохистолошки је верификован добро диференциран аденокарцином дебелог црева.

Закључак Појава имплантационих метастаза на месту увођења катетера због малигне билијарне опструкције није ретка компликација после ПТБД и обично има лошу прогнозу. Ипак, преглед литературе показује да у одабраној групи болесника радикална операција са ексцизијом катетерског тракта, која захтева и хепатектомију, омогућава дуготрајније преживљавање.

Кључне речи: колоректални карцином; малигна билијарна опструкција; имплантациона метастаза; перкутана трансхепатична билијарна дренажа

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Basal cell carcinoma – principles of treatment

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SUMMARY
Basal cell carcinoma (BCC) is one of the most common malignant tumors in human medicine and the most common skin malignancy, with the largest number of lesions found on exposed parts of the skin, on the face, head, and neck. The average age of the patients is 60 years, with an increasing incidence in younger ages and an increased incidence in males.
The incidence of BCC is increasing and doubles every 25 years. Annually, there are approximately 1,000,000 newly diagnosed cases worldwide. The frequency of malignant skin tumors depends on the influence of external factors such as ultraviolet radiation and other biological properties of the skin with a higher incidence in fair-skinned people (Fitzpatrick type I and type II skin types).
BCC is a slow-growing malignant tumor that arises from the basal layer of the epidermis, the outer layer of hair follicles, or the sebaceous glands. BCC can be locally invasive and, if neglected, can infiltrate surrounding structures (muscles and cartilage) and vital structures, which can ultimately lead to death. The clinical presentation is very diverse and dependent on the histological subtype. Prevention is the most important and effective approach towards reducing the burden of BCC. The best treatment for BCC is surgical excision with confirmation and verification of surgical margins. The therapeutic goal is oncologic radical resection of the tumor, followed by reconstruction of the affected area for structure and optimal aesthetic result.
Keywords: carcinoma; basal cell; therapeutics; prognostic factors

INTRODUCTION

Malignant epithelial skin tumors, also known as non-melanocytic skin cancer (NMSC) are one of the most common malignant neoplasms in human medicine. NMSCs comprise 95% of all skin cancers and are considered to have the most favorable prognosis with a high 5-year survival rate [1, 2]. It is predicted that more than 50% of people over the age of 50 will be diagnosed with some type of skin tumor [3]. Biological behavior varies where most often they will have a relatively benign course, while others progress to extreme morbidity, mutilation, metastasis, and even death [2 ,4, 5].

In 2020, there were 1,198,073 new cases of NMSCs recorded worldwide. Of that number, 80%, or approximately 1,000,000 cases, were classified as basal cell carcinomas (BCC) [1, 2]. In 2018, the number of newly discovered NMSCs within the territory of the Republic of Serbia for males and females were 1830 and 1715, respectively [3].

EPIDEMIOLOGY

It has been widely accepted that 80% of NMSCs are BCC, and up to 20% are classified as squamous cell carcinoma (SCC) [1, 4, 5].

The greatest number of BCC lesions are located on areas of exposed skin [i.e., face (particularly on the upper two-thirds), head and neck (90% of neoplasms)] [4, 5, 6]. The average age of patients is 60 years, with an increasing incidence in younger ages, and a higher incidence in males [7–11].

NMSCs are predominantly found in the Caucasian population (> 99%) among a fair-skinned population (Fitzpatrick type I and II skin types, which are more prone to solar burns), in geographic regions with a high level of insolation [incidence is directly proportional to the degree of exposure to ultraviolet (UV) radiation] [6].

Over the past 30 years, the incidence of NMSC has increased by 20–80%, and continues to increase at a rate that doubles every 25 years [1, 6, 8]. The number of newly diagnosed cases annually in Europe ranges 40–80 per 10,000 inhabitants in Scandinavian countries and Mediterranean counties, respectively [8]. In Australia, skin neoplasms account for 50% of all tumors in the white population with an incidence of 1600 per 10,000 people [8].

ETIOLOGY

The most important etiological factors are the biological properties of the skin (i.e., skin

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type) and the influence of external factors, primarily exposure to ultraviolet radiation, natural and artificial (i.e., sunbeds). Among other external factors that play a role in cutaneous carcinogenesis are: X-ray radiation, alpha and beta radiation, chemical agents (tar, resins, soot, arsenic, aniline, asbestos), atmospheric pollutants, psoralen and nitrogen mustard, and immunosuppressive therapy [1, 9]. Neoplasms can appear in areas of chronic skin diseases such as actinic keratosis, degenerative skin atrophy, and scars, most often after burns. Neoplasms can also occur as part of a syndrome, such as xeroderma pigmentosum, nevoid basal cell carcinoma syndrome, also known as Gorlin–Goltz syndrome, and albinism [1, 12]. Furthermore, the dose of UVB exposure during childhood and adolescence is directly proportional to the risk of developing BCC [1, 12, 13].

In regard to the pathogenesis of NMSCs, only the wavelengths in the UV spectrum (100–400 nm) are of clinical significance. The sun is a natural source of UV radiation; however, the long-term effects of exposure to sunlamps and sunbeds cannot be ignored, which may also explain the increasing incidence of NMSC in younger women, with the highest relative increase in women aged 40–49 (246%) and 30–39 (191%) [14, 15]. The amount of radiation produced by fluorescent lamps and other "cold" UV light sources is not clinically significant [16].

Solar radiation that penetrates to the Earth's surface generally does not contain UVC radiation. More than 95% of solar UV radiation is within the ultraviolet-visible (UVA) wavelength range, while the small remaining amount of UVB is responsible for acute sunburn, as well as most chronic sun damage and malignant degeneration of human skin. A condition known as *erythema ab igne*, caused by chronic exposure to radiant heat, strongly simulates chronic UV injury [4, 5, 17].

Actinic damage, direct and local or indirect and systemic, is the only universally recognized risk factor for the development of all types of NMSCs [7, 8]. The distribution of NMSCs and individual susceptibility to UV-induced tumors is inversely proportional to the melanocyte content of the skin [the highest density of melanocytes are on chronically photo-exposed parts (i.e., face), the smallest density of melanocytes are typically on unexposed skin regions (i.e., soles and stomach)] and the constitutive higher production of melanin pigment that is transported to keratinocytes to provide protection from UV radiation [6, 16]. UVB rays are most carcinogenic and have the greatest immunological impact, but UVA rays are known to significantly accentuate the acute damage caused by UVB rays and enhance their carcinogenic effect [17, 18, 19].

The process of UV radiation-induced skin damage starts at birth, accumulates over time, and can eventually lead to the emergence of BCC [19, 20, 21]. Given the role that sun and wind exposure play in the carcinogenesis of BCC, there is an increased risk of BCC in certain occupations associated with extended outdoor exposure (i.e., farmers, sailors) [22, 23].

PATHOGENESIS

BCC was first described by Jacob in 1827 using the term "rodent ulcer" [20]. In 1903, Krompecher later described the histological characteristics of what was initially considered a true epithelial carcinoma [21, 24, 25].

BCC is a slow-growing malignant tumor that arises from the basal layer of the epidermis, the outer layer of the hair follicle, or the sebaceous glands. It grows locally, infiltratively, and destructively, affecting the adjacent skin and subcutaneous tissue, which can lead to significant functional and cosmetic defects. Locally, BCC can be very invasive. If neglected, it can infiltrate the surrounding structures (muscles, cartilage, bone), develop a superimposed infection, and even lead to death [1]. Furthermore, BCC metastasizes extremely rarely (0.0028–0.55%) [26, 27].

BCC, when experimentally transplanted without dermal tissue, does not survive [28]. A possible explanation is that the altered stroma of scar tissue helps pluripotent cells transform into malignant cells, which would further explain the appearance of tumors in areas of trauma, ulceration, and burns [29, 30].

Experimentally, BCC has previously been induced in rats through the use of chemical carcinogens; however, no tumor occurrences were ever observed when there was only exposure to UV light. One-third of all BCCs occur in areas of the body with little to no exposure to the sun [30, 31].

CLINICAL PRESENTATION

BCC has a very diverse clinical presentation manifesting macroscopically as macular, papular, nodular, and in the form of a solid plaque, sometimes accompanied by ulceration (skin-color or transparent), lightly erythematous, with raised edges where telangiectasias can be observed (Figure 1) [1].

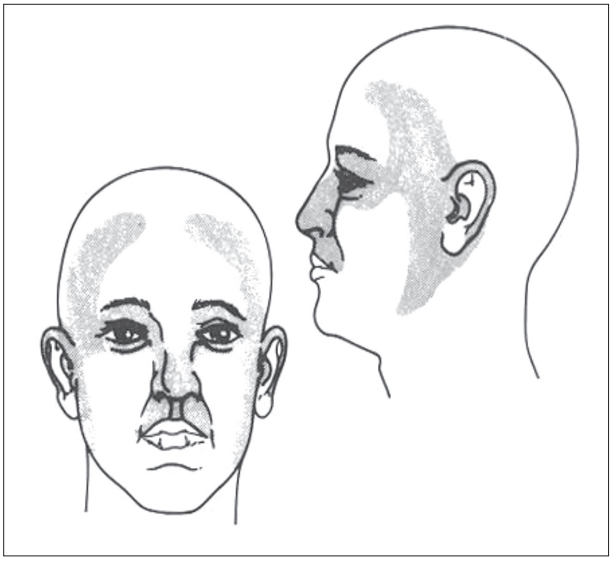

Figure 1. Weber "H" zone; used with permission from: Dimitrijević MV. Epitelni maligni tumori kože. In: Dimitrijevic MV, et al. Maksilofacijalna hirurgija. Medicinski Fakultet, Beograd; 2020. p. 165–70

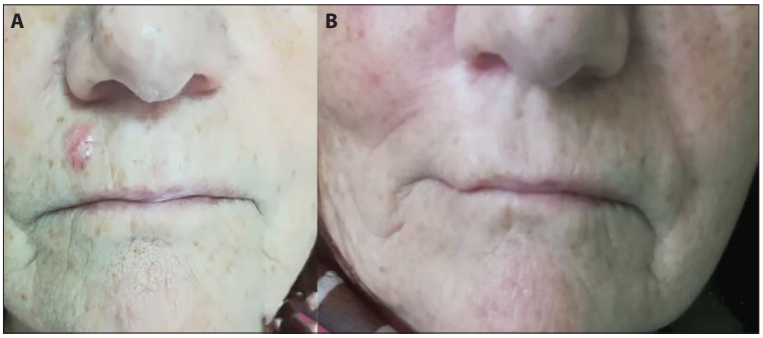

Figure 2. Basal cell carcinoma pre-operative (A) versus post-operative (B); from the private collection of Dimitrijević MV

Table 1. TNM classification of malignant skin tumors (Union for the International Cancer Control, 2017)

TX	Tumor cannot be assessed
T0	No evidence of primary tumor
TIS	Carcinoma *in situ*
T1	Tumor is up to 2 cm in the greatest diameter
T2	Tumor is > 2 cm but < 4 cm in the greatest diameter
T3	Tumor is > 4 cm in the greatest diameter, or with minimal bone erosion, or with one or more high risk parameters (i.e., invasion into the deeper superficial layers of the skin, perineural invasion)
T4A	Tumor with significant invasion to the bone: core and/or marrow
T4B	Tumor with skull base or axial skeleton invasion including foraminal involvement and/or vertebral foramen involvement to the epidural space

T – primary tumor site; N and M categorization corresponds to the same categories of other localized malignant tumors of the head and neck [Brierley JD, Gospodarowicz MK, Wittekind CH (eds). TNM Classification of Malignant Tumours (8th edition). Oxford, UK: Wiley-Blackwell, 2017]

Aggressive BCCs are characterized by a combination of potential clinical manifestations such as subclinical growth, aggressive local spread, incomplete excision, and recurrence [1, 19, 20].

In 1996, Weber defined the "H" zone of the face, the at-risk region where BCCs are found most frequently (Figure 1). BCC can be divided into two categories: low and high risk of recurrence after therapy. The key clinical features used to make this distinction are location, size, margins, immune status of the patient, and histopathological parameters (subtype, depth of invasion, perineural, and perivascular invasion) (Figure 2) [21, 22].

Tumor staging is determined using TNM staging classifications. In Europe, staging is used for all skin regions according to Union for the International Cancer Control 2017, while in the US the American Joint Committee on Cancer 2017 system uses the classification only for tumors of the head and neck region (Table 1).

BCCs in certain anatomical locations, such as the periorbital, perinasal, and periauricular regions, often recur (20–25%) [1, 17, 32]. This may be due to embryonic fusion planes, the tendency for the tumor to spread peripherally below the superficial layers of the skin (i.e., subclinical extension), and the difficulty in accurately assessing the extent of tumor extension during surgical excision (i.e., the lack of adequate margins) [33, 34].

Recurrent BCC becomes more aggressive as the number of treatments increases, as evidenced by a change in tumor histology from nodular to infiltrative with each new treatment attempt [34]. Tumor size greater than 5 cm is associated with a 25% increased risk for metastasis, and tumors that are greater than 2 cm in size are associated with a poorer prognosis [35, 36].

HISTOPATHOLOGICAL CHARACTERISTICS

BCC is characterized by cells with a scant cytoplasm, smaller hyperchromatic (darkly stained) nucleus without a prominent nucleolus. The peripheral layer of the tumor typically forms a palisade arrangement with a cleft that forms the adjacent stroma. BCC can be divided into low- and high-risk types based on certain histopathological characteristics that have prognostic significance (Table 2), primarily in relation to local recurrence, given that metastasis is extremely rare. Superficial BCC, despite always being classified as low-risk, tends to recur due its multifocal growth pattern (Figure 3a), with some peripheral nests. Due to their small dimensions, they can often be clinically undetected and found beyond the tumor's margins typically manifesting in the form of erythematous macules.

Table 2. Histopathological prognostic parameters of basal cell carcinoma

1. Histopathological subtype
1.1 Low risk
1.1.1. Superficial
1.1.2. Nodular and variants
1.1.3. Rarer types (infundibulocystic/harmartomatous, fibroepithelioma of Pinkus)
1.2 High risk
1.2.1. Infiltrative
1.2.2. Morpheaform (morphologic, sclerotic)
1.2.3. Micronodular
1.2.4. Basosquamous (metatypical)
2. Depth (level) of invasion
3. Perineural invasion
4. Vascular invasion
5. Status of resection margins

Modified from references No. 27 and 37

Variants of nodular BCC (i.e., cystic, adenoid) can sometimes cause dilemmas in differential diagnosis due to the similarities with certain adnexal tumors. According to the World Health Organization classification of low-risk tumors, pigmented BCC is also noted, which is essentially not a separate histopathological subtype, but rather a clinical presentation of different (usually superficial and nodular) types of BCC that exhibit brown pigmentation and can simulate melanocytic lesions, including melanoma. The pigment is most often found in melanophages, less often in BCC cells or in multiple dendritic melanocytes in BCC

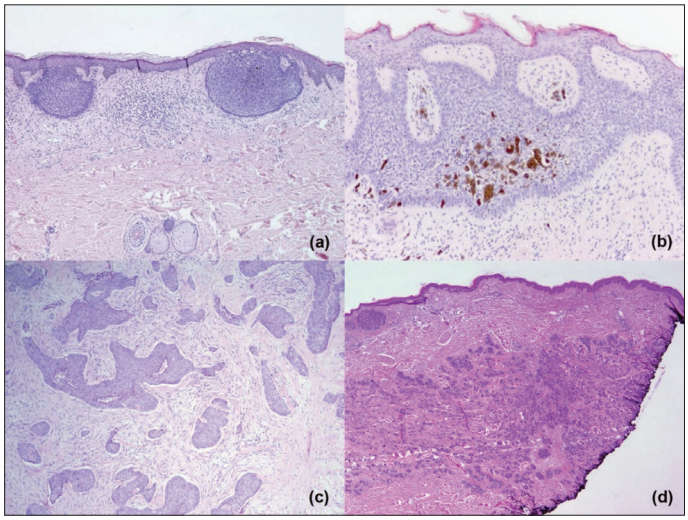

Figure 3. Histopathological types of basal cell carcinoma; superficial (a) with multifocal, small, superficial nests; pigmented (b) with melanin pigmentation in the stromal melanophages and tumor cells; infiltrative (c), and micronodular (d), which is found on the peripheral lines of the resection marked by the black edges (H&E staining, magnification 40× (a, d), 100× (b, c); from the private collection of Brašanac DČ

nests (Figure 3b). Sometimes, clinically visible pigmentation represents the accumulation of hemosiderin.

Infiltrative BCC is characterized by irregular nests (Figure 3c), while morpheaform BCC grows in the form of narrow bands spanning the width of several rows of cells, in a dense collagenous stroma, making it difficult to clinically distinguish the border in relation to scar tissue in recurrences. Micronodular BCC features small nests of basaloid cells (up to 0.15 mm in diameter) that often extend in depth and width beyond clinically detectable margins (Figure 3d) [27, 37]. Basosquamous (metatypical) BCC shows fields of atypical squamous cells with the appearance of SCC which has a higher recurrence rate and metastatic capacity.

BCC often exhibits different types of structural features within the same tumor, from the same risk category (e.g., superficial peripherally and nodular centrally; infiltrative and morpheaform, or in combination with micronodular or basosquamous), or from different categories (usually superficial nodular and deeper in tissue infiltrative or micronodular). There are no clear indicators for the tendency of recurrence in BCC exhibiting a combination of types from different risk groups; however, it is best to determine the risk according to the most aggressive component [37]. More aggressive variants are usually found deeper in the tissue, making it difficult to perform adequate dermatoscopy [38].

Deep invasion (i.e., beyond the subcutaneous fatty tissue or > 6 mm from the granular layer of the surrounding skin) is a factor that transforms T1 or T2 tumors into T3. However, thickness as a risk for local recurrence and metastasis was determined by study performed on SCC [39].

Unlike perineural, vascular invasion is not always mentioned as a factor influencing the staging of skin tumors, primarily for the basosquamous type, although in practice it can also be observed in other variants of BCC [37].

The status of resection margins can be defined as tumor presence at the resection line, close to the resection line (< 1 mm), and tumor-free (> 1 mm) [37].

DIAGNOSIS

Clinical presentation and a thorough patient history of exposure to risk factors and duration of lesion presence are often sufficient for the diagnosis of BCC. Examination to evaluate the location and size of the lesion is necessary, and dermatoscopy can also be used to accurately assess skin lesions. Definitive diagnosis is established via biopsy and pathohistological verification. Smaller lesions are removed entirely with surrounding parts of the healthy skin and subcutaneous tissue, which is commonly the case and a definitive treatment. For larger lesions, a punch biopsy is performed [1, 4, 5].

Computed tomography and magnetic resonance imaging are only used in more advanced tumors. Timely detection and therapy are associated with an excellent prognosis (high cure percentage and low recurrence rate) [1, 5].

TREATMENT

Prevention is the most important and effective therapeutic approach. It consists of education, the use of protective creams, and maximum reduction of sun exposure, particularly during hours with a high UV index [33, 34]. Premalignant lesions should be treated before the full clinical form of the tumor develops. An initially suspected BCC lesion should be biopsied for histological confirmation [1].

The goal of treatment is radical resection of the tumor, reconstruction of the function of the affected region for structure and optimal aesthetic result [1, 17].

Therapeutic modalities can be classified into two categories: surgical methods and non-surgical methods [1, 4, 5, 9]. Surgical treatment is the modality of choice since it has significant advantages, mainly in regard to histological control and the lowest frequency of recurrence (Table 3). Surgical techniques include classic surgical excision with postoperative determination of surgical margins, Mohs micrographic surgery, and destructive surgical techniques [1, 19, 20].

Table 3. Advantages of surgical treatment

1. Histological verification and confirmation that the tumor is excised completely to "healthy" level
2. Tumor can be removed regardless of location and size
3. Aesthetic results are superior and is a significant factor in the regions of the head and neck
4. Shorter duration of therapy
5. Treatment expenses
6. The induction of new tumors is avoided unlike in radiotherapy

Factors that affect the treatment decision are heavily dependent on the tumor size, its location, histological type,

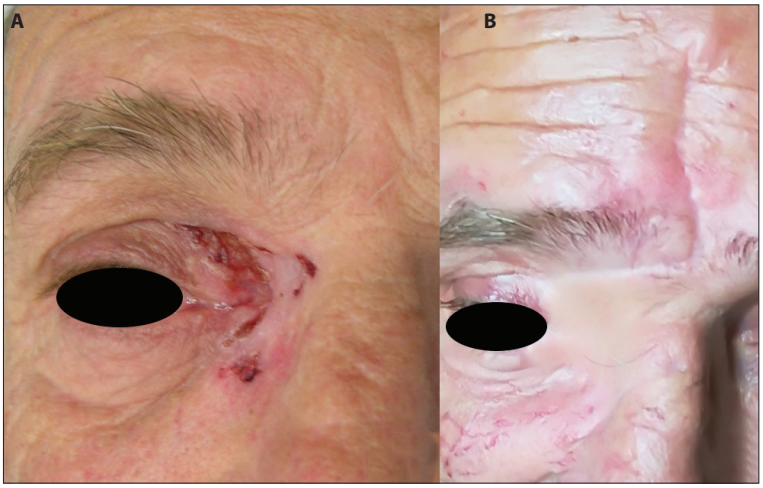

Figure 4. Basal cell carcinoma pre-op (A) versus post-op (B); from the private collection of Dimitrijević MV

Table 4. Criteria for successful treatment of basal cell carcinoma

1. Prevention (patient education, UV protection)
2. Early diagnosis
3. Planned therapy – surgical intervention
4. Regular follow-ups

invasion to surrounding structures, in depth spread, patient age, number of tumors, clarity of edges, whether it is a primary tumor or recurrence, and any previous therapy. Surgical treatment of facial skin cancer after tumor excision also includes reconstruction of the defect (Figure 4) [1, 19]. When the surgical excision is incomplete, a reexcision must be performed taking into account the patient's general health status, tumor type, and location [1, 17, 19].

Surgical techniques

Surgical treatment via excision with histological confirmation or Mohs surgery with confirmed surgical margins is considered the standard therapy for BCC [4, 5, 40]. Surgical excision enables histological confirmation of tumor removal and leads to good results in both low-risk and high-risk tumors. For BCC, the primary plan is surgical excision to reduce the risk of recurrence. The recommended surgical margins for well defined, low-risk tumors (< 2 cm in size) are 4–5 mm and 6–10 mm for high-risk tumor types as well as larger lesions and recurrences [1, 19, 20].

Mohs surgery is a surgical technique by which, with the help of three-dimensional microscopic control, complete excision of the cancerous lesion with the entire subclinical extension of the tumor can be achieved allowing for histological control of margins while achieving maximum therapeutic effect and tissue preservation [40]. Depending on the tumor size, histological type, and recurrence, Mohs surgery is recommended for high-risk tumors in regions of the face. A disadvantage is that it is time-consuming, requires special training, and significant financial resources [19, 20].

Destructive techniques

Other surgical therapies include more destructive techniques such as electrodesiccation and curettage, cryosurgery, "shave excision," and laser therapy [28, 30]. These techniques are typically used in elderly patients with multiple lesions located primarily on the body and extremities, but rarely on

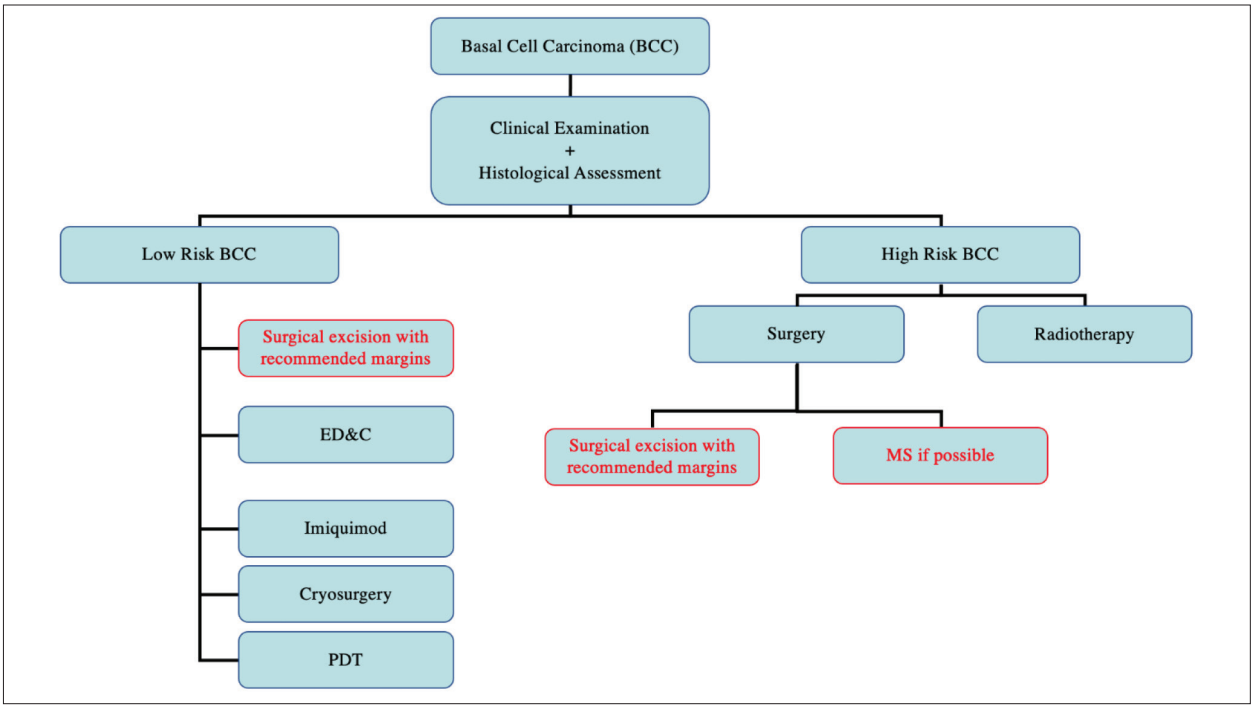

Figure 5. Basal cell carcinoma treatment modalities; ED&C – electrodesiccation and curettage; MS – Mohs micrographic surgery; PDT – photodynamic therapy; red text – standard therapy; black text – other therapeutic modalities; modified from reference No. 23

the face. A major drawback to these techniques is that they do not provide complete histological confirmation and have low therapeutic effect [21, 22, 23, 41].

Non-surgical therapeutic modalities include the following: photodynamic therapy, radiotherapy, local therapy [intralesional application of interferon, and pharmacologic therapy (imiquimod, retinoids, 5-fluouracil)]. For more advanced cases, Hedgehog signaling pathway inhibitor drugs such as vismodegib and sonidegib are used [42]. The disadvantage of destructive and non-surgical modalities is the loss of histological control and a higher percentage of recurrence compared to surgical treatment modalities [1, 25, 26].

All patients with a history of BCC require lifelong follow-ups, regardless of treatment modality (Figure 5). The risk of developing another BCC lesion is the highest in the first three years after initial treatment. Patients with low-risk tumors and histologically negative margins can be followed-up annually, while patients with high-risk tumors without histological confirmation of negative margins, or those that have been treated with non-surgical methods, with recurrent tumors, positive surgical margins or have an increased risk of developing BCC, should be followed-up more frequently. All patients with BCC must be educated about sun protection, trained in self-examination, and be advised to have annual exams performed by their dermatologist [1, 43].

PROGNOSIS

BCC has an excellent prognosis, with a high cure rate and low recurrence rate, with timely detection and therapy. Worldwide mortality attributed to BCC is estimated to be 10,000 people annually. Well planned treatment is over 95% effective. In 50% of patients, there is a possibility of developing a second BCC within five years. Recurrences occur in 5% of patients, most often within 4–12 months after initial treatment. The likelihood of recurrence is dependent on the histological subtype and therapy (i.e., positive surgical margins) [1]. Similar to other chronic conditions, for successful treatment outcome affirmation of prevention (education and UV protection), early diagnosis, and planned therapy – surgical treatment and regular follow-ups are pivotal [41, 44].

CONCLUSION

NMSCs are one of the most common malignant neoplasms in the human population, of which BCC makes up 80% of skin tumors.

The most important etiological factors are genetic predisposition, exposure to UV radiation, radiotherapy, and chemical agents. BCC can also occur in scarred areas, on sebaceous nevi, after long-term immunosuppression, and as part of syndromes and genetic anomalies (i.e., xeroderma pigmentosum, Gorlin–Goltz syndrome, and albinism).

Diagnosis of BCC is highly reliant on the clinical presentation and definitive diagnosis is established by pathohistological verification.

The goal of treatment is radical resection and reconstruction of the affected region for structure, function, and optimal aesthetic result, as a one-stage procedure. Surgery is the treatment of choice, offering significant advantages over other treatment modalities, due to histological control, lowest recurrence rate, and cure rate of up to 95%.

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Ethics: The manuscript has been written in accordance with the ethical standards of the respective institution and the journal.

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Базоцелуларни карцином – принципи лечења

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САЖЕТАК

Базоцелуларни карцином је један од најчешћих малигних тумора у хуманој медицини и најчешћи малигном коже. Највећи број лезија налази се на експонираним деловима коже, на лицу, поглавини и врату. Просечна старост болесника је 60 година с тенденцијом појаве у све млађој животној доби и чешће код мушкараца.

Инциденца је у порасту и удвостручује се на 25 година. Број новооткривених случајева на годишњем нивоу у свету је приближно 1.000.000. Учесталост малигних тумора коже је зависна од утицаја спољних фактора, на првом месту УВ зрачења, а потом и биолошких својстава коже (особе светле пути, тип I и II по Фицпатрику).

Базоцелуларни карцином је спорорастући малигни тумор који настаје из базалног слоја епидермиса, спољног слоја фоликула длаке или себацеалних жлезда. Може бити локално инвазиван и, уколико се занемари, може инфилтрисати околне структуре (мишиће, хрскавицу) и виталне структуре, што може довести до смрти.

Клиничка слика је веома разнолика и зависи од хистолошког подтипа. Превенција представља најважнији и најефикаснији начин лечења. Хируршко лечење ексцизијом с хистолошком потврдом и провером хируршких маргина је стандардна терапија базоцелуларног карцинома. Циљ лечења је онколошки радикално уклањање тумора, реконструкција функције захваћене регије – структуре и оптималан естетски резултат.

Кључне речи: базоцелуларни карцином; лечење; прогностички параметри

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Palliative care – illness, dying, and death as biological-medical and socio-cultural phenomena

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SUMMARY

Introduction In modern global times, the answer to the question of how to live well is palliative care. It is a type of care that is dedicated to creating circumstances in which the process of dying, and death itself, becomes a dignified and acceptable moment. The palliative approach is based on empathy, understanding, on preserving the dignity of the patient, on open two-way communication, as well as on caring for the patient's family.

Objective This article comprises three aims. The first aim refers to introducing our professional milieu to the basic concepts and philosophy of palliative care. The second aim points out the importance of multidisciplinary and comprehensive care (physical, psycho-social, and spiritual) in palliative care in general. The third aim is to critically consider various obstacles and resistance that exists in our environment regarding the organization of palliative care, through the prism of various models of palliative care around the world, particularly in Europe and in the regional countries.

To search the literature, we used the following databases: Web of Science, PubMed, SCIndeks, Google Scholar; by the following keywords: palliative care, neonatal palliative care, nursing, palliative pain, national palliative care program. We presented the analyzed data using a descriptive method.

Conclusion If the right to palliative care is seen as a special human right, it can be concluded that our country lags significantly behind developed countries in this regard. Hence, the preoccupation of the author in this paper is the theoretical foundation of palliative care, with special emphasis on the multidisciplinary team. The purpose of this paper is to point out the connection between palliative care and the phenomenon of the quality of life, as something that is extremely important not only to each individual but to the society as a whole.

Keywords: palliative care; quality of life; social protection; multidisciplinary team

INTRODUCTORY CONSIDERATIONS

In addition to beautiful things, the constants in people's lives are dying, death, grief. The development of medicine in many aspects as well as technological advances have led to the prolongation of human life [1]. However, death still finds its way to people, which is considered a frightening reality and a failure of medicine and doctors. Ever since the fourth century AD, to when the word hospice dates back, in ancient Greece (Latin *hospitium* – home, hospitality), we have been trying to understand the psychosocial and spiritual aspect of the needs of sick and dying members of society [2].

Man of the modern age reacts to the challenge of death with greater fear than man from the past. Thus, for the sake of illustration, the famous philosopher Epicurus tries to bypass the mortal escape of life, defining death as "supposedly the most terrible evil." According to him, "death does not concern us, because as long as we exist, there is no death, and when death comes, then there is no more of us. Death therefore does not concern the living or the dead, because it does not apply to the former" [3].

When curative medicine exhausts all possibilities in the treatment of a patient with active, progressive, and advanced disease, palliative medicine continues with appropriate medical care. Palliative care of patients is a treatment, namely a series of specially adapted treatments that eliminate (as much as possible) the discomfort, symptoms, and stress that severe illness itself brings to the patient, i.e., pain, fatigue, nausea, loss of appetite, depression, insomnia, anxiety, vomiting, stool problems, etc. The emphasis is on interdisciplinary treatment of patients and family members from the moment of learning about the diagnosis of an incurable disease to the period of mourning after the death of an individual. For these reasons, in addition to doctors and nurses, the team is joined by social workers, pharmacists, nutritionists, psychotherapists with specializations in the field of palliative care, theologians, i.e., specially trained spiritual advisors.

The quality of life is increasingly considered the ideal of modern medicine from the aspect of bio-psycho-social point of view [4, 5, 6]. The question arises – what is the quality of life [7, 8]. One of the most comprehensive definitions

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of quality of life is stated by Felce and Perry [9]. They define the quality of life as an overall, general well-being, which includes objective factors and subjective evaluation of physical, material, social and emotional well-being, together with personal development and purposeful activity. Everything is valued through a personal set of moral norms of the environment [8, 9]. Keith [10] and Schalock [11] say that quality of life can be used as a "sensitive term that provides recommendations and guidance," as a "social assembly," as an "organizational concept" or "all together" as: "a systemic framework which can be seen in the work aimed at improving the lives of individuals" [8]. There are connections and differences between the quality of life and similar concepts such as well-being, pleasure, satisfaction, functional status, and health condition [8].

DEFINITIONS OF PALLIATIVE CARE

In Serbia, palliative care is still in its infancy [12, 13]. There are ambiguities about the terminology and the content of the hospice and palliative care [14]. "Hospice care is always palliative, but not every palliative care is hospice care." The terms palliative care and palliative treatment are most commonly used. It is often considered that palliative care is only for the elderly, only for cancer patients, only for the last few days of life.

The definition and meaning of the word "palliative" (from the Latin word *palliativus*) heals only temporarily; solves an issue only seemingly, only for a while, which covers up, mitigates [15, 16, 17]. Palliative treatment is a treatment that seemingly removes only the external signs of the disease, and not the disease itself and its cause [16].

However, there are opinions that the term "palliative" comes from the Latin word *pallium*, which means cloak, mantle, or blanket [2]. This term is also translated as 'mask.' It seems that this is the reason why the word 'palliative' has a negative connotation in our environment, in the sense of concealing the real cause of a negative phenomenon. Such an opinion, which palliative care implies in terms of a procedure that eliminates only the symptoms and not the disease itself and its cause, is logically correct, but it can cause the essentially positive aspects and contributions which the palliative care program contains to be overlooked [18].

In medicine, the term palliative medicine is introduced by Balfor Mount of Montreal, who founded the first palliative care unit at the Royal Victoria Hospital in 1975 [19]. The etymology of the word palliative indicates that such care deals with symptoms that are "wrapped" or "alleviated" by treatment whose main goal is to improve the quality of life of patients under existing circumstances, and never to hide the effects of incurable diseases [20].

The evolution of palliative care can be roughly divided into three phases. In the first, earliest phase of development, the focus was on caring for terminally ill patients by establishing a place to die. The second, middle and key phase of development is related to the development of the modern hospice movement in Great Britain in the second half of the 20th century and Ms. Cicely Saunders. The third, modern phase refers to the present period, which is still developing [21]. The beginnings of modern hospice and palliative care are linked to Dame Cicely Saunders, who in 1967 founded the first modern hospice, St. Christopher's in London. The Hospice Movement, through the promotion of the principle of palliative care, has had a global impact and has led to improved standards of care for people facing a serious or incurable disease [14]. At that time, she opposed the dominance of the medical, conventional model by developing the so-called bio-psycho-social and spiritual holistic model for monitoring the needs, care, and nursing of dying patients. From that period begins the development of the so-called hospice of medicine and philosophy of caring for dying people [22, 23].

In the foreign literature in the field of palliative care, the following terms are distinguished: life-limiting illness, life-threatening illness, and terminal phase of the disease. In free translation, life-limiting illness refers to life-limiting diseases, i.e., all those diseases that lead to inevitably premature death (for example, Dischen's muscular dystrophy). Life-threatening illness includes all those illnesses that lead to premature death but possible long-term survival (for example, children treated for certain malignant diseases in childhood). The terminal phase of the disease describes children or adults who are in the dying phase [2, 24].

The definition of palliative care has been developed over the years as the medicine has evolved [25, 26]. The World Health Organization has most succinctly defined this term as active, overall care for patients whose disease is incurable. The World Health Organization changed the definition on several occasions, so palliative care was a holistic and active approach, and comprehensive procedures applied in caring for the physical, psychological, social and spiritual needs of the seriously ill, as well as providing psychosocial support to the family to achieve the best quality of life for terminally ill patients and their families through pain control and other patient symptoms [24].

The more recent 2002 definition is as follows: "Palliative care is an approach that improves the quality of life of patients and their families by tackling life-threatening problems through prevention and alleviation of suffering through early detection and unmistakable assessment and treatment of pain and other symptoms of illness: physical, psychosocial, and spiritual" [24]. The patient suffers not only physically but also psychologically (fear of the unknown and unpredictable), spiritually (feelings of worthlessness, meaninglessness, and hopelessness) and socially (abandonment). Thus, the notion of quality of life can only be determined by the patient himself [8]. In the field of hospice care, the quality of life is very often explained by the "Kalman gap." In 1984, Dr. Kalman graphically presented the quality of life and guidelines for improving it (Figure 1). According to him, the quality of life is good if the expectations are in line with the current circumstances. The bigger the "gap" between expectations and possibilities, the worse the quality of life. Therefore, if we want to improve the quality of life, we must change our expectations in accordance with the possibilities.

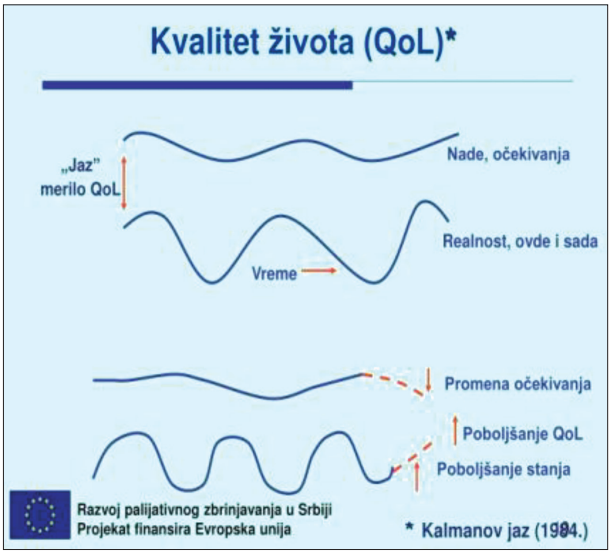

Figure 1. Kalman gap – graphically presented quality of life; source: https://www.slideserve.com/zamir/eti-k-e-dileme-u-palijativnom-zbrinjavanju-dr-john-ely

Palliative care can be seen as active, holistic care for the seriously ill by a multidisciplinary team [27, 28]. Palliative care is there to provide the best quality of life until the moment of death [29]; and that by the following [21, 30]:

- relieving pain and other symptoms of the disease,
- affirming life and all its values, but vieing death as a normal process,
- not accelerating or delaing death,
- integrating psychological and spiritual aspects of patient care,
- offering support to patients so that they can live as actively as possible until the moment of death,
- offering a system of support and assistance to the family,
- using a team approach in identifying the needs of patients and their families.

MULTIDISCIPLINARY APPROACH IN PALLIATIVE CARE

Holistic palliative care is provided by a multidisciplinary team, with the patient and family members being key team members. The composition of the team varies, depending on the stage of program development or the specific needs of the patient at a given time [14]. Multidisciplinary approach implies elaborated team cooperation between experts of different education profiles (doctors of different subspecialties, nurses / pediatric nurses, dieticians, nutritionists, clinical pharmacologists, psychologists, psychotherapists and psychiatrists, social workers, theologians, i.e., specially trained spiritual counselors, educators, educator volunteers), who underwent training for palliative care [31]. Trust, respect, honesty and support are considered to be the characteristics of a good team.

Von Gunten defined the basic skills that all members of a multidisciplinary palliative care team should possess, such as adequate symptom management, empathetic communication with parents, and inter-professional cooperation [31]. Quality palliative care includes the following [14, 32, 33]:

- good communication,
- psychosocial and spiritual support,
- good control of disease symptoms,
- knowledge of ethical principles and ability to make decisions in accordance with those principles and existing circumstances.

PSYCHO-SOCIAL AND SPIRITUAL ASPECTS IN PALLIATIVE CARE

Psycho-social help and support in the field of palliative care refers to the effort to meet the psychological, social, and spiritual needs of patients and their families [34].

The quality of psycho-social assistance provided depends on the way in which assistance is provided and created according to the individual and developmental needs of each child or adolescent separately [2, 35], on the personality traits of the support professional, and on teamwork cooperation and atmosphere that exists within the team dealing with palliative care [36, 37].

There is a growing trend that family members of seriously ill people, especially in the advanced stages of the disease, turn for help to institutions and providers of social protection services [5, 6]. Social work is focused on personal and social changes that have occurred as a result of the disease, and affect the patient, his family, social network, and community. A social worker should help establish stability in the family. Social work is often cited as a profession that helps to guarantee the basic human rights, such as the right to care with choice and preservation of dignity, while respecting different cultures and traditions and insisting on the availability of social life. Social workers are often, among other things, the voice of the poor and marginalized in society [14]. Therefore, quality care of medical patients must be accompanied by social work and the main emphasis is on the need for a significantly larger number of qualified social workers in health care institutions. As research shows, the existing number of social workers does not meet even 10% of the needs. Therefore, there must be an initiative to create a master's program of social work in health care institutions or to include subjects that would provide the necessary knowledge and skills to future social workers in this field through the existing curricula. The goals of social protection are the following [14]:

1. achieving the minimum material security and independence of the individual and the family in meeting the needs of life,
2. ensuring the availability of services and the exercise of rights under social protection,
3. creating equal opportunities for independent living and encouraging social inclusion,
4. preserving and improving family relations, as well as promoting family, gender, and intergenerational solidarity,

5. preventing abuse, neglect or exploitation, eliminating their consequences.

These goals are achieved through the provision of social protection services and other activities that prevent, reduce, or eliminate the dependence of individuals and families on social services. This type of support also includes practical aspects of care such as finances, running a household and helping with daily life.

Spiritual support in palliative care means respecting the uniqueness of each individual and accepting their values [38], beliefs, doubts, dilemmas, concerns, which is provided through the personal relationship and contact between the spiritual counselor and the individual. Such support for the patient facilitates acceptance and preparation for death, assessment of life and completion of the life story, acceptance of one's own illness and inevitable death, facilitates parting, and brings a sense of calm [39]. Useful questions that can help open various topics related to spirituality are contained in the acronym BELIEF (belief, ethics and values, lifestyle, involvement, education, future event [39].

According to research conducted in the USA, a connection has been proven between higher levels of spirituality and improved symptoms in depression and addiction, heart disease, diabetes, immune system disorders, and coping with chronic diseases [40]. Based on the established facts in numerous researches, spiritual support has become an important part of quality palliative care.

In that context, the joint palliative care commissions from various parts of the world and the World Health Organization called for recognizing the patients' needs of spiritual nature, as well as educating medical and other staff involved in recognizing the patients' spiritual needs. In their recommendations, they state that spiritual support should be at the heart of the health composition.

VALUES AND ETHICAL PRINCIPLES OF PALLIATIVE CARE

The ethical principles that guide and respect palliative care in practice are as follows [41]:

- autonomy, i.e., the right and ability of a person to make independent decisions;
- justice, i.e., fair use of available resources, moral and social principles on which respect for justice is based without discrimination or prejudice;
- benefit, i.e., doing good;
- *primum non nocere*, i.e., not to harm.

Solving dilemmas and making the right medical and ethical decisions requires medical knowledge and skills, as well as understanding and accepting basic moral values and ethical principles. As professionals, we are obliged to ask ourselves whether our decisions are in accordance with the patient's will (autonomy) and in their best interest (welfare), and whether we will in any way harm the patient (*primum non nocere*) or society (justice) [41].

Considering that neither a successful cure nor recovery is expected in the seriously ill, the following question arises – what can palliative care offer? Control of pain and other physical symptoms are basic interventions because it is important that the patient feels as comfortable as possible, which means no pain, no unpleasant symptoms, ability to sleep, to ingest fluids and food (depending on the stage of the disease), with maintaining hygiene. Pain as the dominant symptom in many conditions/diseases requires continuous monitoring. The assessment of pain and discomfort in patients has its own specifics, given that the self-assessment of the suffering they experience is often lacking. There are many and varied signs of the patient's suffering: painful grimacing observed on a furrowed and/or bulging forehead, wrinkling of the nasolabial fold, squeezing of the eyes, disturbance of physiological parameters (increase in heart rate, decrease or increase in respiration per minute, altered respiratory amplitude, apnea crises, decrease in hemoglobin oxygen saturation, increase in arterial tension, increased intracranial pressure), altered body position and movement patterns in bed, altered sleep dynamics. Also, biochemical markers that appear in response to painful experience, suffering and stress can be measured: cortisol level, endorphin level, glycemic value, and other parameters [31, 42]. These inadequately manageable symptoms are called refractory symptoms and differ from other difficult-to-treat symptoms in that, contrary to the advice of many experts, they cannot be treated without compromising the patient's consciousness [43]. Palliative/terminal sedation can be defined as the use of sedatives (usually benzodiazepines) with or without complementary opioids given intravenously or subcutaneously to lower the level of consciousness deep enough to alleviate unbearable and persistent symptoms in patients whose life is coming to an end. These symptoms cannot be controlled in any other way. Sedation in this case is not a side effect of symptom control [41, 43].

The Ethical Working Group of the European Palliative Care Association considers palliative sedation to control symptoms justified if used temporarily [41, 44, 45], if the patient is monitored regularly, and, when clinically indicated, for assisted hydration and feeding [46].

Knowledge and understanding of ethical principles and acting in accordance with them not only contributes to improving the quality of life of patients and their families, but also contributes to the satisfaction of professionals during their hard work, stress prevention, and the prevention of occupational burnout syndrome [41].

INSTEAD OF CONCLUSION

The mutual philosophy of palliative care is based on an empathic approach, comprehensive consideration and assessment of needs and conditions with the aim of alleviating suffering and providing multidisciplinary assistance to people with life-threatening diseases, during all phases of illness and treatment. Palliative care seeks to ensure the quality of life of the patient until death, to preserve the dignity of every human being.

It should not be forgotten that one of the main obstacles to achieving quality palliative care is financial. Health

systems in most countries face the problem of how to set priorities given the limited budget allocated to health. What gives hope is that in the future more and more individuals will receive good and quality care at the end of life, given the accelerated growth in the aging population, especially noticeable in Western countries. The very philosophy of palliative care lies in the set of common values that are recognized throughout European countries. One of these values is the dignity of the patient, which in the first place means that palliative care should be performed in a decent, open, and sensitive way, and special attention should be paid to personal, cultural, and religious values of the individual.

No matter how palliative care takes place – within health systems that have developed national palliative care programs or in those where this is not yet the case – it should never be forgotten that the shared time that a family and their sick family member have at their disposal can be very short and limited – when missed, it disappears forever and the loss is irreparable; only painful memories remain.

NOTE

The authors declare that the article was written according to the ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

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Палијативно збрињавање – болест, умирање и смрт као биолошко-медицински и социјално-културолошки феномени

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САЖЕТАК

Увод У савремено глобално време одговор на питање како квалитетно живети даје палијативно збрињавање. Реч је о таквој врсти неге која је посвећена стварању околности у којима процес умирања а и сама смрт постају достојанствен и прихватљив тренутак. Палијативни приступ је утемељен на емпатији, разумевању, очувању достојанства болесника, отвореној двосмерној комуникацији, али и бризи о породици болесника.

Циљ Овај рад обухвата три главна циља. Први циљ се односи на упознавање наше стручне јавности са основним концептима и филозофијом палијативног збрињавања. Други циљ подразумева указивање на важност мултидисциплинарне и свеобухватне бриге (физичке, психо-социјалне и духовне) у палијативном збрињавању уопште, док је трећи циљ био да се кроз призму различитих модела палијативног збрињавања који постоје у земљама света, Европе и региона критички осврнемо на различите препреке и отпоре који постоје у нашој средини у вези са организовањем палијативног збрињавања. За претраживање литературе користили смо следеће базе: *Web of Science*, *PubMed*, *SCIndeks*, *Google Scholar*, према кључним речима: *palliative care*, *neonatal palliative care*, *nursing*, палијативно збрињавање, бол, национални програм за палијативно збрињавање. Анализиране податке приказали смо користећи дескриптивни метод.

Закључак Уколико се на право на палијативно збрињавање гледа као на посебно људско право, може се закључити да наша земља значајно заостаје за развијеним земљама по том питању. Отуда је преокупација аутора у овом раду теоријско утемељење палијативности, са посебним давањем значаја мултидисциплинарном тиму. Сврха овог рада је указати на повезаност палијативног збрињавања са феноменом квалитета живота, као нешто што има изузетан значај не само за сваког појединца већ за целокупно друштво.

Кључне речи: палијативно збрињавање; квалитет живота; социјална заштита; мултидисциплинарни тим

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Historical aspects of diabetes, morbidity and mortality

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SUMMARY
It has been an entire century since the introduction of insulin into clinical practice, which, among other, led to improvements of fertility and pregnancy outcomes of women suffering from gestational diabetes. The prevalence of diabetes worldwide and in Serbia is high and tends to increase as a consequence of modern lifestyle. Nevertheless, modern diagnostic and therapeutic approaches enable people with diabetes to achieve and complete pregnancies without adverse outcomes. Gestational diabetes can be considered as non-communicable disease and efforts should be made to determine its effects on offspring. In the context of COVID-19 pandemic, diabetes mellitus was identified as an important risk factor for severe forms of the disease.
Keywords: diabetes; history; discovery; insulin

INTRODUCTION

Just under 100 years ago, in 1923, Banting and MacLeod received a Nobel Prize for the discovery of insulin and just over 100 years ago, Banting reported the discovery to the American Society of Physiology [1]. This marked the end of the so-called pre-insulin era in diabetes [1]. Although, the terminology of diabetes mellitus is commonly believed to originate from ancient Greece, it was in 1822 in Britain that it was recognized as a separate clinical entity [2]. The word 'diabetes' did find its place in the ancient Greek medical literature in the second century AD, and famous Avicenna gave the description of the increase in appetite and diabetic gangrene at the end of the first millennia AD, and the Aretaeus was thought to be the one introducing the term 'mellitus' [1, 2].

The discovery of the pathophysiological mechanisms in diabetes and insulin itself was a long process, which included numerous anatomists, pathophysiologist, and biologists. The reports on diabetes after the pancreatectomy in dogs fueled the further discoveries on the topic [2]. This work has paved the route for Banting and Best and has enabled the discovery of such an important and widely used treatment that insulin is today [1, 2].

THE SLOW DECREASE IN MORTALITY AFTER THE INTRODUCTION OF INSULIN

Before the discovery of insulin, the diagnosis of diabetes, especially of type 1 diabetes, was a death sentence for the patients, with only vague ideas on the possibilities for treatment and control. The study conducted in the 1960s also showed significantly higher mortality rates among patients with diabetes compared to the general population, decades after the introduction of insulin [3]. Nonetheless, throughout the decades after the introduction of insulin, the mortality declined, but between 1945 and 1967 in which the above-mentioned study was published, the decline in mortality was not observed [3]. The improvement in treatment led to the astonishing decline in mortality from 827 per 1000 before the insulin era to 1 per 1000 in the 1960s [4]. Significant factor that attributed to the decline in mortality from diabetes was the discovery and widespread use of antibiotics, so the infections, as a cause of death among patients with diabetes were reduced [4]. As diabetes became chronic and more controlled illness, most of deaths in the 1960s were associated with cardiovascular and renal illnesses, whose proportion in the total death among patients with diabetes increased from around 25% to more than 75%, mainly due to reduction in deaths from acute causes, like ketoacidosis and infections [4].

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Newer data shows the further decline in diabetes mortality, although its incidence is rising [4]. The introduction of the intensive insulin treatment has significantly contributed to this, although the effect of socioeconomic status, still cannot be ignored [4].

RECOGNITION OF DIABETES IN PREGNANCY

Even before the discovery of insulin, diabetes in pregnancy was described and recognized as a risk factor for the late pregnancy losses, macrosomia and different congenital malformations [5], especially in the late 19th century, including both gestational diabetes and pre-gestational diabetes although their clinical distinction was not well established.

Pre-gestational diabetes was in the pre-insulin era associated with typical symptoms of poor glycemic control: primary or secondary amenorrhea, oligo or hypomenorrhea, hypoplasia of the genital tract, and functional sterility. The fertility of these patients was about 2%, while today it is equal to that of women without diabetes of the same age [6].

Diabetes in pregnancy was described in the 19th century as well, in a 22-year-old Berlin woman who developed symptoms of diabetes during her fifth pregnancy, as she had given birth to unusually large children in the previous two pregnancies. The child was born alive, unusually large, but died shortly after birth, while the mother gradually recovered [7].

At the turn of the century, researchers had already begun describing the diabetes in pregnancy in published literature, and until 1908, there were total of 57 cases described. Diabetes in pregnancy during the late 19th and early 20th century was associated with high maternal and high neonatal mortality, with the half of the mothers and two thirds of newborns dying at childbirth [8].

THE FIRST STEPS IN TREATMENT OF DIABETES IN PREGNANCY

As the discovery of insulin changed the lives of all patients with diabetes, it changed lives of patients with diabetes in pregnancy as well, and in the first decade of the introduction of insulin, the first report of total of 43 successful term pregnancies among women with diabetes was published in 1927 [9]. Nonetheless, in took decades to achieve the adequate and timely diagnosis and introduction of treatment among pregnant women with diabetes to achieve the low mortality that we have today. Maternal mortality is very low nowadays, but in the 1940s, one out of 20 pregnant women with diabetes died during pregnancy and/or childbirth, which decreased to 1–3% in the 1950s. Neonatal mortality was even higher and was around 40% in the 1940s and decreased to 5% in 1980s. Nowadays, women with diabetes are considered conditionally healthy, with normal fertility, with a slightly higher risk of morbidity and mortality compared to healthy pregnant women, but we are still fighting to decrease the perinatal mortality as it remains statistically significantly higher even in the most optimal conditions [10].

DEVELOPMENT OF THE DIAGNOSTIC CRITERIA

The first set of gestational diabetes diagnosis criteria were introduced in 1964, by O'Sullivan and Mahan, with the aim of establishing the risk of diabetes after pregnancy, using the 100g Oral Glucose Tolerance Test [5]. These criteria included the establishment of cut-off for blood glucose levels at the more than two standard deviations above the population mean [5]. These criteria were later changed and improved in 1980 and 1982, first by Metzger and then by Carpenter and Coustan [5]. This was followed by the International Workshop conferences on diabetes in pregnancy and, more recently, The Hyperglycemia and Adverse Pregnancy Outcomes Study, that specifically aimed to shift the established criteria for diagnosis of gestational diabetes mellitus (GDM) from the risk of development of maternal diabetes later in life to assessment of the level of hyperglycemia associated with the adverse pregnancy outcomes, both maternal and neonatal [5].

This was in accordance with the knowledge established even earlier when Karlsson and Kjelmer [11] showed the association between the poor neonatal outcomes and the hyperglycemia in pregnancy with the association between the perinatal outcomes and increase in maternal glycemic values. The pathophysiological basis of macrosomia was also described at the time.

DIABETES IN PREGNANCY TODAY

Along with the increase in the prevalence of diabetes in the general population worldwide, there is the increase in the prevalence of diabetes in pregnancy and data shows that one in every six pregnancies worldwide is affected by maternal hyperglycemia [12]. Today, the research is focused on the most adequate approach to screening, diagnosing and treatment of diabetes in pregnancy, especially lowering the future risks for both mother, and neonates, through direct effects of hyperglycemia, but also through its epigenetic effects.

The possibilities for diagnosis or prediction of maternal diabetes earlier in pregnancy have been examined including the commonly used biomarkers like hemoglobin, hematocrit, fasting blood sugar, and red blood cell count [13], but also newly developed methods, like the possibilities for the use of artificial intelligence algorithms [14]. The possibilities for preventing the development of GDM through introduction of Mediterranean diet and exercise in early pregnancy along with the use of metformin and even probiotics are also examined in order to minimize the possible effects of all levels of maternal hyperglycemia on neonatal outcomes [15].

In the context of current Coronavirus Disease 2019 (COVID-19) pandemic a many questions have been raised

about its potential harm to the mother and fetus. Moreover, some parallels between the increase in prevalence of non-communicable disease after the 1918 Influenza pandemic were made in the context of current COVID-19 pandemic [16]. A large multinational study proved that insulin-dependent GDM was associated with COVID-19, while diabetes and obesity were risk factors for COVID-19 diagnosis in pregnancy [17] Another multicenter study investigated whether GDM represents an independent risk factor for adverse pregnancy outcomes in women with SARS-CoV-2 and confirmed same results [18]. These findings have shed additional light on pathophysiology of COVID-19 in adult population.

CONCLUSION

The prevalence of diabetes is increasing worldwide and in Serbia, as a consequence of poor diet, lack of exercise, and chronic stress. Nevertheless, modern diagnostic and therapeutic approaches enable people with diabetes to achieve and complete pregnancies without adverse outcomes. GDM can be considered as a non-communicable disease and efforts should be made to determine its effects on the offspring. In the context of COVID-19 pandemic, diabetes mellitus was identified as an important risk factor for severe forms of the disease.

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Историјски аспекти дијабетеса, морбидитет и морталитет

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САЖЕТАК

Прошло је тачно 100 година од открића инсулина и његовог увођења у свакодневну клиничку праксу, што је довело до повећања фертилитета жена са дијабетесом, и побољшања исхода трудноћа компликованих дијабетесом. Преваленција дијабетеса у свету и у Србији је висока са тенденцијом пораста, што је последица савременог начина живота. Ипак, захваљујући савременим приступима у дијагностици и терапији, жене са дијабетесом могу да остваре трудноће без нежељених исхода. Гестацијски дијабетес се може посматрати као хронична незаразна болест и потребно је уложити напоре да се одреде његови утицаји на потомство. У контексту пандемије ковида 19, дијабетес мелитус је препознат као значајан фактор који је повезан са тешким формама болести.

Кључне речи: дијабетес; историја; откриће; инсулин

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Metabolism of the mother, placenta, and fetus in diabetes

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SUMMARY
Metabolic changes occur due to the effects of placental hormones such as human chorionic gonadotropin and human placental lactogen in normal pregnancies. These effects enable the development of insulin resistance among all pregnant women, significantly pronounced in the third trimester. In pregnancies complicated by pre-gestational or gestational diabetes mellitus, these changes are more intensive as they affect the fetoplacental unit. In pregnancies complicated by diabetes the increased number of placental macrophages leads to the increased production of different cytokines which include leptin, tumor necrosis factor alpha, and interleukins. This review addresses placental vascular changes that lead to adverse pregnancy outcomes, along with the effects of the maternal hyperglycemia and fetal hyperinsulinemia.
Keywords: pregnancy; pre-gestational diabetes; gestational diabetes; insulin resistance; placental structural abnormalities

INTRODUCTION

Physiological pregnancy is associated with numerous physiological changes, among which there are changes in metabolism, in biochemical parameters, immunological and hematological systems [1]. Among the metabolic changes, the insulin resistance is pronounced, resulting in changes in the glucose utilization [1]. Insulin secretion increases in healthy pregnant women and, as the pregnancy progresses, its values are gradually elevated before meals [2]. Insulin efficiency decreases by 50–70% in the third trimester, which is evidence of increased insulin resistance in healthy pregnant women. Insulin resistance during pregnancy increases in parallel with the growth of the fetoplacental unit and the level of placental hormones (human placental lactogen, progesterone, cortisol, etc.). This adaptation helps the utilization of carbohydrates by the growing foetus and stimulates the use of fats for energy for the healthy pregnant woman [1].

HORMONES AND INSULIN RESISTANCE AND INSULIN SENSITIVITY

The hormones contributing to the increase in insulin resistance during pregnancy are estrogen, progesterone, human placental lactogen [1]. Some other factors, such as cortisol, tumor necrosis factor alpha (TNF-α), and interleukins can interfere with the insulin pathway and be associated with insulin resistance during pregnancy [1]. However, these adaptations are exhausted in pregnancies with diabetes and insulin resistance in diabetes is associated with the changes in the fetal and placental development.

It is worth noting that certain hormones improve insulin sensitivity, including human chorionic gonadotropin (HCG), produced by the syncytiotrophoblast. Its effects are based on slowing down the enzymatic breakdown of insulin in the placenta or increasing peripheral sensitivity to insulin, both among healthy pregnant women and women with diabetes, so much so that the need for insulin is lowered in the first trimester among women with pregestational diabetes [3, 4]. Higher HCG levels in early pregnancy are associated with lower likelihood for development of gestational diabetes later in pregnancy [3].

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BLOOD GLUCOSE AND PLACENTA

Changes in the blood glucose levels in pregnancy with diabetes are associated with changes in the structure and function of the placenta. These changes include the increase in surface area of the villi, thickening of the trophoblastic basement membrane, and the changes in the collagen structure of the basement membrane, with collagen IV predominance [5]. In contrast, the basement membrane of the endothelium cells is thinner in pregnancies with diabetes [5].

Both metabolic and endocrine functions of the physiological placenta are interrupted in the pregnancy with diabetes as the increased number of placental macrophages leads to the increased production of different cytokines, which include leptin, TNF-α, and interleukins [5]. Additionally, the changes in the placental capillary network, such as proliferation of the capillary network, are also described [5].

Along with the increase in fetal weight, there is an increase in the placental weight in the pregnancies with diabetes and the increase in the placental-to-fetal weight ratio, meaning that the weight increase in more pronounced in the placenta than in fetus [6]. The placental growth can be associated with fetal insulinemia as well, as it was observed among women with diabetes and good glycemic control as well [7, 8].

The thickening of the trophoblast basement membrane seems to have more significant effect on the diffusion of antipyrine and L-glucose compared to the effect of the thinning of the endothelial basement membrane and their diffusion is decreased in pregnancies with diabetes [7–10]. This further leads to fetal hypoxia that stimulates the angiogenesis. Angiogenesis is stimulated by hypoxia through the stimulation of the secretion of factors like fibroblast growth factor type 2, vascular endothelial growth factor, and placenta growth factor [7–10].

The diabetes in pregnancy is a well known factor associated with a decreases in placental blood flow, including both uteroplacental and cord blood flow. Different mechanisms have been described to cause these changes; however, most described are changes in secretion of thromboxane and prostacyclin, and the increase in their vasoconstrictor activity. Acute atherosis of uteroplacental blood vessels can lead to obstruction and thus to impaired blood flow through the intervillous space, mainly due to the increase in the parenchymal tissue of the villi and a reduction in the volume of the intervillous space. Some other factors can also contribute, such as the changes in the production of nitric oxide [7, 8, 11].

GLUT 1 and GLUT 3 glucose transporters mediate the glucose transport through the placenta, and they are in the syncytiotrophoblast and endothelium. Among women with diabetes, studies have found a decrease in the number of GLUT 1 transporters, which is considered an evolutionary adaptation with the aim of protecting the fetus from the excessive maternal glucose. *In vitro* studies have shown the reduction of the level of GLUT 1 ribonucleic acid in placenta due to hyperglycemia, which could indicate that in pregnant women with diabetes there is reduced regulation and control over the expression and activity of glucose transporters (GLUT 1) [12]. These effects seem to be the more permanent and persist despite the strict glycemic regulation after the diagnosis of gestational diabetes has been established [12].

This is why it is very important that in the modern approach to monitoring pregnancy with diabetes, the mother's glycemia is regulated as accurately as possible and control of the development of the fetus is established early and continuously. Glycosylated hemoglobin values need to be normalized before conception, as once the arrangement and the quantity of glucose transporters is created, no clinical monitoring or intervention methods available can change it [13, 14].

Additionally, placenta in women with diabetes has the increased glycogen levels, which is not associated with either glucose nor insulin in the trophoblast, which indicates that the synthesis of glycogen is being conducted in cells other than trophoblast. The histochemical analyses showed that glycogen is located predominantly around the fetoplacental blood vessels, especially in type 1 diabetes. Since the endothelium is rich in glucose transporters, primarily the high-affinity glucose transporter GLUT3, endothelial cells have a molecular mechanism by which they withdraw glucose from the fetal circulation and store it in the form of glycogen. Regarding the return flow of glucose from the fetal circulation to the placenta, it can be assumed that the placenta could have a buffer function for excess glucose, and thus the ability to protect the fetus from glucose overload. This phenomenon is not fully understood, but it may explain why sometimes in circumstances of moderate maternal hyperglycemia the fetus is of normal weight, while in others the fetus is macrosomic [15, 16].

In pregnancies with diabetes, the third-trimester fetal hyperinsulinemia is common even among the eutrophic fetuses, as it was shown that there is a significantly higher difference in the blood glucose concentration between the umbilical vein and umbilical artery, suggesting the significant glucose utilization by the fetus [17]. Importantly, fetal hyperinsulinemia is associated with the higher likelihood for the intrauterine fetal death, due to impaired metabolism and hypoxia and consequential hyperplasia of the beta cells in the fetal pancreas. The beta cells hyperplasia is associated with the well-described neonatal complication among infants of diabetic mothers – hypoglycemia. However, it can also be associated with the metabolic acidosis in the full-term neonates, and the risks of prolongation of pregnancy after the 38th week of gestation. Although some authors suggest individual approach to each patient with diabetes in pregnancy in terms of planning and timing of delivery, based on individual maternal and fetal data [18, 19], the diabetes in pregnancy is still one of the leading causes associated with the late pregnancy loss and stillbirth [20].

CONCLUSION

Placental hormones such as HCG and human placental lactogen cause complex changes in the metabolism of carbohydrates during normal pregnancies. Fetoplacental unit is mostly affected by those changes in diabetic pregnancies. The mechanism behind them lays in placental vascular modifications directed by different cytokines such as TNF-α and leptin, as well as up- and down-regulation of various GLUT transporters. Maternal hyperglycemia and fetal hyperinsulinemia lead to adverse pregnancy outcomes.

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Метаболизам мајке, плаценте и фетуса код дијабетеса

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САЖЕТАК

Метаболичке промене настају услед деловања плацентних хормона као што су хумани хорионски гонадотропин и хумани плацентни лактоген у физиолошким трудноћама. Дејство ових хормона омогућава развој инсулинске резистенције код свих трудница, која је најизраженија током трећег триместра трудноће. У трудноћама компликованим прегестацијским или гестацијским дијабетесом мелитусом ове промене су интензивније и утичу на фетоплацентарну јединицу. Повећан број макрофага у плаценти доводи до повећане продукције цитокина који укључују лептин, фактор некрозе тумора алфа и интерлеукини. Овај прегледни рад се бави васкуларним променама плаценте које доводе до неповољних исхода трудноће, као и утицајем који имају хипергликемија мајке и хиперинсулинемија фетуса у дијабетесу мелитусу.

Кључне речи: трудноћа; прегестацијски дијабетес мелитус; гестацијски дијабетес мелитус; инсулинска резистенција; структурне абнормалности плаценте

UDC: 61:061.2(491.11)

The 1st Forum of the Academy of Medical Sciences of the Serbian Medical Society

The Academy of Medical Sciences of the Serbian Medical Society (Academy) gathers our most famous scientists and experts from various fields of medicine and dentistry. During the 46 years of its activity, the Academy has held over 850 scientific and educational meetings, a large number of lecture cycles for the general population at the Ilija M. Kolarac Endowment, it has published dozens of monographs and thus contributed to the promotion of the scientific and professional work of distinguished members of the Academy, as well as to the education of doctors and the popularization of science.

On March 24, 2023, the Academy will organize the *1st Forum of the Academy of Medical Sciences of the Serbian Medical Society*. It will be a scientific meeting where the research results of the members of the Academy and their coworkers will be presented and discussed with the aim of promoting their scientific work and realizing their better cooperation in subsequent studies. The topic of the *1st Forum of the Academy* is "Prevention, Diagnosis, Treatment and Control of Mass Infectious and Non-Infectious Diseases." It has been our opinion that such a broad topic would attract experts from various fields and thus illustrate the wide-ranging field of scientific interest and work of the Academy members.

Twenty-four papers have been submitted for the *1st Forum of the Academy*. The greatest attention is paid to COVID-19. The results and experiences of the clinical presentation, course, treatment, and outcome of the disease are presented, as well as the results of experimental research and presentations of various post-COVID disorders. These results and experiences are valuable guideposts for planning measures in future epidemics that infectologists and epidemiologists have been warning us about.

In addition to COVID-19, lectures will also be delivered on some important infectious diseases that are not given enough attention, and which can seriously threaten health and life. These are, above all, tuberculosis and fungal diseases, which will be discussed by our well-known experts.

The meeting dedicated to mass diseases could not but include lectures on malignant diseases. Significant results of the study of modern drugs will be presented, as well as research of insufficiently studied tumors that require additional education of doctors, especially concerning the diagnosis of these diseases.

At the Forum, there will be an opportunity to find out the results of clinical and experimental studies on cardiovascular diseases, such as pharmacogenetic studies and analyses of the results of modern methods of cardiovascular diseases treatment.

Studies from the field of dentistry confirm that modern research requires a multidisciplinary and interdisciplinary approach, which has made it possible to achieve exceptional progress in the treatment of oral and dental diseases.

In addition to the presentation from the aforementioned fields, results from ophthalmology, anesthesiology, and otorhinolaryngology will also be presented. The wide range of topics and the quality of submitted abstracts confirm that Academy members contribute significantly to professional and scientific work and the continuous progress of all fields of medicine.

We hope that at the *1st Forum of the Academy*, doctors from different specialties, as well as experts from other related fields, will gather and that a lively discussion will contribute to the quality of the meeting. We wish to all the participants that the Forum fulfills the expectations of us all – the organizers, the authors of the presented studies, and the audience – and that it will be an incentive for the Academy Forum to become a regular annual scientific meeting of the Academy.

Prof. Ljubica Đukanović, MD, PhD
President of the Academy of Medical Sciences of SMS
President of Scientific Board of the 1st Forum of AMS SMS

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ABSTRACTS

THE 1ST FORUM OF THE ACADEMY OF MEDICAL SCIENCES OF THE SERBIAN MEDICAL SOCIETY

March 24, 2023
Serbian Medical Society, Belgrade, Serbia*,#

Update on diagnostic criteria for fungal rhinosinusitis and new methods for detection of fungi in sinuses

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Introduction The persistent fungal exposure and presence of chronic rhinosinusitis (CRS) area certain threat for the development of fungal rhinosinusitis (FRS). The aim of this study was to consider the potential diagnostic criteria for FRS, and to evaluate the newly formed methods for detection of fungi in upper parts of respiratory tract.
Methods The study is the combination of clinical experiment and a case-series of 218 patients with CRS. We designed new methodological algorithms for detection of fungi in the sinuses: (i) induction of the sinonasal secretion (ISNS) with lavage and aspiration of sinonasal secretions, (ii) processing of nasal polyps (NP) to single cell suspension and (iii) interpretation of the findings of fungi.
Results The next prevalence has been found in the group of CRS patients: asthma 130/218 (59.6%), NP 101/218 (46.3%), FRS 50/218 (22.9%), out of whom 24/218 (10.9%) are allergic-FRS (AFRS) and 26/218 (12%) are non-allergic FRS. The results obtained with new methods were evaluated by comparison of 10 predictive criteria for FRS (FRS index) and the mycological findings. The highest specificity and sensitivity are shown in ISNS_comb method (lavage+aspiration) with nasal pre-treatment (89%; 96%), highest positive and negative predictive value (PPV/NPV) are shown for ISNS_comb method (94%;93%) and ISNS_lavage method (93%;87%) with pre-treatment.
Conclusion The proposed sampling protocol facilitates extraction of fungi from muco-purulent secretion in patients with FRS and improves fungal detection and isolation rate. Lavage with hypertonic-NaCl should be included in the everyday hygiene routine in an effort to decrease fungal load and antigenic exposure.
Keywords: fungal rhinosinusitis; chronic rhinosinusitis; nasal polyps; fungi
Conflict of interest: None declared.

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Clinical presentation, therapy and outcome of COVID-19 patients treated in the temporary COVID hospital "Karaburma"

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Introduction/Objective The WHO declared the COVID-19 pandemic on March 11, 2020. The temporary COVID hospital "Karaburma" started operating 19 days later and a total of 4083 COVID patients were treated there until February 22, 2022. The aim of this study was to analyze the first 500 patients and compare the results obtained with the same parameters in all patients treated in COVID hospital „Karaburma".
Methods This study included 4083 COVID patients (65% men, 35% women) average age 59.5 years. Demographic data, clinical picture, treatment and outcome of all patients were analyzed.
Results Out of the first 500 patients, 226 (45.2%) had bilateral pneumonia, 23 (4.6%) ARDS, of which 43 (8.6%) were treated in the ICU, compared to 612 (15.0%) of 4083 included in the study. Comorbidities (heart disease, diabetes, etc.) were registered in 235 (47%) of 500 patients. Out of the first 500 patients, 350 (65%) received antibiotics, 214 (42.8%) hydroxychloroquine, 72 (14.4%) oxygen, 150 (30%) anticoagulant therapy, and 64 (12.8%) corticosteroids. Among them, 18 (3.6%) received non-invasive and 21(4.2%) mechanical ventilation. After the first pandemic wave, all patients received anticoagulant therapy, and dexazone only patients who received oxygen. Out of 4083 patients, 144 (3.52%) received Tocilizumab, and 86 (2.1%) Baricitinib. In the first pandemic wave, 32 (6.4%) of 500 patients died, while mortality in the entire group was 363 (8.9%) patients, of which 262 (72.2%) were men.
Conclusion The severity and outcome of COVID-19 depend on the pandemic wave and the patient's comorbidities. A multidisciplinary approach plays a very important role.
Keywords: COVID-19; treatment; outcome
Conflict of interest: None declared.

Invasive fungal infections – analysis of non-neutropenic patients with invasive aspergillosis treated at the Military Medical Academy in the period from 2008 to 2022

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Introduction/Objective Invasive fungal infections (IFIs) are a significant cause of morbidity and mortality in patients in intensive care units (ICUs) and after solid organ or hematopoietic stem-cell transplantation (HSCT). Diagnosis of IFIs before death is successfully established in only 12% of patients. The number of patients with invasive aspergillosis (IA) is increasing, especially in ICU, and it is the leading cause of death among IFIs. The aim of this report is to present the clinical characteristics, therapy applied and comorbid conditions in non-neutropenic patients with IA.
Methods A total of 57 non-neutropenic patients with IA, of average age 56 years (26 women, 31 men) were treated in the clinic in the period from 2008 to 2022. The diagnosis of IA was established on the basis of radiological procedures, biological material, serology tests and histopathological findings.
Results Pulmonary IA was found in 46 (80.7%), rhino-orbital cerebral IA in 4 (7.0%), IA of the paranasal sinuses in 5 (8.7%) and IA of the skin in 2 (3.51%) patients. In these patients various comorbidities were found (tumors, bronchiectasis, IgA immunodeficiency, corticosteroid therapy, diabetes mellitus and others). Treatment was carried out with itraconazole, voriconazole or echinocandins, and 31 (54.4%) patients underwent surgical procedures. By the beginning of 2023, 18 (31.58%) patients had died.

Conclusion Diagnosis and therapy of IA in non-neutropenic patients must be accompanied by malignancy and/or immunodeficiency tests. It is possible to increase the survival rates of these patients with regular clinical, microbiological and morphological monitoring.
Keywords: invasive aspergillosis; diagnosis; treatment; risk factors
Conflict of interest: None declared.

Prophylaxis and treatment for viral infections in 355 patients with hematopoietic stem cell transplants treated at the Military Medical Academy

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Introduction/Objective Viral infections are a significant cause of morbidity and mortality in patients with allogeneic hematopoietic stem cell transplants (allo-HSCT).Underlying disease, conditioning regimens, source of stem cells, and immunosuppressive therapy after allo-HSCT favor different infections in the post- transplant period. The aim of this work is to present the frequency and type of viral infections in patients with allo-HSCT at the Military Medical Academy and the modalities of treatment.
Methods The study included 355 patients (144 women, 211 men) of mean age 29 years, who underwent allo-HSCT at the HSCT Center of the Hematology Clinic of the Military Medical Academy between 1995 and 2017. Allo-HSCT was performed in 130 patients with acute myeloid leukemia, 102 with acute lymphoblastic leukemia, 22 with multiple myeloma and 101 patients with other malignant hemopathies. The type of donor, source of hematopoietic stem cells, type of conditioning regimen and GvHD prophylaxis were analyzed.
Results Reactivation of VZV infection was registered in 21 (6.0%) patients, hemorrhagic cystitis associated with BK polyoma virus (BKPyV) in the early phase in 21 (6.0%), in the late phase in 15 (4.2%), influenza in 2 (0.5%) patients, CMV reactivation in 63 (17.3%), reactivation of HBV infection in 6 (1.69%) and HCV infection in 2 (0.56%) patients. Antiviral drugs were used for prophylaxis, pre-emptive and targeted therapy.
Conclusion Viral infections are a significant cause of morbidity and mortality in patients after allo-HSCT. This requires monitoring and the application of prophylactic, pre-emptive or direct therapy.
Keywords: viral infections; allo-HSCT
Conflict of interest: None declared.

Antioxidant and free radical species in the aqueous humor of patients with age-related cataract

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Introduction/Objective Age-related cataract is a significant cause of visual impairment worldwide. Oxidative damage and the effects of free radical species are considered important in the etiopathogenesis of cataracts. The aim of this study was to evaluate antioxidative capacity and oxidative stress in the aqueous humor of patients with age-related cataracts of different maturity.
Methods The clinical and biochemical investigation involved 55 patients with age-related cataract. According to cataract maturity, the patients were classified into incipient (cortical C,18 pts, nuclear N, 20 pts) and mature (M17 pts) groups. The antioxidant activity of aqueous humor was measured by the reduction power (RP) method and the activity of glutathione peroxidase (Gpx) spectrophotometrically. Changes in the concentrations of hydroxyl and ascorbyl radicals were detected by electron spin resonance spectroscopy.
Results Both RP and GPx activity were significantly ($p < 0.001$) reduced in group N compared to group C and in group M compared to group N. Concentrations of hydroxyl (29.45 ± 1.01% in group C, 38.12 ± 1.29% in group N and 74.14 ± 2.52% in group M) and ascorbyl radicals (26.12 ± 0.89% in group C, 41.15 ± 1.39% in group N and 83.56 ± 2.84% in group M) increased significantly ($p < 0.001$) with progression of age-related cataract. Significant negative correlation ($r = -0.759$, $p < 0.001$) was detected between the concentrations of hydroxyl radicals and GPx activity.
Conclusion Our data support the hypothesis that antioxidant capacity decreases with production of reactive hydroxyl radicals that are involved in the aetiology of age-related cataract.
Keywords: cataract; antioxidant enzyme; hydroxyl radical; ascorbyl radical
Conflict of interest: None declared.

Experiences Acquired During the COVID-19 Pandemic for the Future Organization of Work in Medical Education and Scientific Research

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Introduction/Objective With the beginning of the pandemic and the announcement of the state of emergency, the healthcare system faced two unfamiliar issues. One was the treatment of the infected people and the timely receipt of protocols for assessment, triage, testing and therapy, and the other one was the protection of healthcare professionals. The aim of this study is to present experiences acquired during the COVID-19 pandemic and emphasize the importance of continuous medical education and multidisciplinary approach for work organization of healthcare professionals.
Methods The authors analyze the identified problem comprehensively, from a comparative law perspective, analyzing the legislation of European states and national laws.
Results In the first part of the study, we analyze the inclusion of certain medications in the treatment of patients with COVID-19 in EU countries and Serbia, while in the second part we analyze whether the right to safe and healthy working conditions was denied to healthcare professionals in Serbia during the pandemic. Since both of considered issues cause serious disputes between doctors and lawyers, it was necessary to prepare special strategies and protocols for the triage of the most vulnerable patients in case of a new pandemic, and to dedicate special attention to the organization of the work of healthcare professionals. We have determined that the human rights of healthcare workers have been violated and identified in which spheres of human rights violations have occurred in particular.
Conclusion Continuing medical education, respecting the rights of healthcare professionals and ensuring working conditions are necessary elements for a successful fight against health-related crises.
Keywords: COVID-19; healthcare workers; treatment protocols; the right to safe and healthy work
Conflict of interest: None declared.

Intratemporal facial nerve paralysis: morphologic basis, clinical and microsurgical implications

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Introduction/Objective Facial nerve palsies are a common and significant presentation, specifically to ear surgeons, but also in general medical practice. They result from facial nerve dysfunction due to different etiologic factors (trauma, infections, tumors). Inflammatory processes of the middle ear may involve the facial nerve at any point, especially where exposed. The aim of this study is to present our experience with facial nerve paralysis of otitic origin from the anatomical, histopathological and surgical aspects.
Methods Anatomical studies were performed on a large collection of temporal bones (2000) to evaluate variations of the facial canal (course, dehiscence, protrusion). The histopathological analysis was done on temporal bones with chronic otitis media to investigate a pathological process involving the facial canal and nerve with and without clinical facial impairment. The clinical studies included 64 patients treated for facial paralysis due to chronic otitis media.
Results The most significant anatomical finding in the fallopian canal was the high incidence of dehiscence (defect >0.4 mm) in the tympanic segment close to the oval window (60%). In temporal bones with chronic otitis media, the affected facial nerve showed degenerative changes (demyelinization, hypertrophy, proliferation of Schwann cells) and an area with small dark globules resulting from cellular and myelin degeneration. The pathological process was commonly localized in the destroyed tympanic part of the facial canal and the exposed nerve and correlated with clinical and surgical findings.
Conclusion Otitis media may be associated with degenerative changes in the facial nerve without clinical impairment of its function, but may have the potential for development of facial paralysis.
Keywords: facial nerve paralysis; clinical implication
Conflict of interest: None declared.

The first presenting feature of long COVID 19 infection and acute inflammatory demyelinating polyneuropathy

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Introduction/Objective Certain severe neurological illnesses associated with long COVID-19 include immune neuropathies like Guillain-Barré syndrome (GBS) and exacerbation of pre-existing chronic inflammatory demyelinating polyneuropathy (CIDP). The aim of this study was to determine the prevalence of pain in patients with acute inflammatory demyelinating polyneuropathy (AIDP) and to analyze sociodemographic, clinical predictors for the occurrence of pain, clinical phenotype and course of the pain.
Methods A total of 124 patients with recently diagnosed long COVID-19 infection presented at the Pain Clinic, UCC of Vojvodina, Novi Sad. The research was conducted with the consent of the Ethics Committee of the Faculty of Medicine, University of Novi Sad. Data were collected monthly for one year.
Results The patients had pain, bilateral lower extremity weakness, mute reflexes and sensory loss. Pain was present in 62 patients, 3 months after the onset of symptoms, but only five patients had neuropathic pain. More pronounced deficits, age, female gender, the presence of protein in cerebrospinal fluid, occurrence of sensory symptoms and dysautonomia were recorded as predictors for maintaining pain. When comparing types of pain, non-neuropathic pain was more frequent but less intense and had fewer consequences on the mental health of the sufferer. Musculoskeletal pain persisted for up to 2 years in as many as 1/3 of the patients.
Conclusion Neuropathic pain in AIDP was experienced by 3.72% of the total number of patients; 50% of all patients mentioned pain as a symptom. After 3 months, neuropathic pain was recorded in less than 10% of the total number of patients.
Keywords: COVID-19; inflammatory demyelinating polyneuropathy; acute; chronic
Conflict of interest: None declared.

Gender difference in the perception of acute postoperative pain in laparoscopic surgery and application of multimodal analgesia

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Introduction/Objective Multimodal analgesia consists of nonsteroidal anti-inflammatory drug application 1 hour before surgery, followed by nerve block, skin infiltration with a local anesthetic, use of opioids perioperatively, infusion of the local anesthetic into the peritoneal cavity, and regular fluid and electrolyte therapy. This study was aimed at examining gender differences in the perception of acute postoperative pain after laparoscopic surgery.
Methods This prospective, randomized study included 220 patients who underwent laparoscopic surgery between January 2021 and January 2022 at the Clinic for abdominal, endocrine and transplantation surgery (UCC of Vojvodina, Novi Sad). The local anesthestic levobupivacaine (0.25%) was administered in the region of the cholecyst, on the right side of the diaphragm, intraperitoneally and in incisional wounds. The patients were included voluntarily in the study, with written consent. The investigation had the consent of the Ethics Committee of the Faculty of Medicine, University of Novi Sad and the Department of Surgery.
Results There were 70 male (31.8%) and 150 female (68.2%) patients in separate groups. A gender difference in pain perception was noted early in the postoperative period and was statistically significant in the first hour ($t = 1.9$; $p < 0.05$) and second hour ($t = 2.05$; $p < 0.05$) postoperatively. Female patients reported greater pain intensity compared to male patients, and they required a higher dose of opioid analgesics postoperatively.
Conclusion Intraperitoneal application of the local anesthetic, 0.25% levobupivacaine, during laparoscopic cholecystectomy significantly lowers the intensity and duration of acute postoperative pain. Female patients require a higher dose of this anesthetic due to differences in hormonal status between males and females.
Keywords: laparoscopic cholecystectomy; multimodal analgesia; pain
Conflict of interest: None declared.

Physical and biological properties of TiN doped with Ag and Cu by combined methods of cathodic arc evaporation and DC magnetron sputtering

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Introduction/Objective Recently, one of the most important complex issues in medicine is the deposition of protective nanocoatings on the surface of medical implants using various plasma procedures. By combining cathodic arc evaporation and magnetron sputtering, a hard TiNx nanocoating can be obtained. This functions by protecting the human body from penetration by any released heavy metal ions present in the composition of different types of implants. In addition, in order to improve the antimicrobial properties of medical implants, an antimicrobial nanocoating is usually applied to their surface, those containing Cu and Ag ions.
Methods The methods used for characterization of these coatings include: X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), ellipsometry, inductively coupled plasma (ICP) and a procedure for determination of the wetting angle together with antimicrobial tests.
Results TiNx thin films of thickness 29 to 176 nm, were obtained by a combination of cathodic arc deposition and DC magnetron sputtering. The phase composition of the films was determined by a combination of XRD, XPS, FTIR, ellipsometry. Thickness and morphology of the coatings were determined using a combination of ellipsometry and SEM methods. The wetting angles showed that a TiNx nanocoating covered with Cu is superhydrophobic, while that covered with Ag is superhydrophilic. Therefore, rates of release of Cu and Ag ions differ greatly, which leads to differences in their antimicrobial properties.

Conclusion TiNx nanocoatings in combination with Cu or Ag nanocoatings possess very important barrier and antimicrobial properties, which are extremely relevant in the development of new medical implants in dentistry and orthopedics.
Keywords: cathodic arc evaporation; DC magnetron sputtering; nanocoatings; ellipsometry; XRD
Conflict of interest: None declared.

Out-of-hospital cardiac arrest before and after the COVID-19 pandemic – a comparison

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Introduction/Objective Pandemic coronavirus disease 2019 (COVID-19) caused much disruption in the functioning of health care systems throughout the world. As a consequence, significant deterioration of health of the population was observed. The aim of this study was
to determine if the COVID-19 pandemic affected management of cardiac arrest (CA) and the survival rate of patients with out-of-hospital CA (OHCA) in this area.
Methods An observational before-and-after study was carried out to determine the effects of COVID-19 pandemic on the survival of patients with OHCA, who were given cardiopulmonary resuscitation (CPR) by the emergency medical services (EMS) teams. The study was conducted between 1 March 2018 and 1 March 2022, with two equal observation periods: prior to the outbreak of the pandemic (Group I) and after it (Group II).
Results A total of 958 patients formed Group I (434 pts; 45.30%) and Group II (524 pts; 54.64%) ($p < 0.05$). No significant difference was found for age, sex, time of arrival of the EMS teams, initial rhythm and adrenaline administration between them. However, patients in Group I were more often intubated ($\chi^2=8.737$; df=3; p=0.033). Moreover, amiodarone ($\chi^2=6.508$; df=1; p=0.011) and saline solution ($\chi^2=5.510$; df=1; p=0.019) were administered to relatively more patients in this group. Return of spontaneous circulation (ROSC) and prehospital survival rates were significantly higher in Group I (18.4%) than in Group II (12.6%)($\chi^2=5.685$; df=1; p=0.017).
Conclusion The COVID-19 pandemic led to an increase of OHCA. ROSC and prehospital survival rates were higher in the prepandemic period. The management of OHCA by EMS teams may have affected the results.
Keywords: coronavirus disease 2019; cardiopulmonary resuscitation; sudden cardiac arrest; survival
Conflict of interest: None declared.

The first recognized human case of multilocular echinococcosis in Serbia

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Introduction *Echinococcus multilocularis*, causes multilocular or alveolar echinococcosis, which differs from infection caused by *Echinococcus granulosus* in clinical presentation in humans. The most common definitive hosts for *E. multilocularis* are foxes and jackals, while domestic mammals like dogs and cats are rare. Humans are rare and accidental intermediate hosts. Cystic echinococcosis in humans is endemic in Serbia, while more severe alveolar echinococcosis has not yet been recorded.
Case Outline We present a case of a 67-year-old female from a small village in Sremska Mitrovica municipality. The offset of symptoms began a few years ago. The main one was liver pain which progressed over time. Differential diagnoses included benign liver tumors like haemangioma, cystic echinococcosis and abscess formed in the cystic echinococcal lesion. Left lateral hepatectomy was performed, and S II /III liver segments were removed. Pathological examination clearly showed multilocular echinococcosis with numerous small and empty vesicle spaces with chitin membrane without protoscolices, surrounded by massive fibrosis and infiltrative type of growing into the liver parenchyma. Surgical margins were found positive for echinococcal vesicles showing that echinococcal tissue was not completely removed. Thus albendazole therapy was recommended. Epidemiological interview revealed that the patient lives in an endemic region of multilocular echinococcosis, in a house with two hunting dogs, and back yard where contamination of soil with fox faeces is possible.
Conclusion This is the first recorded human case of multilocular echinococcosis in Serbia. Therefore, we must improve prophylactic and diagnostic procedures and surgical techniques to cure this zoonotic disease.
Keywords: *Echinococcus multilocularis*; human case; Serbia; Srem region;, Mačva region; Vojvodina Province
Conflict of interest: None declared.

Activity of essential oils against the most important endodontic pathogen – *Enterococcus faecalis*

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Introduction/Objective In the resistance-growing era, the necessity to modulate oral biofilm with nature-based products including essential oils (EOs) is recognized. The aim of this systematic review is to summarize the antibacterial activity of numerous EOs against the main infected root canal pathogen – *Enterococcus faecalis*.
Methods Minimal inhibitory concentrations (MIC) of 56 EOs were determined in the microdilution assay. The data was processed in Microsoft Excel and analyzed in IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).
Results Among tested EOs, 20 achieved ultrahigh antibacterial effect (MIC < 0.5 mg/mL), nine achieved high effect (MIC ≥ 0.5 mg/mL), nine achieved moderate effect (MIC ≥ 1 mg/mL), while 18 had the weakest potential (MIC ≥ 2 mg/mL). A high abundance of oxygenated monoterpenes was observed in EOs with stronger antibacterial effects (80%, 66.7%, and 77.8% of total content within EOs with ultrahigh, high, and moderate potential, respectively). EOs possessing weak potential had a lower share of oxygenated monoterpenes (44.4%), but the share of monoterpene hydrocarbons (27.8%) was notable. The most dominant constituents in the EO group with ultrahigh effect were 1.8-cineole (4/20), thymol (3/12), and geraniol (2/20), while in the weakest effect oils the most abundant were α-pinene (3/18), 1.8-cineole (2/18), and β-pinene (2/18).

Conclusion Although interactions among constituents should not be underestimated, EOs rich in oxygenate monoterpenes and especially thymol (3/12) and geraniol (2/20) seem to have promising antibacterial potential. Furthers studies are required to estimate to possibility of their implementation in dentistry.
Keywords: essential oils; *Enterococcus faecalis*; minimal inhibitory concentration; oral bacteria; oral biofilm
Conflict of interest: None declared.

Clinical presentation, diagnosis and outcome of tuberculous meningitis: our experience in the treatment of 31 patients

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Introduction/Objective Tuberculous meningitis (TM) is characterized by a course leading to the emergence of permanent sequelae or death, especially in cases where specific therapy was not started early. The goal of this study was to evaluate the clinical presentation, therapy and outcome of patients with TM treated at our Clinic over a 27-year period.
Methods This retrospective study included 31 patients with TM of average age 36.3±17.2 years. Diagnosis was made on the basis of an appropriate clinical presentation, cytological and microbiological findings and the subsequent response to anti-tuberculous therapy (ATT). Disease outcome was defined as recovery, recovery with sequelae and death.
Results The time from onset of TM to hospital admission was 15 (2-120) days. The number of lymphocytes in cerebrospinal fluid (CSF) was predominant in 28 (90.3%) patients. The CSF and blood glucose ratio was lower than 0.5 in 28 (90.3%) patients. *M. tuberculosis* was detected in CSF in 4 (12.9%) cases, while this microorganism was isolated in cultures from 12 (38.7%) patients. Continuous ATT was initiated in 29 (93.5%) patients with adverse effects registered in 22 (75.9%) cases. Corticosteroids were given to 23 (74.2%) patients. Complete recovery was recorded in 18 (58.1%) patients. Another 9 (31.0%) patients recovered with permanent neurological squeal. A fatal outcome was noted in 4 (12.9%) cases, 2 (6.5%) of which occurred before diagnosis of TM.
Conclusion Early diagnosis and correct therapy of TM can significantly reduce the high morbidity and mortality associated with this illness.
Keywords: tuberculous meningitis; treatment; outcome
Conflict of interest: None declared.

Gender difference in inflammatory and coagulation factors in hospitalized patients with COVID-19

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Introduction/Objective Male sex is associated with greater severity and mortality from coronavirus disease 2019 (COVID-19), although infection is equally distributed between men and women. The study aimed at investigating sex differences in the hyperinflammatory immune response to SARS-CoV-2 infection and consequent thrombosis using the linked cytokine profile and blood laboratory data.
Methods The observational cohort study involved 99 COVID-19 patients (69 males and 30 females), hospitalized between March 2021 and April 2022. Their clinical/laboratory data were collected to examine sex differences in oxidative stress, neutrophil extracellular traps (NETs) formation and plasma cytokines at hospital admission and up to 5 months of recovery.
Results Dihydrotestosterone (DHT) levels were transiently reduced, while sex hormone binding globulin levels decreased continuously in male post-COVID-19 patients after the rise at diagnosis. Pro-inflammatory interleukin-6 (IL-6) and

interferon-gamma were generally increased at diagnosis, while IL-6 level fell in post-COVID-19 patients. Tumor necrosis factor-alpha exhibited a 5-fold increase in females at diagnosis. The chemokines IL-8 and monocyte chemoattractant protein-1 and the coagulation markers intercellular adhesion molecule-1 and E-selectin were consistently upregulated in female COVID-19 and post-COVID-19 patients, in contrast to vascular cell adhesion molecule-1 and P-selectin. DHT increased reactive oxygen species (ROS) in neutrophils of male patients, while estrogen decreased ROS in female patients. NET markers, such as circulating DNA and myeloperoxidase, were significantly increased in the plasma of patients. Sex hormone levels were positively correlated with coagulation markers.
Conclusion Markers of chemotaxis, endothelial dysfunction and inflammation are still detectable and partially sex dependent in COVID-19 patients 5 months after hospital admission.
Keywords: COVID-19; sex hormones; neutrophil extracellular traps; oxidative stress; cytokines
Conflict of interest: None declared.

Advanced colorectal adenomas in healthy members of families with Lynch syndrome

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Introduction/Objective Lynch syndrome (LS) is an autosomal dominant disorder characterized by early age of onset, the development of cancers in different organs and microsatellite instability. According to Amsterdam criteria at least three relatives may have colorectal or LS-associated cancer. Synchronous and metachronous tumors are common in LS. The aim of our study was to analyze the colonoscopy findings in healthy members of families with LS knowing that colorectal cancer arises from colorectal adenomas.
Methods Complete colonoscopies up to the cecum were performed in 68 healthy members of 16 families with LS. All colorectal polyps were removed by snare polypectomy or mucosectomy. Advanced adenomas (AA) were defined by a villous structure and/or high-grade dysplasia and/or a diameter of 10 mm or more.
Results In 33 (48.5%) healthy members, 42 adenomas (5-25mm) were detected in all colorectal segments. Thirty (71.4%) adenomas were detected proximal to the splenic flexure. AA were found in 10 (14.7%) healthy members, 6 of them in the cecum and ascending colon, 2 in the transverse and 2 in the descending colon. One healthy member had synchronous AA in different segments of the colon.
Conclusion AA are common in healthy members of families with LS. Complete colonoscopy to the cecum is the diagnostic method of choice. Polypectomy or mucosectomy of adenomas, particularly AA breaks the last link in the chain from adenoma to colorectal carcinoma and thus prevents the development of colorectal carcinoma.
Keywords: Lynch syndrome; advanced adenoma; colonoscopy
Conflict of interest: None declared.

Women's oral health as a public health indicator (Case Study Serbia)

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Introduction/Objective Prevalence of oral diseases is over 90% and gender also plays an important role. Women show better preventive behavior in oral health than men, but their dentition is often incomplete.
The aim of this study was to examine the state of women's oral health in Vojvodina and the use of dental health care (demographic, socio-economic determinants, dental anxiety) to assess the impact of oral health on women's quality of life.
Methods The research was conducted as an epidemiological cross-sectional study. Questionnaires: on general and dental health status, on the impact of oral health on quality of life (OHIP-14), for the assessment of dental anxiety (DAS), and the Modified Oral Health Assessment Form for Adults of the World Health Organization, were used.
Results 1900 women aged 16 years and over were included. The results showed that the better dental and periodontal status of women was negatively correlated with age (t=24,242; p=0,000) and positively correlated with education (χ2test; χ2=70,919; p=0,000), material condition (χ2 test; χ2=67,716; p=0,000) and employment status (χ2 test; χ2=30,630; p=0,000). The most important predictors of good oral health of women were a high level of education and financial status, employment, the existence of partners and social support. The coverage of women with regular dental examinations was less than 20%.
Conclusion This research confirmed the public health importance of women's oral health. Our results are useful for future research and the creation of programs for prevention of oral diseases and improvement of oral health in women.
Keywords: oral health; women; public health
Conflict of interest: None declared.

Subacute thyroiditis (De Quervain) during the COVID-19 pandemic

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Introduction/Objective Dysfunction of the thyroid gland, usually transient, was observed in approximately 15% of patients with mild to moderate symptoms of COVID-19 since the receptor for ACE2, used by SARS-CoV-2 virion for cell entry, is highly expressed in thyroid tissue. Subacute thyroiditis (SAT) is an inflammatory disorder of the thyroid gland associated with viral infection (direct viral toxicity or an inflammatory response to the virus): mumps, measles, rubella, coxsackie, and adenoviruses. There is increasing evidence that SARS-CoV-2 can also be considered responsible for causing subacute and atypical thyroiditis. The aim of the study was to analyze the effect of COVID-19 infection on appearance and disease course of SAT.
Methods In the period 2006–2021, a total of 66 patients were treated for SAT at our clinic. During the COVID-19 pandemic (years 2020 and 2021), seven new patients with SAT were presented. In year 2022 no new patients were registered at our clinic. The diagnosis was made on the basis of the anamnesis (pain in the neck and thyroid gland), high erythrocyte sedimentation rate and CRP, ultrasound, and occasionally thyroid aspiration cytology (epithelioid cell findings).
Results In four out of seven patients who previously had COVID-19, SAT had an atypical course: two patients had normal ultrasound findings, one patient had anamnesis of a painful neck, while the palpation findings during the ultrasound examination of the thyroid gland were normal, and one suffered a relapse of SAT after five years.
Conclusion SAT after SARS-CoV-2 infection has presented an atypical course in four out of seven patients treated at our institution. More investigation is required in order to associate the atypical course of SAT with SARS-CoV-2 infection.
Keywords: COVID-19; thyroid gland; subacute thyroiditis;, SARS-CoV-2 infection
Conflict of interest: None declared.

Development of the TAVR program at the Institute for Cardiovascular Diseases of Vojvodina

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Introduction/Objective Aortic stenosis is the most common valvular heart disease in elderly patients. In patients with high risk for surgical aortic valve replacement, TAVR (transcatheter aortic valve replacement) is method of choice. Since its introduction in 2002, the number of TAVR has been growing exponentially. We present the results of the TAVR program at the Institute for Cardiovascular Diseases of Vojvodina.
Methods During 2022, the procedure was performed in 23 patients with symptomatic severe aortic stenosis and high risk for surgical aortic valve replacement. The decision to perform the TAVR procedure was made by the TAVI team.
Results Mean age of the patients was 75.6 years and 57% of patients were men. Medtronic Evolut R valve was implanted in 16 patients (69.5%), the Abbott Portico valve in 7 patients (30.5%). Direct valve implantation was performed in 56.5% of patients. Valve predilatation was performed in 43.5% of patients, while valve postdilatation in 17.4% of patients. In all patients, the procedure was performed through a transfemoral access. Ultrasound-guided puncture was performed in 65.2% of patients, and in 34.8% of patients it was guided by angio-guidewire-ultrasound. Aortic regurgitation was not registered in 52.6% of patients, and mild aortic regurgitation was registered in 47,4% of patients. The average peak to peak gradient is 7 mmHg. The mean value of maxPg was 16.8 mmHg. In one patient, a pacemaker was implanted after the procedure. Vascular complications were noted in 11.8% of patients.
Conclusion Results indicate a low percentage of complications with favorable outcome in patients treated with the TAVR procedure.
Keywords: aortic stenosis; TAVI; transfemoral access
Conflict of interest: None declared.

Effects of metformin and its combinations with other repurposed drugs on fibrosarcoma in hamsters

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Introduction/Objective Many drugs registered for various other indications can act selectively on tumor receptors, signaling pathways, metabolic processes, bioenergetic factors, enzymes, proteins and genes that regulate proliferation, apoptosis and neoangiogenesis of the tumor without affecting these activities in healthy cells. The introduction of new drugs is a very long, complex and expensive process of research. Detecting an anticancer effect in drugs already registered for other indications and forming combinations, may directly reduce the time and cost of such research.
Methods The anticancer efficacy of metformin and its combinations with caffeine, itraconazole, nitroglycerin and mebendazole was tested on fibrosarcoma experimentally induced by BHK21/C13 cells in Syrian golden hamsters (6 animals per group, randomly allocated to control and experimental groups, doses equivalent to usual human doses). After animal sacrifice, tumors were excised and their size, biophysical characteristics, histology and immunohistochemistry were assessed. Blood samples were collected for hematological and biochemical analyses and the main organs were toxicologically analyzed. Statistical significance was determined by one-way ANOVA followed by the Student-Newman-Keuls post hoc test.
Results Only two-drug combinations of metformin with caffeine or itraconazole or nitroglycerin showed significant antitumor effects on hamster fibrosarcoma compared to control, regarding all tested tumor parameters ($P < 0.05$) without toxicity.

Conclusion Administration of metformin in combination with caffeine or itraconazole or nitroglycerin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment.
Keywords: metformin; caffeine; itraconazole; nitroglycerin; hamsters; fibrosarcoma
Conflict of interest: None declared.
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Experimental evaluation of the effects of anticancer modulation therapy on MAPK/PI3K/AKT/mTOR/NF-kB signaling with non-toxic drugs

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Introduction/Objective The large diversity in molecular mechanisms of cancer regulation allows some marketed pleiotropic non-oncological non-toxic pharmaceuticals to be used in oncology, which may reduce the duration and cost of research on novel anticancer treatment. At present, there are no published results *in vivo* on the anticancer effects of certain combinations of non-oncological pleiotropic drugs (disulfiram, diclofenac, nitroglycerin, metformin, deoxycholic acid, mebendazole) that influence MAPK/PI3K/AKT/mTOR/NF-kB signaling.
Methods The anticancer effects of the aforementioned repurposed drug combinations at 20-50% LD_{50} (equivalent to the usual human dose) were assessed by fibrosarcoma growth kinetics (measured daily *in vivo* with calipers) and tumor apoptosis markers (COX4, cytochrome C) in hamsters, randomly allocated to control and experimental groups (6 animals per group). The animals were sacrificed 15-18 days after BHK-21/C13 tumor inoculation. Tumors were excised, measured and blood collected. Biophysical, pathohistological, toxicological, hematological, biochemical and statistical analyses were performed.
Results Disulfiram with metformin, disulfiram with deoxycholic acid and deoxycholic acid with metformin were combinations that showed significant antitumor effects on fibrosarcoma growth kinetics and tumor apoptosis markers in hamsters ($P < 0.05$). All examined drugs in efficacious combinations could inhibit MAPK/PI3K/AKT/mTOR/NF-kB signaling. Addition of the NF-kB stimulator, mebendazole, to effective two-drug combinations rescued cancer growth, indicating that these pathways may be responsible for the antitumor action.
Conclusion The combinations of non-oncological drugs: disulfiram with metformin, disulfiram with deoxycholic acid and deoxycholic acid with metformin have the potential to be used as effective non-toxic adjuvant anticancer therapy in oncology.
Keywords: disulfiram; deoxycholic acid; metformin; hamsters; BHK-21/C13; fibrosarcoma; signal pathway
Conflict of interest: None declared.
Acknowledgment. This study was supported by the Provincial Secretariat for High Education and Scientific Research, AP of Vojvodina, grants no.142-451-2676/2021 (JM), 142-451-2626/2021 (DL) and Ministry of Education, Science and Technological Development , Republic of Serbia, grant no. 451-03-68/2022-14/200114.

Cardiovascular precision medicine – the role of pharmacogenetic testing

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Introduction/Objective Dual antiplatelet therapy (DAPT) with aspirin plus a $P2Y_{12}R$ inhibitor (clopidogrel or 3rd generation drugs such as ticagrelor or prasugrel) is the standard of care after percutaneous coronary intervention (PCI) to reduce the risk of major adverse cardiovascular events (MACEs). Clopidogrel is a prodrug that requires CYP2C19-catalyzed metabolism to its active form. The gene for the enzyme CYP2C19 is highly polymorphic, so we may distinguish normal metabolizers (NM), intermediate metabolizers (IM) and poor metabolizers (PM). The main objective of our study was to identify IM and PM patients who should be treated with ticagrelor or prasugrel and NM patients, who should receive clopidogrel after PCI for prevention of MACEs.
Methods Using the PCR method in DNA from whole blood of patients after PCI, we analyzed the genotype of 70 patients of average age 66.89 years.
Results The results of our study are as follows: among female patients 71.43% were NM and 28.57% were PM; among male patients 80% were NM, 17.14% were IM and 2.86% (one patient) PM.
Conclusion According to our results that are compatible with genotyping data from other studies on the European population, we may conclude that more than 70% of our patients ≥65 years old are NM and may receive genotype-guided long-term clopidogrel therapy for prevention of MACEs after PCI. This pharmacogenetic testing enables a precision approach in cardiovascular medicine, as for older patients clopidogrel shows a safer profile (lower rate of bleeding events) in comparison with prasugrel and ticagrelor.
Keywords: dual antiplatelet therapy; pharmacogenetic testing; percutaneous coronary intervention; clopidogrel; precision medicine
Conflict of interest: None declared.
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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.

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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.

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